Differential microglial activation in neurodegenerative diseases

Peixuan Pey

Imperial College London
Department of Medicine
Charing Cross Campus
St Dunstan's Road
London, W6 8RP

This thesis is submitted for the degree of Doctor of Philosophy

May 2013

Abstract

Inflammation is thought to be a pathological feature that drives the neuronal degeneration in Alzheimer (AD) and Parkinson's diseases (PD). This is normally associated with classically activated microglia, which release pro-inflammatory cytokines. Recently, the heterogeneous nature of microglial activation states has been acknowledged and the focus has been shifting to the alternative activation state, an immunosuppressive state that promotes tissue maintenance and repair. This project examined microglia phenotypes in AD and PD, focusing on alternative activation and phagocytosis. Traumatic brain injury (TBI) cases were also studied to provide a comparison between an acute inflammatory reaction and chronic inflammation observed in AD and PD.

An initial test using a battery of antibodies against microglia e.g. MHCII, Iba1, CD68, MRC1 was used to identify interesting targets for characterization in AD and PD. CD163 and CD14, both of which are thought to be exclusive to perivascular macrophages, were detected in parenchymal microglia in AD and PD, with a much more florid reaction observed in AD. Many of these microglia were associated with extracellular pathology i.e. Aβ plaques and extracellular Lewy Bodies. Experimental evidence from AD, PD and TBI cases suggest that these microglia were of local and systemic origin. A number of clinicopathological correlations were found in PD cases. Upregulation of CD14+ microglia in the substantia nigra was significantly correlated with absence of gait and balance problems as an onset symptom. Upregulation of CD163+ microglia in the cingulate cortex, entorhinal cortex, and locus coeruleus was significantly correlated with a lack of anxiety. In TBI cases, positive association was found between the upregulation of CD163+ microglia and survival time. My study demonstrates differential microglial activation in AD and PD, emphasizes the heterogeneity of microglia phenotype, and provides an insight into the influence of systemic inflammation on microglial activation.

Declarations

I declare that this thesis is solely my own work and effort, and has not been submitted elsewhere for any other qualifications. All other sources of information used in this thesis have been acknowledged.

Copyright statement

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution-Non Commercial-No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or distribution, researchers must make clear to others the licence terms of this work.

Acknowledgements

My deepest gratitude to my supervisor Professor Stephen Gentleman for giving me the opportunity to do this PhD. He is a terrific teacher and mentor, and has given me fantastic advice, help and care during my time as his student. I truly appreciate that he has been generous with his time despite being very busy. He is also very patient and is certainly one of the nicest and kindest person I have ever met.

I would also like to thank Dr Ronald Pearce and Dr Magdelena Sastra, both of whom have been most approachable and helpful. I am very grateful to Dr Pearce for his expert guidance with regards to my project and his counsel for my health and well-being. Dr Sastre is unbelievable in her attention to detail and her guidance in the laboratory has made it one of the most enjoyable and informative laboratory sessions. I am also very grateful to her post-doctorate- Dr Amy Birch, for her help.

The staff of the UK Parkinson's Disease Society tissue bank have been extremely competent and kind. I would like to thank Dr Gervic Djordje (manager), Mrs Louisa McGuiness, and Ms Julia Stiles for their support and friendship. I would also like to thank Dr Federico Roncaroli and Dr Illaria Bravi who have been very helpful whenever I approached them for advice.

John Defelice, our laboratory manager, has provided indispensable support in the laboratory. He is most efficient and well-organized, making my laboratory experience a lot easier. I am very grateful for his help and friendship.

Dr Chiara Marini Bettolo and Dr Hannah Rogers have given me unbelievable encouragement. Chiara has been most wonderful and caring, and I am fortunate to have her as a friend. I have been extremely lucky to meet Dr Hannah Rogers. She has given me life-changing advice and support, for which I cannot thank her enough.

My family has played the biggest role in supporting me emotionally and financially through this PhD. Both my sisters have provided me with immense support, love, company, care and laughter. My deepest gratitude to my grandpa for his love and generosity; without his help I would never have been able to do this PhD. Last but not least, to my mom and dad, who have been tremendously sacrificial and supportive through these years, and have done so much more than can be expected of parents. I would also like to thank my pet, who has always been there for me.

Finally, I would like to thank Imperial College London for its great student welfare, financial support and the opportunity to do this PhD.

Table of Contents

Title page **1**
Abstract **2**
Declarations **3**
Acknowledgements **4**
Table of Contents **5**
List of tables **11**
List of figures **12**
List of abbreviations **15**
Chapter 1: Introduction **19**
1.1 Inflammation and Microglia 19
1.1.1 Microglia and Macrophages 19
1.1.2 Defining the phenotypes and roles of microglia and activation states 20
1.1.3 Pro-inflammatory function of microglia 24
1.1.4 Anti-inflammatory function of microglia 25
1.1.4.1 Alternative activation of microglia 26
1.1.4.2 Acquired deactivation of microglia 26
1.1.5 Pro-inflammatory vs Anti-inflammatory functions of microglia 28
1.1.6 Acute vs chronic inflammation 28
1.1.7 Systemic inflammation and its effects in the brain 29
1.1.8 Differentiating between peripheral and parenchymal macrophages 30
1.2 Alzheimer's Disease 31
1.2.1 Epidemiology of AD 31
1.2.2 History of AD 31
1.2.3 Neuropathology of AD 32
1.2.4 Aβ plaques and APP 33
1.2.4.1 Structure and properties of APP 33
1.2.4.2 APP processing and formation of Aβ 33
1.2.4.3 Structure and formation of Aβ plaques 34
1.2.4.4 Correlation to clinical symptoms 36
1.2.5 Cerebral amyloid angiopathy 36
1.2.6 Tau pathology 37
1.2.6.1 Staging of neurofibrillary pathology 40
1.2.6.2 Relationship between Aβ and Tau pathology 41
1.2.7 Causes of AD – Sporadic and Genetic 42
1.2.8 Clinical criteria and ratings for AD and dementia 44

1.3 Neuroinflammation in Alzheimer's Disease 45
1.3.1 Relationship between microglia and Aβ 45
1.3.2 Relationship between microglia and neurofibrillary pathology 46
1.3.3 Alternative activation in AD 47
1.3.4 Treatment – Immunization in AD 48
1.3.5 Effect of NSAIDs on AD 48
1.4 Parkinson Disease 50
1.4.1 Epidemiological aspects of PD 50
1.4.2 History of PD 51
1.4.3 Neurotransmitter Deficits in PD 51
1.4.4 Clinical aspects of PD 52
1.4.4.1 Motor deficits 52
1.4.4.2 Non-motor Symptoms 53
1.4.4.3 Dementia 54
1.4.4.4 Anxiety and Depression 54
1.4.4.5 Psychosis 55
1.4.5 Neuropathology of PD 56
1.4.6 Neuronal loss in PD 56
1.4.7 Lewy inclusions and α-synuclein 56
1.4.8 Ubiquitin-proteosome system 60
1.4.9 Staging of Lewy related α-synuclein pathology 60
1.4.10 Causes of PD 61
1.4.10.1 Genetic causes of PD 61
1.4.10.2 Toxins 62
1.5 Neuroinflammation in Parkinson's Disease 63
1.5.1 Inflammation caused by α-synuclein 64
1.5.2 Inflammation caused by neuromelanin 64
1.5.3 Oxidative Stress 65
1.5.4 Trophic support in PD 65
1.6 Traumatic brain injury 67
1.6.1 Pathophysiology of TBI 67
1.6.2 Focal brain injury 68
1.6.3 Diffuse brain injury 68
1.6.3.1 Diffuse Axonal Injury 69
1.6.4 Chronic Traumatic Encephalopathy 70
1.6.5 Long-term effects of TBI 70

1.7 Neuropathology of TBI 71
1.7.1 Aβ 71
1.7.2 Tau 71
1.7.3 Inflammation 72
Hypothesis 73
Microglia phenotype, an indication of classical and alternative activation states, differs in AD, PD and TBI. **..... 73**
Aims 73
Chapter 2: Materials and Methods 75
2.1 Tissue specimens 75
2.1.1 Clinical assessments of cases 75
2.1.2 Neuropathological assessment of cases 76
2.1.3 Sectioning and processing of formalin-fixed, paraffin-embedded tissue 76
2.2 Antibodies 77
2.3 Methodology of immunohistochemistry 78
2.4 Methodology of double immunofluorescence 79
2.5 Analysis of immunohistochemical staining 80
2.6 Protein extraction and quantification 82
2.6.1 Protein extraction 82
2.6.2 Protein quantification 82
2.7 Western Blotting 83
2.7.1 Gel preparation 83
2.7.2 SDS-PAGE gel electrophoresis 83
2.7.3 Transfer and Detection 83
2.7.4 β-actin normalization 84
2.8 Enzyme-linked immunosorbant assay 85
Chapter 3: Evaluating microglia phenotypes in AD 86
3.1 Introduction 86
3.1.1 Non-specific markers 86
3.1.1.1 MHCII 86
3.1.1.2 CD68 87
3.1.1.3 Iba1 87
3.1.1.4 MRC1 87
3.1.2 Classical activation markers 88
3.1.2.1 IL-1α 88
3.1.2.2 TNFα 88

3.1.3 Alternative activation markers 89
3.1.3.1 Arginase 1 and 2 89
3.1.3.2 CD36 89
3.1.3.3 BDNF 90
3.1.3.4 IL-10 90
3.1.3.5 CD163 and CD14 91
3.2 Aims 91
3.3 Materials and methods 92
3.3.1 Case selection 92
3.3.2 Immunohistochemistry 94
3.3.3 Pretreatments 94
3.4 Results 97
3.4.1 Negative results using IHC on paraffin-embedded, formalin-fixed tissue 97
3.4.2 Markers for identifying microglial activation are uniform within AD 97
3.4.3 Astrocytic labeling by IL-1α 102
3.4.4 Markers exclusive to perivascular macrophages are observed in the diseased brain parenchyma 103
3.5 Discussion 106
Chapter 4: Characterization of CD14 immunoreactivity in AD and LBD 108
4.1 Introduction 108
4.1.1 Structure of CD14 108
4.1.2 Functions of CD14 109
4.1.3 Inflammatory roles of CD14 109
4.1.4 Expression of CD14 109
4.2 Aims 110
4.3 Materials and Methods 111
4.3.1 Case selection 111
4.3.2 Immunohistochemistry 115
4.3.3 Double Immunofluorescence 116
4.3.4 Semi-quantitative assessment of CD14+ microglia 116
4.3.5 Statistical analysis 118
4.4 Results 119
4.4.1 Mild upregulation of CD14 immunoreactivity by parenchymal microglia in control cases 119
4.4.2 PVM and parenchymal microglia are CD14 immunopositive in AD and LBD 119
4.4.3 CD14 immunoreactivity is more extensive in AD than in LBD cases 119

4.4.4 Age at death correlates positively with immunoreactivity of CD14 in AD 122
4.4.5 Correlation between CD14 load and clinical and motor symptoms.....122
4.4.6 CD14+ microglia are associated with Aβ plaques and α-syn pathology, but not tau pathology.....125
4.5 Discussion.....128
Chapter 5: Characterization of CD163 immunoreactivity in AD and LBD 130
5.1 Introduction 130
5.1.1 Structure of CD163 130
5.1.2 Functions of CD163 132
5.1.3 Inflammatory roles of CD163 132
5.1.4 Expression of CD163 132
5.2 Aims 133
5.3 Materials and methods.....134
5.3.1 Case selection.....134
5.3.2 Immunohistochemistry 137
5.3.3 Double Immunofluorescence 137
5.3.4 Semi-quantitative assessments of CD163+ microglia.....139
5.3.5 Western Blot.....140
5.3.6 ELISA.....140
5.3.7 Statistical analysis.....140
5.4 Results 141
5.4.1 CD163 immunoreactivity is restricted to PVM in majority of control cases 141
5.4.2 PVM and parenchymal microglia are CD163 immunopositive in AD and LBD 141
5.4.3 CD163 immunoreactivity is more extensive in AD than in LBD cases 144
5.4.4 Do CD163 parenchymal microglia originate from the periphery? 146
5.4.5 Correlation between CD163 %area and clinical symptoms of LBD cases 146
5.4.6 CD163+ microglia are associated with Aβ plaques, but not NFT or α-syn pathology.....152
5.4.7 Cytokines and neurotrophin levels do not correlate with CD163+ microglia load 152
5.4.8 Comparison between different methodologies of assessing CD163 immunoreactivity 152
Figure 35: CD163 quantification using Western blot.....156

5.4.9 Co-localisation of CD163 and CD14 immunoreactivities within the same microglial cells 157
5.4.10 CD163 immunoreactivity in the parenchyma of a haemorrhage 157
5.4.11 CD163+ microglia in non-lesional sites of TBI 157
5.4.12 Presence of β-amyloid protein in TBI cases relates to increasing age at death 157
5.4.13 Survival times and age at death correlate with CD163+ microglia load in TBI cases 162
5.5 Discussion 163
Chapter 6: General discussion 166
6.1 Evaluation of microglia quantification methods 166
6.2 Functions and inflammatory states of CD163 and CD14 immunoreactive microglia 167
6.3 Heterogeneity of microglial phenotype 170
6.4 Origin of CD14 and CD163 immunoreactive microglia 170
6.5 Distribution of CD14 and CD163 immunoreactive microglia in AD, LBD and TBI 171
6.6 CD163 immunoreactive microglia associated with anxiety symptoms in LBD patients 171
6.7 Future studies 172
References 174
Appendix I 228
Buffers and Solutions 228
Appendix II 230
CD163 %area assessment in AD cases 230
CD14+ microglia load assessment in AD cases 231
Appendix III 232
Clinical data for LBD patients 232
CD163 %area assessment in LBD cases 233
CD14+ microglia load assessment in LBD cases 234
Appendix IV 235
CD163+ microglia load assessment in TBI cases 235
Publication arising from this work 236
Conferences 236

List of tables

Table 1. List of secondary antibodies used in the study……..…..…..78

Table 2. Age, gender and causes of death of AD cases……....93

Table 3. List of antibodies used for characterization of microglial phenotype…....95

Table 4. Additional antibodies and pretreatments tested for characterization…….96

Table 5. Age, gender, causes of death and BNE α-synuclein staging of cases with LBD……..112

Table 6. Age, gender and causes of death of control cases……....114

Table 7. Primary antibodies used with CD14 in double immunofluorescence…...115

Table 8. List of traumatic brain injury cases…….135

Table 9. Primary antibodies used with CD163 in double immunofluorescence….138

List of figures

Figure 1. Morphologies of microglia ……21

Figure 2. Perivascular macrophages……..……22

Figure 3. Astrocytes……23

Figure 4. Abeta accumulation in AD……38

Figure 5. Tau pathology in AD……..……39

Figure 6. Lewy pathology in PD……59

Figure 7. Blot transfer arrangement…….……84

Figure 8. Iba1 as a pan-microglia marker……...99

Figure 9. MHCII immunoreactivity in diseased brains……100

Figure 10. CD68 immunoreactivity in AD cases…….…...101

Figure 11. Immunostaining of astrocytes by IL-1α……..……..102

Figure 12. MRC1 immunoreactivity exclusive to brain macrophages……..……...104

Figure 13. CD14 and CD163 positivity in brain macrophages and parenchymal microglia…….…105

Figure 14. X-ray crystal structure of CD14 (human) ……..…....108

Figure 15. Figure legend of CD14+ microglia load……..……117

.

Figure 16. Immunoreactivity of CD14 in AD, PD, and control cases……..…120

Figure 17. Close association of CD14+ macrophages with blood vessels……....121

Figure 18. Comparison of CD14+ microglia load in various brain regions of AD and LBD cases……...123

Figure 19. Boxplot showing CD14+ microglia load in the substantia nigra in relation to gait and balance impairment as a motor onset symptom in LBD patients……..…….…..…..124

Figure 20. Double immunofluorescence of CD14 with Aβ pathology……..126

Figure 21. Double immunofluorescence of CD14 with tau pathology……..…127

Figure 22. Double immunofluorescence of CD14 with α-synuclein pathology…..127

Figure 23. Schematic representation of the structure and domain organization of membrane-bound CD163……..…131

Figure 24. IHC detection of CD163…….……..142

Figure 25. Range of CD163 immunoreactivity in microglia of AD cases……..…...143

Figure 26. Comparison of percentage area of CD163+ microglia in various brain regions of AD and LBD cases…….……..145

Figure 27. Double immunofluorescence of CD163 with microglia/macrophage markers Iba1 and MRC1…….……..……..147

Figure 28. Immunofluorescence staining against fibrinogen……..……..……148

Figure 29. Double immunofluorescence of CD163 with fibrinogen…….……149

Figure 30. Double immunofluorescence detection of CD163 with CAA……....150

Figure 31. Level of CD163 immunoreactivity in LBD patients with anxiety symptoms……..…..151

Figure 32. Double immunofluorescence of CD163 with Aβ……..…..153

Figure 33. Double immunofluorescence of CD163 with CD68,
tau and α-synuclein pathology…….....…..…154

Figure 34. Scatter plot of expression levels of IL-10 against TNFα
in LBD cases……..……...155

Figure 35. CD163 quantification using Western blot…….……156

Figure 36. Double immunofluorescence of CD163 and CD14……..…..158

Figure 37. CD163 immunoreactivity in a haemorrhage……159

Figure 38. CD163 immunoreactivity in non-lesional areas of TBI……..……160

Figure 39. Boxplot showing the association between the presence of
Aβ plaques and age at death of TBI patients…….……..….161

Figure 40. Boxplot showing the association between survival time and
CD163+ microglia load in the cortices of TBI patients……..…..162

List of abbreviations

+:	Immunopositive
%area:	Percentage area
[^{11}C]PiB:	Pittsburgh compound B
6-OHDA:	6-hydroxydopamine
Aβ:	Amyloid beta peptide
ABTS:	2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium
AD:	Alzheimer's disease
ADL:	Activities of daily living
Ala:	Alanine amino acid
APP:	Amyloid precursor protein
Arg1/2:	Arginase 1/2
α-syn:	Alpha synuclein
BBB:	Blood brain barrier
Bcl:	B-cell lymphoma
BDNF:	Brain-derived neurotrophic factor
BNE:	BrainNet Europe
BSA:	Bovine serum albumin
CA1-4:	Cornu Ammonis 1-4
CAA:	Cerebral amyloid angiopathy
CD:	Cluster of differentiation
CDR:	Clinical dementia rating
CERAD:	The Consortium to Establish a Registry for Alzheimer's Disease
CNS:	Central nervous system
COX-2:	Cyclooxygenase-2
CPM:	Choroid plexus macrophages
CSF:	Cerebrospinal fluid
CTE:	Chronic traumatic encephalopathy
C-terminus:	Carboxyl terminus
CVA:	Cerebrovascular accident/ Stroke
DA:	Dopaminergic
DAB:	3,3'-diaminobenzidine
DAI:	Diffuse axonal injury
DAMP:	Damage-associated molecular pattern
DAPI:	4',-6-diamidino-2-phenylindole
DIF:	Double immunofluorescence

DLB: Dementia with Lewy bodies

DMV: Dorsal motor nucleus of the vagus

DRN: Dorsal raphe nuclei

ECM: Extracellular matrix

EDH: Extradural haematomas

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme-linked immunosorbant Assay

FIZZ1: Inflammatory zone 1

FTDP: Frontotemporal dementia

GABA: Gamma-Aminobutyric acid

GDNF: Glial-derived neurotrophic factor

GDS: Global deterioration scale

GI: Gastrointestinal

GM-CSF: Granulocyte macrophage colony-stimulating factor

H_2O_2: Hydrogen peroxide

hAPP: Human Amyloid precursor protein

Hb: Haemoglobin

HIV: Human immunodeficiency virus

HIVE: Human immunodeficiency virus encephalitis

Hp: Haptoglobin

HP-tau: Hyperphosphorylated tau

HRP: Horse-radish peroxidase

Iba1: Ionized calcium binding adaptor molecule 1

ICD: International Classification of Diseases

ICH: Intracerebral haemorrhages

ICP: Intracranial pressure

IFNγ: Interferon gamma

Ig: Immunoglobulin

IHC: Immunohistochemistry

IL: Interleukin

IMS: Industrial methylated spirit

iNOS: Inducible nitric oxide synthase

JNK: c-Jun N-terminal kinase

LB: Lewy body/bodies

LBD: Lewy body disease

LC: Locus coeruleus

L-dopa: Levodopa

LN: Lewy neurites

LPS: Lipopolysaccharide

MAP: Microtubule-associated protein (MAP)

M-CSF: Macrophage colony stimulating factor

MHCII: Major histocompatibility complex II

MM: Meningeal macrophages

MMP: Matrix metalloproteinase

MMSE: Mini-mental state examination

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MPP+: 1-methyl-4-phenylpyridinium

MRC1: Mannose receptor complex I

MRI: Magnetic resonance imaging

MS: Multiple sclerosis

MT: Microtubule

NADPH: Nicotinamide adenine dinucleotide phosphate

NBM: Nucleus basalis of Meynert

NGF: Nerve growth factor

NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells

NFT: Neurofibrillary tangles

NIH: National Institutes of Health

NM: Neuromelanin

NMS: Non-motor symptoms

NO: Nitric oxide

NOD: Nucleotide-binding oligomerization domain

NSAID: Non-steroidal anti-inflammatory drug

N-terminus: Amino-terminus

NT: Neuropil threads

p: Probability

PAMP: Pathogen-associated molecular patterns

PBS: Phosphate-buffered saline

PD: Parkinson's disease

PDD: Parkinson's disease with dementia

PET: Positron emission tomography

PHF: Paired helical filaments

PIGD: Postural instability and gait disturbances

PINK: PTEN-induced putative kinase

Pro: Proline amino acid

PS: Phosphatidylserine

PSEN: Presenilin

PST: Proline, serine, threonine residues

PVDF: Polyvinylidene difluoride

PVM: Perivascular macrophages

RAGE: Receptor for advanced glycation end products

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SAH: Subarachnoid haemorrhages

SDH: Subdural haematomas

SDS: Sodium dodecyl sulfate

Ser: Serine amino acid

SIV: Simian immunodeficiency virus

SN: Substantia nigra

SNpc: Substantia nigra pars compacta

SPECT: Single-photon emission computed tomography

SRCR: Scavenger receptor cysteine-rich

STAT3: Signal transducer and activator of transcription 3

SVZ: Subventricular zone

TAI: Traumatic axonal injury

TBI: Traumatic brain injury

TGFβ: Transforming growth factor beta

Thr: Threonine

TLR: Toll-like receptors

TMB: 3,3',5,5'-tetramethylbenzidine

TNFα: Tumor necrosis factor

TRAP: Tremor, rigidity, akinesia and postural instability

Trk: Tyrosine-related kinase B

UCH-L1: Ubiquitin-C terminal hydrolase-L1

UKPDSTB: Parkinson's UK Tissue Bank

UPDRS: Unified Parkinson's Disease rating scale

UPS: Ubiquitin-proteosome system

UTI: Urinary tract infection

WB: Western blotting

Ym1: Chitinase 3-like 3

Chapter 1: Introduction

1.1 Inflammation and Microglia

Inflammation is a defense mechanism that kicks in whenever a system is afflicted by mechanical injury, pathogenic infection, toxins, irradiation or autoimmunity. It can destroy affected cells and invading pathogens, remove cellular debris, or isolate, protect, and repair cells and/or the extracellular matrix (ECM)(Correale and Villa 2004). The chief mediators of neuroinflammation are microglia and astrocytes. Transient responses are required when the central nervous system (CNS) is compromised, but chronic inflammation as a result of a persistent aggravator e.g. in neurodegenerative diseases, can cause a self-sustaining cycle that can lead to neuronal death (Tansey and Goldberg 2010).

1.1.1 Microglia and Macrophages

Glia cells include microglia (Figure 1), oligodendrocytes, and astrocytes (Figure 3). In a mature human brain, 10-20% of the glial population are microglial cells (Kreutzberg 1995, Borda et al. 2008, Ouchi et al. 2009). Microglia are derived from myeloid progenitor cells that infiltrate through the undifferentiated blood-brain barrier (BBB), and populate the CNS during embryogenesis (Ransohoff, Kivisakk and Kidd 2003). Differential expression of microglia's immunoregulatory markers is found throughout various brain regions (de Haas, Boddeke and Biber 2008). Microglia have a very long lifespan and a slow turnover rate, both from local self-renewal or recruitment from the bone marrow (Ajami et al. 2007, Mildner et al. 2007).

In a normal healthy condition, microglia are known to be in a resting state. Contrary to terminology, microglia are never "resting". They are constantly surveying their environment for damaged neurons, debris, and infectious agents. Their default morphology is that of a ramified form, with highly motile ciliary processes to aid surveillance. It is hypothesized that these microglia are also giving continuous neurotrophic support. With age, microglia in the human brain were shown to become dystrophic e.g. fragmented cytoplasmic processes, and it has been theorized that neurodegeneration occurs as neurons lose their trophic support and protection from microglia (Streit et al. 2009). Upon activation, microglia secrete cytokines, chemokines, complement proteins, trophic factors, reactive oxygen species (ROS), nitric oxide (NO), and proteolytic enzymes, migrate to the affected area, and exhibit a capacity for localized phagocytosis. There is also *de novo* synthesis or upregulation of cell surface molecules (Lue, Walker and Rogers 2001, Perry, Nicoll and Holmes 2010).

In the CNS, the four main resident macrophages are microglia, meningeal, choroid plexus and perivascular macrophages (PVM). PVM (Figure 2) lie between the endothelial cells of cerebral blood vessels and glial basement membranes, also known as the Virchow-Robin space. By virtue of their location, PVM respond to inflammatory stimuli from both within the CNS and from the periphery (Galea et al. 2005). Unlike microglial cells, they are constantly replenished by peripheral/blood-derived monocytes, and also from proliferation of pre-existing PVM (Lassmann et al. 1993, Fabriek et al. 2005). The turnover rate of PVM is several months. PVM have many functions including phagocytosis, drainage of proteins from the brain (Carare et al. 2008), antigen presentation, maintenance of the blood brain barrier (BBB) and the structural integrity of blood vessels, regulation of endothelial cell proliferation, etc. (Mendes-Jorge et al. 2009)

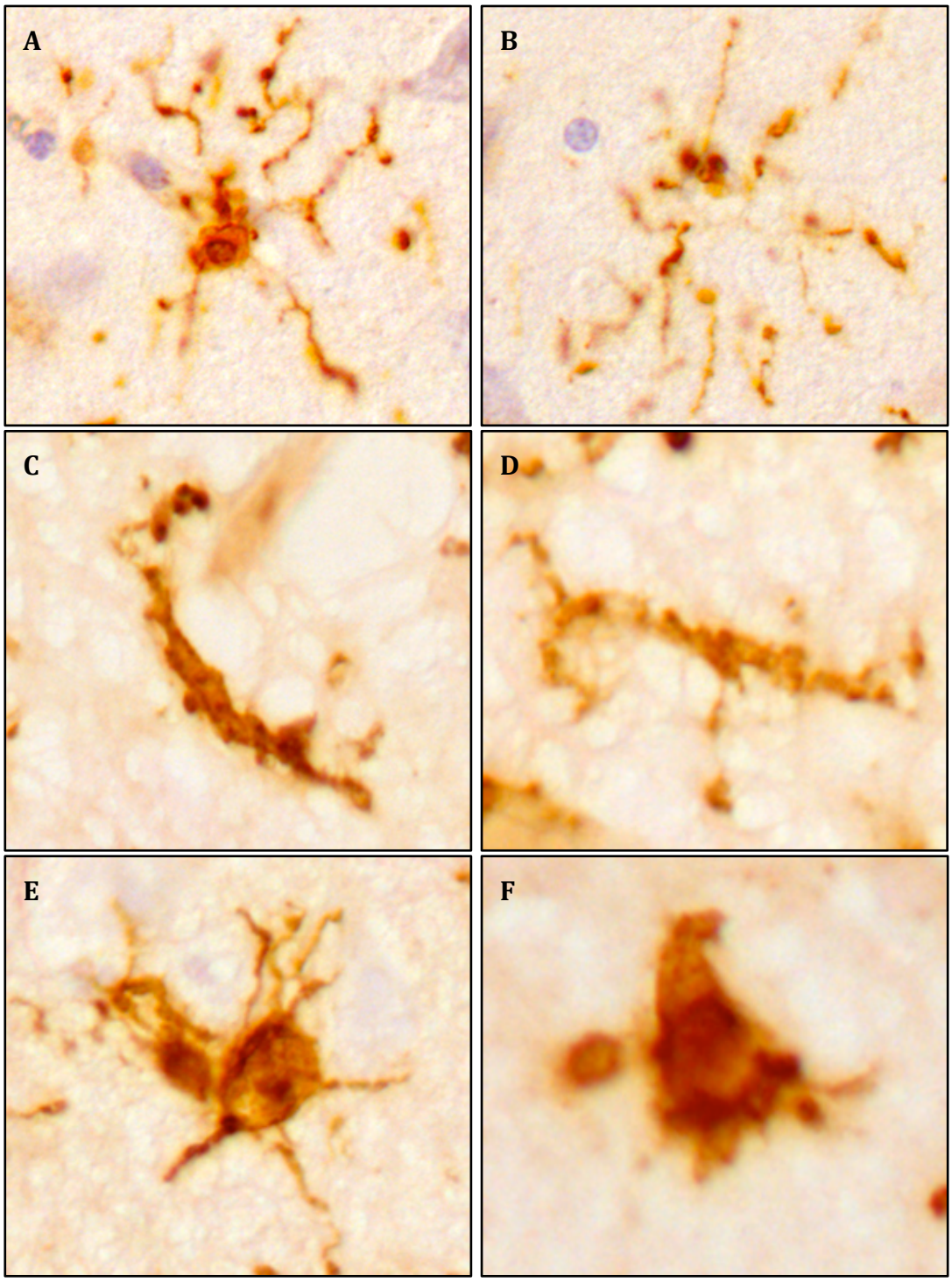
Another type of brain-resident macrophage is the pericyte, which has an elongated and amoeboid morphology that follows the contours of the vascular element. The main difference between pericytes and PVM is their location- pericytes are found within the endothelial basal lamina. It is thought that pericytes are simply intermediates between peripheral monocytes and PVM (Thomas 1999, Vallieres and Sawchenko 2003).

As of yet, there has been no single marker to distinguish between a macrophage and microglia. A combination of three immunological markers can help to distinguish between PVM and microglia. PVM are $CD11b^{+}CD14^{+}$ $CD45^{+}$, while microglia are $CD11b^{+}$ $CD14^{-}CD45^{-}$ (Guillemin and Brew 2004). Some PVM are also not immunoreactive for Iba1, which is a reliable pan-microglia marker (Ito et al. 2001, Mendes-Jorge et al. 2009).

1.1.2 Defining the phenotypes and roles of microglia and activation states

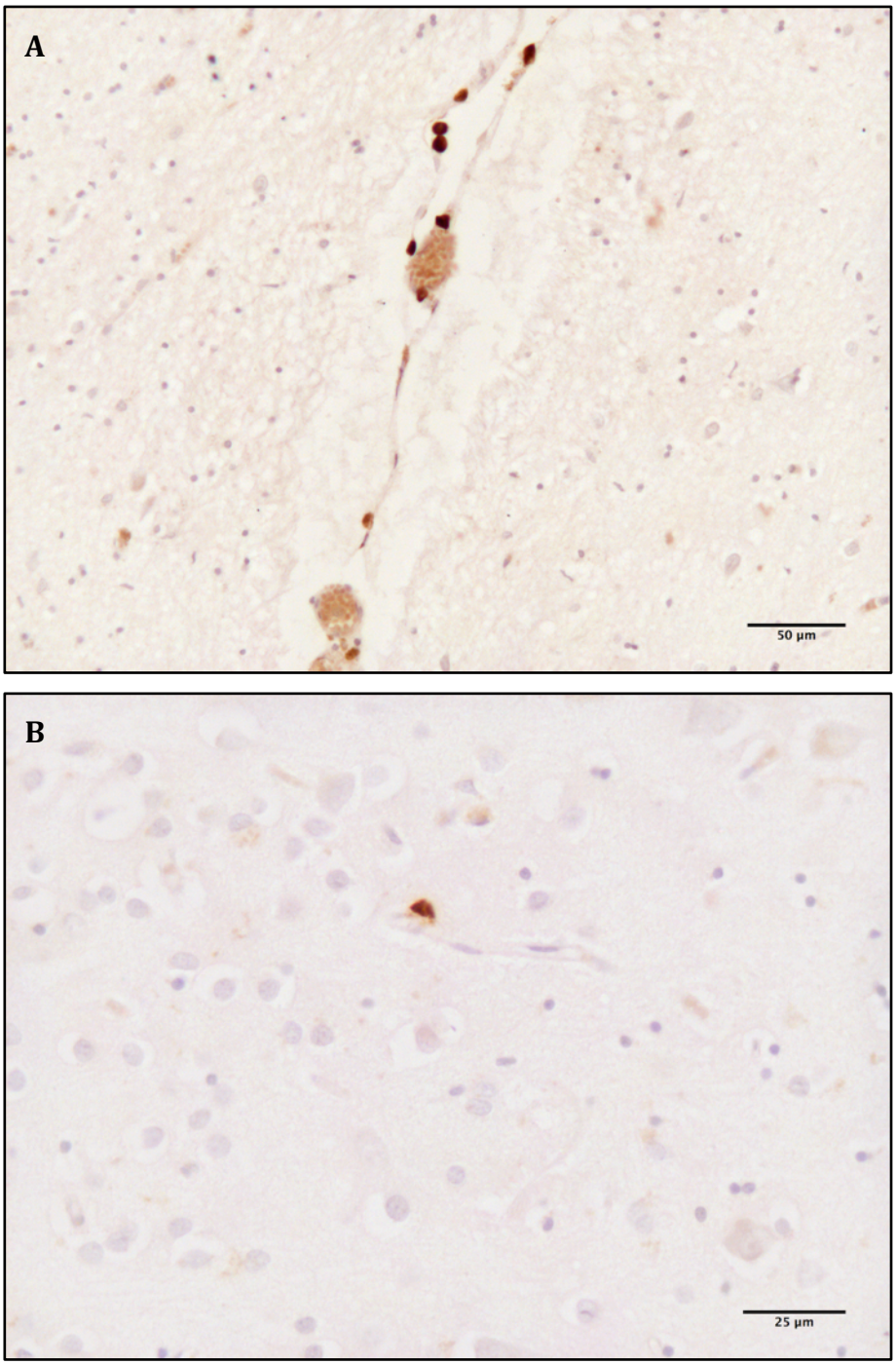
The old school of thought looks at the activation of microglia along a linear scale, from a resting state, to a major transformation when triggered, including hypertrophy of the soma and shortening of the processes into an amoeboid form that is indistinguishable from typical peripheral macrophages. Microglia at their final stages of activation are thought to secrete pro-inflammatory cytokines, reactive oxygen species (ROS) etc., and are capable of phagocytosis. This interpretation of microglial activation is highly inaccurate, as their morphology is a poor indicator of their phenotype. Treatment with pro-inflammatory or anti-inflammatory cytokines results in mixed morphologies (Porcheray et al. 2005). Major histocompatibility complex (MHC)

Figure 1. Morphologies of microglia



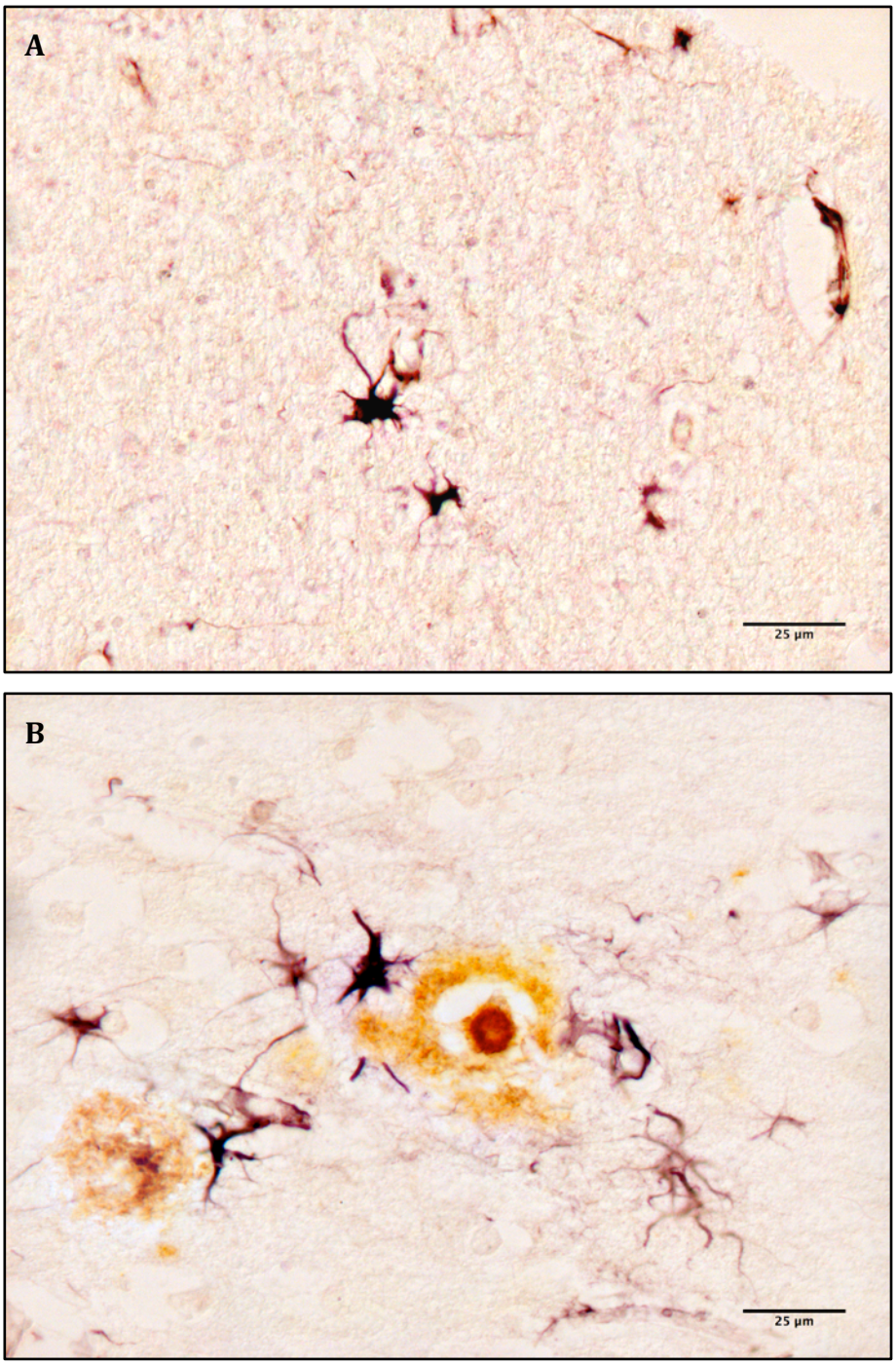
(A&B) Ramified microglia in a control case. Long, thin cell processes are seen extending from the cell body. **(C&D)** Bushy microglia in an Alzheimer case that has an elongated cell body and short, thickened cell processes **(E&F)** Amoeboid microglia in an Alzheimer's case that has a large, round cell body and little to no cell processes. All images were captured at x40 magnification. Anti-Iba1 was used to stain for these microglial cells. Iba1 is a pan-microglial cell marker used to label both resting and activated microglia (Ito et al. 2001).

Figure 2. Perivascular macrophages



(A) Meningeal macrophages in the temporal cortex of an AD case. Magnification x20. **(B)** A perivascular macrophage in the temporal cortex of an AD case. Magnification x40. Anti-MRC1 was used to stain both **A & B**. Anti-MRC1 is a specific marker for perivascular macrophages (Galea et al. 2005).

Figure 3. Astrocytes



(A) Astrocytes in the pons of an AD case. Magnification x40. **(B)** Double staining of astrocytes and Aβ plaques in the pons of an AD case. Magnification x40. Anti-GFAP (glial fibrillary acidic protein) was used to stain for astrocytes in both **A & B**. Anti-GFAP is a marker for astrocytes (Jacque et al. 1978).

class II molecules, which are required for antigen presentation, can be used to distinguish between activated and surveillance microglia (Graeber, Bise and Mehraein 1994). It can be used to label both classical and alternative activation states (Colton and Wilcock 2010).

In recent years, microglia have been found to be heterogeneous with respect to their activation states. A newer approach towards microglia's activation separates them into three categories- classical activation associated with cytotoxicity and the release of pro-inflammatory cytokines; alternative activation and acquired deactivation, both of which are associated with anti-inflammatory cytokines, immunosuppression and tissue repair (Colton 2009). However, it is most likely that their functions, inflammatory and cytokine profiles, and phenotypes overlap to a large extent. The phenotypes acquired by microglia are dependent on many factors, including age, stage of neurological disease, systemic inflammation, and location of misfolded protein – whether it's internal or external to the cell (Perry et al. 2010). Thus, it becomes tricky to use surface antigens and/or morphology to classify/distinguish between different microglia phenotypes.

Macrophages do not have a fixed phenotype and are able to adapt depending on the stimuli (Stout and Suttles 2004). Their functional plasticity disputes the theory that different populations of microglia/macrophages exist- variation in phenotypes might be attributed to preconditioning and their microenvironments. Approaches to studying their progressive phenotypes before and during a neurodegenerative disease becomes a problem when using post mortem brain specimens, which only reflect a static state during the terminal moments of a patient (McGeer and McGeer 2008).

1.1.3 Pro-inflammatory function of microglia

Microglia express several pattern recognition receptors including toll-like receptors, receptor for advanced glycation end products (RAGE) and scavenger receptors, nucleotide-binding oligomerization domain (NOD) proteins, and purinergic receptors (Sterka and Marriott 2006, Salminen et al. 2009). These receptors respond to the presence of damage-associated molecular pattern molecules (DAMPs) which are released from degenerating or injured cells, or pathogen-associated molecular patterns (PAMPs) (Bianchi 2007, Rubartelli and Lotze 2007).

The resulting activation of microglia causes the production of pro-inflammatory cytokines including tumor necrosis factor α (TNFα), interleukin 1β (IL-1β), IL-6, IL-12, interferon γ (IFNγ) (Liu 2006, Colton 2009), reactive nitrogen species (RNS), and reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) and superoxide (Qian and Flood 2008). The non-specificity of these molecules poses a threat to the surroundings and may cause bystander injury. Upregulation of chemokines,

nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, cyclooxygenase-2 (COX-2), secreted and cytosolic phospholipases A2, and other enzymes that generate prostaglandin, act to reinforce and intensify the inflammation reaction (Farooqui, Horrocks and Farooqui 2007).

The chief mediators of cytotoxicity include TNFα (Floden, Li and Combs 2005, Taylor et al. 2005b) and IL-1β (Tikka and Koistinaho 2001), which are able to mediate neuronal apoptosis. These cytokines also act to reinforce the initial microglial response, resulting in an escalating inflammatory reaction (Colton 2009).

Inducible nitric oxide synthase (iNOS) is also upregulated during a classical activation microglia response, generating free radical nitric oxide (NO). Upon reacting with superoxide, a much more lethal oxidant- peroxynitrate, is produced. Recent evidence suggests that peroxynitrate is the main culprit behind cytotoxicity, causing damage by oxidation and nitration of enzymes, lipids, and DNA (Pacher, Beckman and Liaudet 2007).

1.1.4 Anti-inflammatory function of microglia

After an episode of classical activation, microglia/macrophages need to move away from a pro-inflammatory to an anti-inflammatory, reparative phenotype. Resolution of inflammation by reducing pro-inflammatory factors, restoration of tissue homeostasis by promoting repair and replacement of lost or damaged cells, as well as reparation of the extracellular matrix need to take place. This is especially important for the CNS, known to have limited neuroregenerative potential (Colton 2009).

From the study of peripheral macrophages, 4 major anti-inflammatory cytokines have been discovered- IL-4, IL-13, IL-10 and transforming growth factor β (TGFβ) (Martinez, Helming and Gordon 2009). They can be produced by microglia, astrocytes, and/or neurons. IL-4 is produced by astrocytes (Brodie et al. 1998), IL-10 by glial cells (Ledeboer et al. 2000), IL-13 by microglia (Shin et al. 2004) and TGFβ by all three (Finch et al. 1993). These four cytokines enable macrophages to switch from a pro-inflammatory to an anti-inflammatory state by downregulating classical activation pathways and inducing genes and transcribing proteins associated with repair and resolution. This is known as the alternative activation state (Colton 2009).

The classification of the repair/resolution behavior of microglia/macrophages into just one category under alternative activation has been viewed as inadequate. Instead, it has been proposed for the phenotype to be further split into two wide categories: alternative activation and acquired deactivation. In this classification system, alternative activation is microglia's response to IL-4 and IL-13, while acquired deactivation is the

response to IL-10, TGFβ, and/or exposure to apoptotic cells (Gordon 2003, Gordon and Taylor 2005). These two activation states demonstrate similar gene profiles, but can still be differentiated by their type of induction and functions (Colton 2009).

1.1.4.1 Alternative activation of microglia

Alternatively activated microglia has a lower tendency to produce pro-inflammatory cytokines. They are associated with tissue repair and humoral immunity (Gordon 2003). IL-4 and IL-13 share the IL-4 receptor α subchain, and although their signaling pathways diverge, both tend to lead to similar gene profiles (Colton 2009). Both IL-4 and IL-13 suppress production of TNFα, NOS2, COX-2, IL-1β, IL-6 and IL-12 caused by LPS stimulation of microglial/macrophage cell cultures (Ledeboer et al. 2000, Scotton et al. 2005, Colton et al. 2006). Instead, alternative activation state induces an increase in mRNA expression of cytokines and growth factors including IL-10, TGFβ, insulin growth factor, nerve growth factor (NGF), peroxisome proliferation activation receptor γ (PPAR-γ), arginase 1, mannose receptor, inflammatory zone 1 (FIZZ1) and chitinase 3-like 3 (Ym1) (Brodie et al. 1998, Kitamura et al. 2000, Odegaard et al. 2007, Colton et al. 2006, Colton 2009). In mouse microglia cultures, IL-4 opposed the action of IFNγ by inhibiting IFNγ's induction of TNFα and iNOS (Colton et al. 2006).

IL-4 and IL-13 regulate phagocytosis, albeit differently from IL-10 and TGFβ. IL-4 decreases expression levels of CD163 and Fc receptor I and II, while increasing expression of mannose receptor and dectin 1 (Schaer et al. 2002, Gordon 2003, Willment et al. 2003, Nimmerjahn and Ravetch 2006). IL-4 treatment restores phagocytic ability to fibrillar Aβ treated murine microglia even in the presence of LPS and pro-inflammatory cytokines IL-1β and IFNγ (Koenigsknecht-Talboo and Landreth 2005).

1.1.4.2 Acquired deactivation of microglia

Acquired deactivation involves immunosuppression and upregulation of scavenger receptors to enable phagocytosis of apoptotic cells. Both TGFβ and IL-10 induce this state in microglia, while phagocytosis of apoptotic cells stimulates production of IL-10 and TGFβ in a positive feedback manner (Gregory and Devitt 2004). IL-10 exerts its effects downstream via STAT3 (Mosser and Zhang 2008), while TGFβ's signaling is mediated by SMAD (Li et al. 2006). The effects of these two cytokines overlap.

TGFβ and IL-10 act in a positive feedback manner by autocrine and paracrine methods to further increase their production (Colton and Wilcock 2010). They negatively regulate the production of pro-inflammatory cytokines, chemokines, and suppress MHC II protein

expression (Lodge and Sriram 1996, Colton 2009). They also act as growth factors by activating B-cell lymphoma-2 (Bcl-2) and Bcl-X_L (Weber-Nordt et al. 1996, Vivien and Ali 2006). Increased levels of TGFβ and IL-10 are found at tight junctions of the blood-brain barrier (BBB) (Wu et al. 2008).

In LPS-induced rat mesencephelic neuron-glia culture, IL-10 significantly inhibits production of TNFα, NO, IL-1β, IL-6, IL-12 and extracellular superoxide (Lodge and Sriram 1996, Ledeboer et al. 2000, Qian et al. 2006). IL-10 increases expression of IL-1 receptor antagonist, which reduces inflammation by binding and inhibiting the actions of IL-1 (Williams et al. 2002), and CD163, a scavenger receptor most well known for clearing of haemoglobin-haptoglobin complexes (Pioli et al. 2004). IL-10 also upregulates IL-4Rα, a receptor for both IL-4 and IL-13, and IL-4 and IL-10 were found to act synergistically e.g. for the induction of arginase 1 (Biswas et al. 2011). One method by which IL-10 exerts its neuroprotective effect is by activating heme oxygenase 1, which has anti-inflammatory and anti-apoptotic effects (Lee and Chau 2002, Weis et al. 2009).

TGFβ influences many cell functions including cell growth, differentiation, chemotaxis, apoptosis, and hematopoiesis (Qian and Flood 2008). In cell cultures, TGFβ protects 6-hydroxydopamine (6-OHDA) treated neurons (Polazzi et al. 2009, Ruocco et al. 1999). Its neuroprotective capability has also been shown in animal models of ischemia, excitotoxicity, facial nerve axotomy and oxidative stress (Ruocco et al. 1999, Henrich-Noack, Prehn and Krieglstein 1996, Makwana et al. 2007). TGFβ also regulates ROS production in microglia (Herrera-Molina and von Bernhardi 2005). TGFβ is thought to exert neuroprotection directly on neurons, and indirectly via microglia and astrocytes (Qian and Flood 2008). TGFβ negatively regulates pro-inflammatory cytokines TNFα, NO, IL-12 (Lodge and Sriram 1996), and upregulates prostaglandin E2 (Levi, Minghetti and Aloisi 1998). However, TGFβ is an important mediator of cell death in ontogenetic conditions. Ablation of TGFβ in developing chick embryos reduced apoptosis and neuronal loss was significantly decreased (Krieglstein et al. 2000, Dunker, Schuster and Krieglstein 2001).

The task of clearing apoptotic cells in the CNS falls mainly to microglia. There are many scavenger receptors on microglia that serve this purpose, e.g. phosphatidylserine (PS) receptors, CD36 and CD14. The apoptotic cells are recognized by PS on the cell surface. Minghetti et al. 2005 found that microglia co-cultured with apoptotic neurons actively suppressed synthesis of pro-inflammatory cytokines i.e. TNFα and NO, while increasing the release of TGFβ, prostaglandin E_2 and NGF (Minghetti et al. 2005).

1.1.5 Pro-inflammatory vs Anti-inflammatory functions of microglia

While inflammation can act to destroy or protect, there is a misconception that destruction is associated with pro-inflammatory cytokines, while protection is associated with anti-inflammatory cytokines.

The gradual degeneration of neurons often implicated in neurodegenerative diseases has been thought to trigger microglia activation. This can be either classical or alternative activation, or a mixture of both (Colton et al. 2006). Classical activation of microglia causes production and release of cytotoxic and pro-inflammatory cytokines, resulting in a positive feedback loop that give rise to further neuronal degeneration (Akiyama et al. 2000). Apoptosis of neurons in these diseases causes microglia/macrophages to switch to an acquired deactivation phenotype. In both AD patients and transgenic human amyloid precursor protein (hAPP) mice model, levels of pro-inflammatory cytokines are low, and plaques are not removed efficiently, providing evidence for an anti-inflammatory phenotype (Perry, Cunningham and Holmes 2007).

This mixed microglia phenotype observed in neurodegenerative disease results from chronic inflammation where the stimulants for microglia activation are not removed effectively, causing their classical activation state to persist, while apoptosis of neurons fuels microglia to take on alternative activation/ acquired deactivation phenotypes. The inefficient removal of plaques might be attributed to its incessant production and deposition in the ECM. Constant and repeated exposure to Aβ plaques can cause microglia to respond differently to this stimuli (Perry et al. 2007). This phenomenon was observed with repeated stimulation of microglia with LPS, causing a down-regulation of pro-inflammatory cytokines with subsequent stimulations (Ajmone-Cat, Nicolini and Minghetti 2003).

1.1.6 Acute vs chronic inflammation

Acute inflammation comes on rapidly, can be accompanied by pain, and has a spontaneous resolution (Serhan and Savill 2005). In contrast, chronic inflammation persists over the years and continues to exert damage to neurons through release of pro-inflammatory cytokines, chemokines and ROS (Farooqui and Farooqui 2011). Long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) have been found to decrease the risk of developing AD and PD, further emphasizing the role of chronic inflammation in neurodegenerative diseases.

1.1.7 Systemic inflammation and its effects in the brain

Systemic inflammation reaches the CNS via both neural and humoral pathways. There are three routes of communication between peripheral inflammation and the CNS. First, the vagal nerve sensory afferents signal from the thoracic abdominal cavity to the brain. Secondly, circumventricular organs, which lack a BBB, communicate through inflammatory mediators e.g. cytokines via the peripheral blood flow. Finally, these mediators can signal directly through the BBB to PVM by interacting with the endothelial cells (Perry et al. 2007). Microglia and macrophages in the brain react to these signals and in turn release their own inflammatory mediators.

Microglia are long-lived and have an innate memory of challenges and assaults to the brain. Signaling reduction to microglia's immunoreceptor tyrosine-based inhibition motif (ITIM) receptors due to neuronal loss with age or disease, in addition to the release of DAMPs, cause microglia to become 'primed'- they demonstrate an amplified response to systemic inflammation (Perry et al. 2010).

Microglia priming occurs with age, but this simply means a higher probability of having been exposed to pesticides or head trauma, systemic diseases, repeated infection of the brain, or cellular dysfunction and abnormal deposition of protein in the extracellular matrix (Tansey and Goldberg 2010). This may cause a decreased production of neuroprotective molecules and hence a reduction of trophic support for neurons, and amplification of pro-inflammatory reaction to further stimuli. The resulting toxic environment might contribute to the development of brain diseases (Dilger and Johnson 2008, Streit et al. 2009). One study using a triple transgenic mice model of AD found that repeated administration of LPS promoted neurofibrillary tangle formation (Kitazawa et al. 2005).

Microglia from a diseased brain are more sensitive towards systemic challenges compared to a healthy control brain. Systemic inflammation that occurs in a setting of a brain disease has been found to exacerbate disease progression (Perry et al. 2010). This has been proven in both humans and animal models. An intracerebral challenge with LPS in a mouse model of prion disease resulted in a severe pro-inflammatory response, which brought on an exaggerated sickness behaviour and increased neuronal apoptosis (Cunningham et al. 2005). Similar results were observed in a mice model of PD (Pott Godoy et al. 2008).

Delirium is a medical term used to describe an acute, fluctuating syndrome that is transient and reversible. Clinical features are divided into hypoactive- with lethargy, sedation and slow response time; and hyperactive -with agitation, restlessness, hallucinations and delusions; or mixed delirium, where patients exhibit both types of symptoms. It occurs at a higher rate, and accelerates cognitive decline in patients with

dementia. It can be caused by illness and infection, drug complication or surgery. We now know that in certain situations, delirium is not reversible, and can result in functional decline and permanent damage to the brain (Fong, Tulebaev and Inouye 2009). Episodes of infection that do not result in delirium are also associated with an increased risk of developing dementia (Dunn et al. 2005).

1.1.8 Differentiating between peripheral and parenchymal macrophages

Resident microglia migrate into the CNS during embryogenesis and are long lived. Peripheral-derived macrophages, also known as blood-borned macrophages, are recently recruited from the blood, and exert very different functions compared to resident microglia (Simard and Rivest 2004). It is most crucial to be able to differentiate between them and their roles in both healthy and diseased brains.

Resident and peripheral microglia/macrophages exhibit a variety of phenotypes. They can be distinguished by expression of markers, morphology, and function. While one can differentiate a quiescent ramified microglia easily from a rounded macrophage (Figure 1), it becomes virtually impossible to use morphology as a distinguishing factor between a macrophage, and an activated microglia assuming an amoeboid shape with shortened processes and a swelled soma.

Microglia and peripheral macrophages also share many antigens, differing only in their levels of expression. No single marker is capable of distinguishing between the two; instead a combination of three to four of the following markers- CD68, CD45, CD11b, MHCII and CD14, can be utilized using flow cytometry to reliably separate the two populations (Guillemin and Brew 2004).

Macrophages seem more adapt at phagocytosis compared to microglia. An example that demonstrates human macrophages' capability for phagocytosis is during comorbidity of AD with stroke, where β amyloid fibrils were found in the lysosomes of peripheral-derived macrophages. Microglia did not contain any Aβ (Wisniewski, Barcikowska and Kida 1991). Simard et al. 2006 also revealed that blood-derived microglia had the ability to phagocytose Aβ plaques efficiently and restrict their deposition in the ECM. This can be attributed to their phenotype flexibility by virtue of being located in the periphery and having a dynamic life cycle, while brain-resident microglia have a limited range of responses to preserve the fragile and vulnerable environment of the CNS (Gate et al. 2010). Despite their effectiveness at phagocytosis, blood-derived macrophages' entry into the brain is probably restricted, with or without compromise of the BBB (Wenkel, Streilein and Young 2000, Soulet and Rivest 2008).

1.2 Alzheimer's Disease

1.2.1 Epidemiology of AD

Alzheimer's Disease (AD) is the leadng cause of dementia in a population with a rapidly increasing proportion of elderly, and the leading age-related neurodegenerative disorder.

AD is a progressive disease that accelerates neuronal demise, leading to memory loss and decline of other cognitive domains. It first manifests by affecting episodic memory, eventually affecting other cognitive functions e.g. executive function, language, praxis, complex visual processing (Dubois et al. 2007). It is distinguished neuropathologically by extracellular aggregates of β amyloid protein (Aβ plaques), along with intracellular inclusions of hyperphosphorylated tau e.g. neurofibillary tangles (NFT) or neuropil threads (NT), and marked gliosis.

Incidence of AD increases exponentially with age, and reaches a peak around ages 70-90. As of 2012, there are approximately 36 million people with dementia in the world, with 800 000 sufferers in the UK alone. North America and Western Europe take the lead in number of patients afflicted with dementia, followed by Latin America and China (Reitz, Brayne and Mayeux 2011). This figure was projected to reach 115 million worldwide by 2050. With such prevalence and no way for treatment, AD places an enormous strain on the public health system, making it imperative to find a cure, or a method to delay and prevent this devastating disease.

Several factors are known to cause an increased propensity for AD, including cerebrovascular disease (Pendlebury and Rothwell 2009), blood pressure (Kivipelto et al. 2001a, Kivipelto et al. 2001b, Whitmer et al. 2005, Kalaria 2010, Deane, Wu and Zlokovic 2004), type 2 diabetes (Luchsinger et al. 2001, Ott et al. 1999), body weight (Whitmer et al. 2008, Gustafson et al. 2003), smoking (Cataldo and Glantz 2010), depression (Barnes et al. 2006), and traumatic brain injury (TBI) (Guo et al. 2000, Johnson, Stewart and Smith 2010, Plassman et al. 2000). Despite identifying these risks, the molecular mechanisms that underlie the etiology of AD remain vague.

1.2.2 History of AD

AD was first brought to attention when Alois Alzheimer, a German psychiatrist and neuropathologist received a 51-year-old patient exhibiting strange behaviour, including paranoia, aggression, progressive sleep and memory disturbance, and confusion. In 1907, he brought forth his histological findings to the 37th meeting of South-West German Psychiatrists in Tubingen, describing an extracellular 'peculiar material' deposit

on the cortex and fibrils of 'characteristic thickness and peculiar impregnability' within cells (LaFerla, Green and Oddo 2007, Hippius and Neundorfer 2003).

However, this discovery was not received well by the general and scientific community for the next five decades. The National Institute on Aging was established in 1974, but its research on AD did not generate enough scientific interest and consequently, funding. Only in the 1980s did the severity and scope of the disease gained recognition, and research networks and clinical trials were established in response. The histological data from Alois Alzheimer were also revealed to be what we now know are amyloid plaques and NFT respectively.

1.2.3 Neuropathology of AD

There are several gross changes that can be seen in a brain afflicted with AD, without looking under the microscope. These include atrophy of the hippocampus and the cerebral cortex, including the fronto-temporal association cortex, and a symmetrical dilation of the lateral ventricles (Perl 2010).

While these visible changes might give an indication to the presence of AD, a definitive diagnosis can only be made when presence of neuritic Aβ plaques and NFT distributed in specific brain regions is observed. Other pathological changes also abound, including inflammation, oxidative stress and mitochondrial dysfunction (de la Monte and Wands 2006, Gotz et al. 2008, Ferreiro et al. 2012). These changes begin early in life (10 to 20 years before clinical symptoms), and are pervasive even in mildly demented individuals (Perrin, Fagan and Holtzman 2009, Jack et al. 2010).

Though the correlation between moderate/severe AD neuropathology (plaques and tangles) and the occurrence of dementia is robust in patients below the age of 80, this association is attenuated when dealing with the oldest old (older than 95 years), with extensive overlap in the amount of neuropathological changes between those with and without dementia. However, atrophy in the neocortex remains just as robust for differentiating between dementia and non-dementia sufferers throughout the ages (Knopman et al. 2003, Kril 2009).

AD pathology, which includes toxic abnormal protein aggregates and inflammation, eventually culminates as neuronal and synaptic loss (Caselli et al. 2006). Not surprisingly, there is extensive synaptic loss in brains afflicted with AD, as observed by several groups using electron microscopy, stereological techniques, immunohistochemistry e.g. staining for synapsin 1, synaptopodin and synaptophysin, and MRI. The damage is more apparent in certain brain areas like the neocortex and the hippocampus, including frontal cortex (Reddy et al. 2005), temporal cortex (Scheff and Price 1993), molecular and granular layer of the hippocampal dentate gyrus

(Hamos, DeGennaro and Drachman 1989, Scheff, Sparks and Price 1996, Scheff and Price 1998), and the entorhinal cortex, CA1 and CA2, and subiculum (Rossler et al. 2002, Mueller et al. 2010). Synaptic loss is highly associated, and may well be the best correlate with the severity of dementia (Clare et al. 2010). Neuronal loss in CA1 and the subiculum positively correlates with the formation of NFT and ghost tangles (Rossler et al. 2002).

Certain neurons are more vulnerable to degeneration and death, including glutaminergic e.g. in the hippocampus, cholinergic e.g. in the basal forebrain, noradrenagic e.g. in the locus coerelus, dopaminergic e.g. in the striatum and serotonergic e.g. dorsal raphe nucleus (Mann, Yates and Hawkes 1982, Ouchi et al. 2009, Chen et al. 2011, Holtzman, Morris and Goate 2011). Therapy directed at modification of these neurotransmitter systems may offer symptomatic relief.

1.2.4 Aβ plaques and APP

1.2.4.1 Structure and properties of APP

Amyloid precursor protein (APP) is highly expressed in the brain, especially by neurons (Holtzman et al. 2011). It is made up of a large extracellular region and a small cytoplasmic tail, with a transmembrane portion in between (Gralle and Ferreira 2007).

APP is proposed to have a role in cell adhesion and migration due to its localisation to the plasma membrane (Sabo et al. 2001, Soba et al. 2005). Higher concentrations of APP are found in regions with more intensive synaptic modifications (Loffler and Huber 1992), also influences synaptic formation and transmission, as observed in cultured mice neurons lacking APP (Priller et al. 2006). APP knockout mice exhibit behavioral deficits and decrease in spatial learning abilities (Ring et al. 2007).

APP is naturally cleared from the brain either by proteolytic degradation, or via efflux into the periphery. This occurs by protein mediation e.g. low-density lipoprotein receptor-related protein-1 (Sagare et al. 2007) across the BBB, and also by drainage into the interstitial fluid (Lee and Landreth 2010).

1.2.4.2 APP processing and formation of Aβ

Aβ is mostly produced in the secretory pathway and at the plasma membrane, but also in the endoplasmic reticulum, Golgi bodies, and endosomal-lysosomal system (Gouras, Almeida and Takahashi 2005, LaFerla et al. 2007). Their physiological role is still under investigation, but it is most likely that they influence synaptic formation and function by depression of excitatory synaptic currents (Priller et al. 2006). Aβ is naturally cleared from the brain. This occurs via several pathways- namely receptor mediated clearance

and proteolytic degradation, as well as elimination into the periphery via active transport through the BBB, and drainage via interstitial fluid (Charidimou, Gang and Werring 2012).

There are three groups of enzymes involved in the processing of APP, namely α-, β-, and γ-secretases. α-secretase activity has been narrowed down to three enzymes from the ADAM (A disintegrin and metalloproteinase) family (Allinson et al. 2003). BACE (β-site APP-cleaving enzyme 1) has been revealed as the sole contributor to the activity of β-secretase (Vassar et al. 1999). On the other hand, activity of γ-Secretase relies on 4 separate components, presenilin, nicastrin, anterior pharynx-defective 1 and presenilin enhancer 2 (Edbauer et al. 2003).

The non-amyloidogenic pathway for APP processing involves α-Secretase, which activity serves to cleave APP within the Aβ domain, 83 amino acids from the carboxyl-terminus (C-terminus). This produces a secreted form of amino-terminal (sAPPα), while precluding formation of Aβ. sAPPα has a neuroprotective function and is found to stimulate proliferation and increase neurite outgrowth (Milward et al. 1992, Mattson et al. 1993, Caille et al. 2004). The C-terminal is retained in the membrane before being cleaved by γ-secretase to generate p3 (Haass et al. 1993, LaFerla et al. 2007).

The amyloidogenic pathway involves β- and γ -secretases. Cleavage from β-secretase (99 amino acids from the C-term of APP) produces sAPPβ from the N-terminal, and a C-terminal (C99). C99 contains the Aβ sequence, with its N-terminal corresponding to the first amino acid of Aβ. The first 28 amino acids of Aβ come from the extracellular portion of APP, while amino acids 29-43 are acquired from within the membrane region. γ-secretase then proceeds to cleave C99 to determine the length of Aβ, leaving behind Aβ intracellular cytoplasmic domain (Gotz and Ittner 2008). γ-secretase can cleave at various sites of APP within the membrane region, with $A\beta_{40}$ being the most common and $A\beta_{42}$ (~10%) being the most fibrillogenic species due to two additional C-terminal hydrophobic amino acids (Yan et al. 2003, LaFerla et al. 2007). While $A\beta_{42}$ are predisposed to form plaques, $A\beta_{40}$ are more likely to be seen deposited in leptomeningeal and grey matter cortical blood vessels (Perl 2010). $A\beta_{40}$ has been showed to be able to prevent aggregation of $A\beta_{42}$ (Yan and Wang 2007).

1.2.4.3 Structure and formation of Aβ plaques

$A\beta_{42}$ accumulates intraneuronally with increasing age. When observed with immuno-gold electron microscopy in mice, the accumulation occurs within multivesicular bodies especially in synaptic compartments and distal processes (Gouras et al. 2005).

Monomeric Aβ has a tendency to aggregate into multimeric complexes- from low-weight oligomers to protofibrils and fibrils. While monomers are not known to be toxic and (both Aβ40 and Aβ42) actually exhibit neurotrophic effects at low nanomolar concentrations, oligomers have been proven to be most pathological of the Aβ species, compromising neuronal viability more than 10x that of fibrils and ~40x that of monomers (Dahlgren et al. 2002). Ranging from dimers to 24mers, oligomers disrupt long-term potentiation and synaptic function in rodent models, even before the development of plaques and NFT (Walsh et al. 2002, Oddo et al. 2003b, Cleary et al. 2005). They were also found to inhibit proteasome function, causing accumulation of Aβ and tau in transgenic mice (Tseng et al. 2008).

There are several theories involving the formation of β-sheet conformations of Aβ from monomers. This is known to take place intracellularly, as verified by studies in transgenic mouse models (Oddo et al. 2006) and using human neuronal cells (Walsh et al. 2000). Though its formation process is still controversial, one hypothesis involves a straightforward nucleation process starting from monomers, that coalesce to form paranuclei, and then large oligomers followed by protofibrils, before the final fibril product. This is formed either by association of protofibrils, or by annealing of monomers and dimers to protofibril ends, thought to be an irreversible procedure (Walsh et al. 1997, Lomakin et al. 1997, Bitan et al. 2003). One concern about this theory is that the state of oligomers are might be stable enough to prevent spontaneous fibrillogenesis (Arimon et al. 2005).

Diffuse plaques (Figure 4b) are amorphous aggregates of nonfibrillar Aβ peptides that do not contain dystrophic neurites. They do not stain for Congo red, faintly with thioflavin-S or thioflavin–T, but intensely with silver dyes (Patton et al. 2006). These plaques occur quite frequently in the elderly, and do not associate with cognitive impairment (Morris et al. 1996). Diffuse plaques are thought to evolve into neuritic plaques, which then become cored plaques (Sheng, Mrak and Griffin 1997b), though others believe they develop independently of each other.

Neuritic plaques (Figure 4a) are characterized by a central core of β-pleated sheets arranged in radial spikes, and surrounded by a corona of astrocytic processes, and swollen and dystrophic neurites which are often axonal instead of dendritic. These neurites contain dense bodies and clusters of paired helical filaments (PHF). The beta-pleated sheet that makes up these plaques confer its ability to bind to Congo red. Dense-cored plaques, also known as burnt out or end-stage plaques, consist of only a persistent condensed core of Aβ, and do not contain any neuritic or cellular components (Sheng et al. 1997b).

The fibrillar form of Aβ has notoriously been known for their pathological presence in AD (Walsh et al. 1999); another school of thought suggests that they are formed as a defense mechanism to collect soluble toxic Aβ into inert pools (Haass and Selkoe 2007). Aβ plaques evoke microglia activation and production of IL-1β over time, causing chronic inflammation (White et al. 2005). However their presence are not known to correlate with dementia severity (Cortes-Canteli and Strickland 2009). A clinical trial involving immunization with $A\beta_{42}$ was conducted by Elan Pharmaceuticals, demonstrating that although Aβ plaques might be cleared successfully, progression of neurodegeneration was neither halted nor delayed, even in patients with close to complete plaque removal. No improvement was observed in the rate of cognitive decline and survival times of patients (Holmes et al. 2008).

1.2.4.4 Correlation to clinical symptoms

PET imaging for [^{11}C]PiB (Pittsburgh Compound B), cerebrospinal fluid (CSF) $A\beta_{42}$ and autopsy studies all revealed extensive and widespread amounts of Aβ plaques in up to 20-40% elderly who are cognitively intact. It is posit that this is an indication of MCI, and AD pathology starts one to two decades before the onset of clinical symptoms (Price and Morris 1999, Knopman et al. 2003, Mintun et al. 2006, Aizenstein et al. 2008). While Aβ plaque pathology does not seem to significantly impair cognitive function, CSF biomarkers might be a good indication for preclinical AD; there is a reduction of baseline CSF $A\beta_{42}$ levels in patients with MCI who go on to develop AD 1-10 years later (Fagan et al. 2007, Buchhave et al. 2012).

1.2.5 Cerebral amyloid angiopathy

Cerebral amyloid angiopathy (CAA) is a common vessel disorder, affecting more than half of dementia sufferers, and over 90% of AD patients (Jellinger 2002). CAA was found to correlate significantly with the incidence of dementia, and has a compounding effect (together with other AD pathology) on cognitive function (Pfeifer et al. 2002). It is characterized by progressive Aβ deposition in blood vessels, mostly of Aβ40, and occurs more often in arterioles than capillaries, rarely affecting veins. It initially accumulates around the smooth muscle cells of the tunica media and tunica externa, but proceeds to completely enclose the vessel, along with loss of smooth muscle cells. Finally, dissociation of the vessel walls may occur, along with dyshoric changes, e.g. deposition of Aβ in the intermediate parenchyma (Attems et al. 2011). The most frequent and severe CAA is observed in the occipital lobe (Figure 4c & d), followed by the rest of the neocortical regions and the allocortex. The most advanced stages of

CAA would see the basal ganglia, thalamus and cerebellum being affected (Attems et al. 2007, Attems et al. 2011). Blood vessels in the white matter are usually spared.

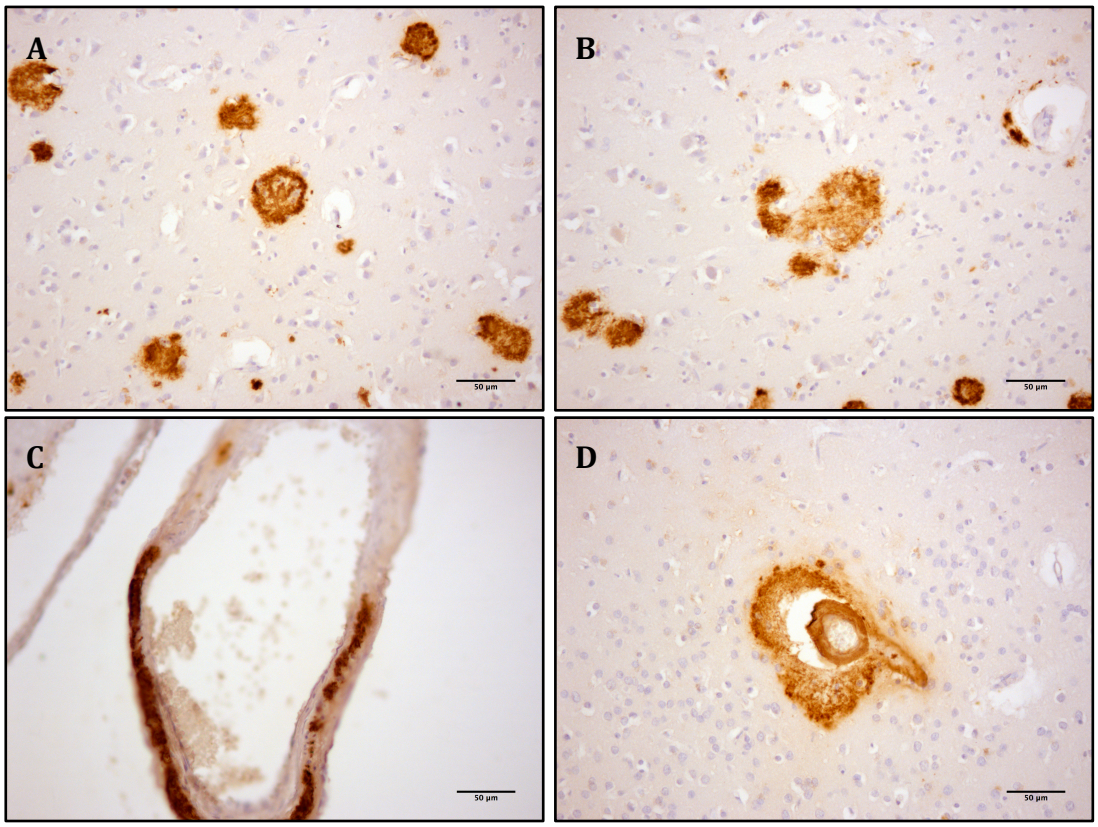
Aβ deposition in vessels is most probably caused by impairment in clearance of Aβ from the CNS via perivascular drainage. Aβ is released into the interstitial fluid, which drains out through the basement membranes of the capillaries and between smooth muscle cells of arteries. The drainage is aided by pulsations of the vessels, which causes Aβ to be trapped and deposited along the vessel walls when hindered, resulting in CAA (Preston et al. 2003, Weller et al. 2009). Increase risk of CAA can result from a higher ratio of $A\beta_{40}$ to $A\beta_{42}$, and the presence of ApoE alleles ε2 and ε4 (McGowan et al. 2005, Revesz et al. 2003).

CAA has extremely adverse effects on the vasculature of the brain, including occlusion of lumen, atrophy of smooth muscle and endothelial cells, causing vessels to become weak and vulnerable to rupture and leakage (Charidimou et al. 2012). It is also known to be a risk factor for ischemic infarctions (Cadavid et al. 2000). CAA varies between cases, some with CAA only in leptomeningeal and cortical vessels, while others with CAA in capillaries as well (Thal et al. 2002). Only capillary CAA correlates with dementia and high Braak stages (Attems and Jellinger 2004). This might be due to the effects of capillary blockage, which is capable of impeding cerebral blood flow, as demonstrated in mice models (Thal et al. 2009).

1.2.6 Tau pathology

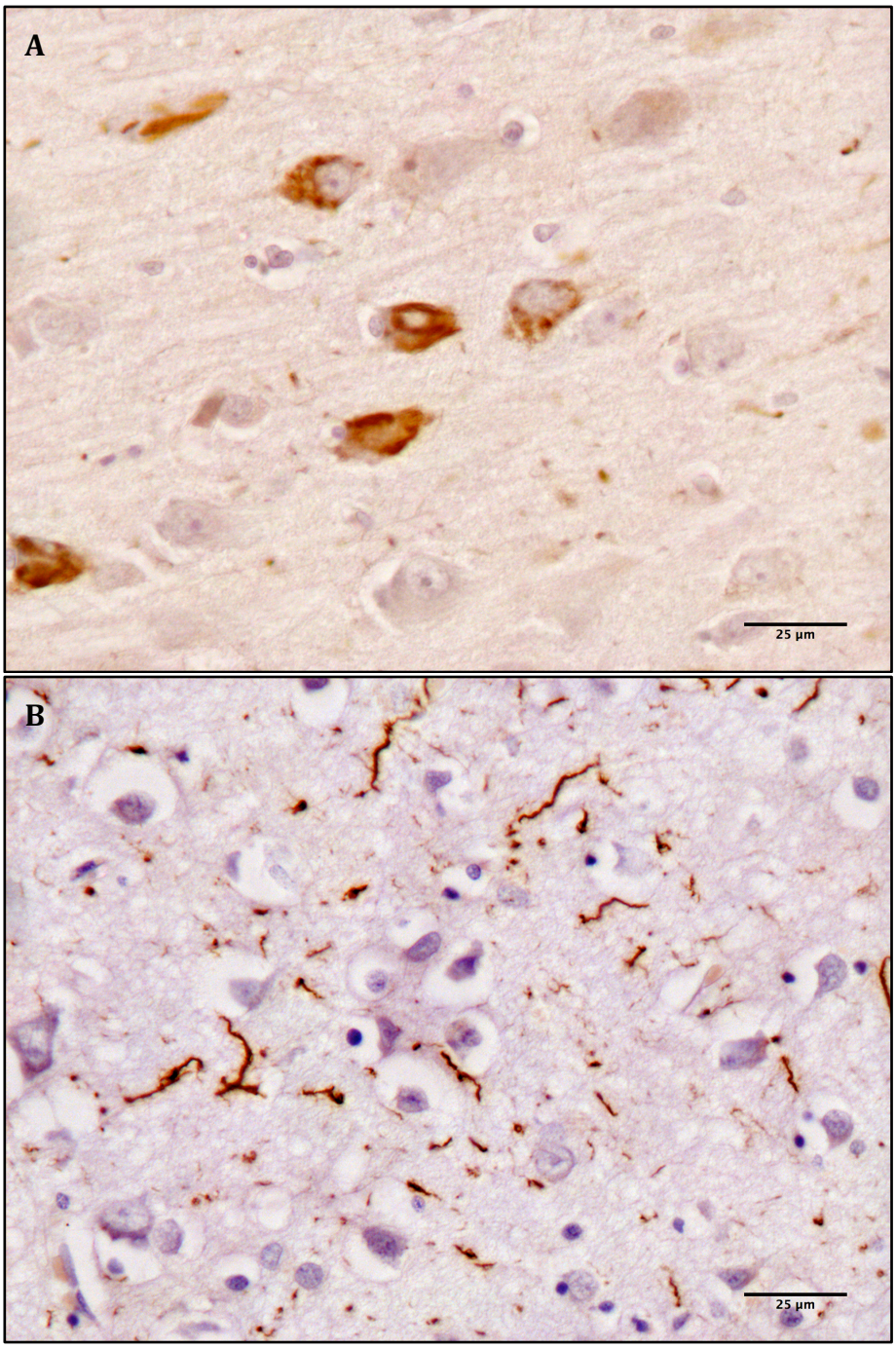
Another pathological hallmark of AD are neurofibrillary lesions, which consist mostly of aggregates of hyperphosphorylated tau (HP-tau). These aggregates usually take on the shape of the neurons they inhabit. They can be found as flame-shaped neurofibrillary tangles (NFT)- thick, parallel fibrils, surrounding the nucleus in the soma and extending into the apical dendrite in pyramidal neurons of the ammon's horn of the hippocampus (Figure 5a); or globoid NFT made up of interweaving fibers in rounder neurons like those in the locus coerulus (LC) or nucleus basalis of Meynert (NBM). They also appear as neuropil threads in distal dendrites (Figure 5b), and as abnormal neurites in neuritic plaques. These fibrils are made up of paired helical filaments, each filament measuring 10nm in diameter, with a periodicity of 80nm (Gotz et al. 2008, Perl 2010).

Figure 4. Abeta accumulation in AD



(A & B) Aβ plaques in the occipital cortex of AD cases. A neuritic plaque with a halo of dystrophic neurites around a central core, and a diffuse plaque with an amorphous form and absence of dystrophic neurites, are seen in the centre of images A and B respectively. **(C)** CAA in a meningeal vessel in the occipital lobe of an AD case. **(D)** CAA in a cortical vessel with dyshoric changes in the occipital lobe of an AD case. All images were taken at magnification x20.

Figure 5. Tau pathology in AD



Neurofibrillary tangles **(A)** and neuropil threads **(B)** in the hippocampus of an AD case**.** Magnification x40.

Like Aβ plaques, thioflavin S, or silver dyes can be used to visualize NFT, which are mainly composed of β-pleated sheets.

Tau is a 50-70 kDa protein that belongs to the microtubule (MT) associated protein (MAP) family. It is mainly localized to the axons under physiological conditions. 6 isoforms of tau exist, differing by the number of inserts in the N-terminal region, and number of MT-binding repeat motifs in the C-terminal region (Lei et al. 2010). It has a role of stabilizing microtubules by binding to tubulin, and also contributes significantly to the regulation of axonal transport via interaction with motor proteins dynein and kinesin (Dixit et al. 2008). It has over 80 sites for phosphorylation, although the role of each distinct site remains to be solved (Hanger, Anderton and Noble 2009). An increase in phosphorylation reduces its affinity to bind to MT. HP-tau in AD is found to be phosphorylated to a greater extent, and at additional sites compared to tau in controls (Pritchard et al. 2011). HP-tau dissociates from MT and amasses in the somatodendritc compartment. They then undergo further phosphorylation, which predispose them to aggregation (Stoothoff and Johnson 2005, Spires-Jones et al. 2011).

Similar to the new theories regarding toxicity of Aβ, soluble tau oligomers, instead of fibrils, are suspected to be responsible for causing neuronal dysfunction. HP-tau is hypothesized to destabilize the integrity of cytoskeletal network and hinder axonal transportation. HP-tau oligomers are seen in the prefrontal cortex of Braak stage 1 patients before the onset of clinical symptoms, and may precede the formation of NFT (Maeda et al. 2007). As seen in mice bearing the non-mutant human tau gene, dying neurons did not contain NFT, while those bearing NFT maintained robust nuclear morphology (Andorfer et al. 2005). HP-tau is found to sequester normal tau, MAP1 and MAP2; polymerization into PHF is actually protective and minimizes its toxicity (Alonso 2006). However, the area occupied by NFT would probably reach a saturation point, after which normal neuronal function would be affected, inevitably causing neuronal death (Spires-Jones et al. 2011, Alonso 2006). NFT, unlike neuritic Aβ plaques, are found to associate strongly with disease duration of AD and dementia severity (Arriagada et al. 1992, Bierer et al. 1995). Measurement of total and phosphorylated tau levels in the CSF give indication 3-4 years before the onset of MCI (Fagan et al. 2007).

1.2.6.1 Staging of neurofibrillary pathology

NFT are distributed in the brain of AD patients in a stereotypical pattern, with regions like CA1 of the hippocampus, layer II of the entorhinal cortex, and the neocortex developing severe tangle pathology (Perl 2010). Staging systems including the Consortium to Establish a Registry for AD (CERAD) strategy, Braak staging in 1991,

and the National Institutes of Health- Reagan (NIH-Reagan) criteria for neuropathological diagnosis of AD which combined both CERAD and Braak systems, had experimental setups based on thick sections, thioflavin S preparation or silver staining, and were not specific nor easily replicable (Alafuzoff et al. 2008a).

A staging system has been developed by Braak and colleagues based on the temporal-spatial distribution of hyperphosphorylated tau protein using an immunostain against AT8, and can be performed on paraffin-embedded sections of 5-15 μm, making it a very useful system that is easy to replicate.

Braak's staging system comprises of six stages. AT8-immunoreactivity is initially detected in the transentorhinal and entorhinal regions in stages 1 and 2, while stage 3 sees the pathology spreading to the allocortex and fusifom and lingual gyri. It then extends into the middle temporal gyrus at stage 4. Finally, the pathology reaches the higher order association areas of the frontal, parietal, and occipital neocortex in stages 5 and 6. The two final stages are differentiated by the expanding of pathology from the peristriate and parastriate areas into the striate area of the occipital cortex (Braak et al. 2006).

1.2.6.2 Relationship between Aβ and Tau pathology

One theory suggests that tangles are formed as a reaction to the presence of plaques, resulting in synaptic and neuronal loss, and ultimately leading to dementia (Hardy and Selkoe 2002, Sommer 2002, Caselli et al. 2006). This is in agreement with the amyloid hypothesis, including Aβ pathology being an event upstream of tau pathology. It has been shown *in vivo* using transgenic mice models that tau and Aβ seemed to be depend on each other to exert their neurotoxicity. In mice models expressing human APP, reduction of endogenous tau ameliorates Aβ induced behavioral deficits, while Aβ levels remain unchanged (Roberson et al. 2007). Tau pathology was worsened when coupled with the predilection for Aβ plaque formation (Gotz et al. 2008). One hypothesis on how this might occur is demonstrated using SH-SY5Y neuroblastoma cell system-tau and Aβ independently affect mitotic changes on a genetic level, resulting in aberrant cell cycle re-entry and apoptosis (Hoerndli et al. 2007). Another theory is that Aβ and tau have high binding affinity both in solution and *in vivo* using extracts of AD and control brains. They have been shown to aggregate intracellularly in the same neurons (Guo et al. 2006).

Yet, neurodegeneration processes that cause Aβ plaques and NFT might not be directly related, as most Aβ plaques are observed in the temporal cortex, in comparison with NFT which occurs in the hippocampus (Valente et al. 2010). Tau pathology first starts in

the hippocampus and spreads to the cortical regions, while the opposition occurs for Aβ plaque deposition (Oddo et al. 2003a).

Furthermore, mutations in the tau gene that result in autosomal dominant frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) show that Aβ plaque formation does not necessarily accompany NFT to cause neurological impairment (Hutton 2000). Other observations further disprove the amyloid hypothesis. For one, the presence of Aβ plaques is common to both AD and non-demented elderly, and the failure of $A\beta_{42}$ immunization trials to allay disease progression (Holmes et al. 2008) indicate that Aβ plaques might not be directly involved in the pathogenesis of AD (Maccioni et al. 2010).

1.2.7 Causes of AD – Sporadic and Genetic

The majority of AD cases is sporadic and has a late onset, i.e. before the age of 65. Approximately 5% of AD patients suffer from an earlier onset, i.e. around the third to fifth decades of their lives. It is essential to note that out of these early onset cases, only 20% (~1% of total AD population) have a familial disorder (Joshi et al. 2012). Though genetic defects probably contribute to cases with late onset, not many have been uncovered (Lambert and Amouyel 2007). More recently, APP and presenilin (PSEN) 1 mutations have also been found in late onset cases (Kauwe et al. 2007, Cruchaga et al. 2012).

Familial AD is passed down via autosomal dominant transmission that involves mutation in one of these three genes: amyloid precursor protein (APP) (Goate et al. 1991), PSEN 1 (Sherrington et al. 1995) and 2 (Levy-Lahad et al. 1995, Cruts et al. 1998), located on chromosomes 21, 14 and 1 respectively.

Attention was first drawn to APP mutations when Down syndrome individuals who are susceptible to early-onset AD had an extra copy of this gene in chromosome 21 (Rocchi et al. 2003). APP mutations cause aberrant APP processing and early Aβ deposition (Miyoshi 2009), e.g. Swedish mutation at APP 670/671, in proximity to the β-secretase cleavage site, causes elevated levels of soluble Aβ peptide (Johnston et al. 1994); Flemish mutation at APP 692 increases the ratio of $A\beta_{42}$ to the less fibrillogenic $A\beta_{40}$, resulting in both presenile dementia and cerebral haemorrhage due to CAA (Haass et al. 1994), London mutation at APP 717 causes increased production of $A\beta_{42}$ by more than two folds (Suzuki et al. 1994).

PSEN mutations cause an increase in the ratio of $A\beta_{42}$ to $A\beta_{40}$. PSEN1 is known to act as a co-factor for γ-secretase (Edbauer et al. 2003, Takasugi et al. 2003). γ-secretase is involved in the cleavage of APP to form Aβ of varying lengths, and mutations

increase $A\beta_{42}$ production, leading to increased propensity for aggregation and plaque formation (McGowan et al. 2005, Holtzman et al. 2011). It is difficult to target γ-secretase specifically using drugs, as it also cleaves other substrates e.g. Notch, which is involved in cell proliferation (Liu et al. 2011b); cognitive decline was worse in patients treated with an inhibitor Semagacestat compared to placebos in a clinical trial by Eli Lily and Elan (Schor 2011). In the past two years, a solution seemed to have arisen via the form of γ-secretase modulators, that while optimizing the production of Aβ forms, does not affect cleavage with other usual substrates (Kounnas et al. 2010).

An unusual type of plaque, also known as cotton wool plaques, is observed with several PSEN1 mutations. It differs from the usual plaques by being eosinophilic and unreactive to thioflavin S. It is formed from $A\beta_{42}$, degenerated synapses, neuropils, but little or no amyloid fibrils (Houlden et al. 2000). This might indicate that pathogenesis of AD is mediated by Aβ in forms other than its fibril form, e.g. molecular or oligomeric form; in agreement with the absence of correlation between the amount of amyloid plaques and clinical symptoms or neuronal loss.

Within the sporadic late-onset cases, dozens of polymorphisms have been found, but only apolipoprotein E (ApoE) variations pose as a substantial risk for developing AD, depending on the allele carried (Rocchi et al. 2003, Chen et al. 2012). ApoE has three major isoforms, ε2, ε3, and ε4. While ε2 allele is associated with the lowest risk of developing late onset AD, ε4 carriers have the highest risk (~3x for heterozygotes, ~12x for homozygotes) and a younger onset age (~5 years per ε4 allele), effecting in a dose-dependant manner (Corder et al. 1994, Holtzman et al. 2011); ε3 being the most frequent and considered neutral (Corbo and Scacchi 1999). ε4 homozygotes suffering from AD also have the fastest rate of cognitive decline when compared with all other allele groups (ε3/4, ε3/3, ε2/3) (Craft et al. 1998). However, only 20% of sporadic AD cases actually carry the ApoE ε4 allele, and individuals positive for ε4 might not go on to develop AD (Williamson, Goldman and Marder 2009).

APOE is a plasma glycoprotein containing 299 amino acids, and is synthesized by neurons, glia cells, macrophages and monocytes (Siest et al. 1995). It has many functions including transportation of cholesterol during neuronal growth and regeneration after injury (Mahley 1988). APOE receptors belong to a low-density lipoprotein receptor family, and have a direct influence on the production and cellular uptake of Aβ (Bu, Cam and Zerbinatti 2006). Individuals with ε4 alleles were found to have more neuritic plaques and higher frequency of CAA (Olichney et al. 1996, Morris et al. 2010). Effect of ε4 on quantity of amyloid deposition was further confirmed to exceed that of ε3 via transgenic mice models that were humanized for pathogenic APP

and APOE ε3 or ε4 genes (Holtzman et al. 2000). Unlike ε3, ε4 does not bind to tau *in vitro*, and accumulation of phosphorylated tau was a great deal more abundant in mice with ε4 genes than ε3 genes, raising the possibility that ε4 might be less neurotrophic and does not confer as much protection against phosphorylation of tau and formation of NFT (Brecht et al. 2004, Strittmatter et al. 1994).

1.2.8 Clinical criteria and ratings for AD and dementia

Current criteria for diagnosis of AD was established in 1984 by the National Institute of Neurological Communicative Disorders and Stroke/Alzheimer's Disease and AD requires dementia with deficits in at least 2 cognitive domains, insidious progression, onset ages between 40-90, and absence of other brain or systemic diseases that might explain the symptoms. A definite diagnosis of AD is only made upon histological confirmation (McKhann et al. 1984). Another set of criteria is based on the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR). It involves the presence of a memory disorder and cognitive impairment that interferes with social functioning and activities of daily living (ADL), and disturbance in at least one additional cognitive domain, excluding causes by any other neurologic, psychiatric, substance-induced or systemic diseases (Laboratories).

At around the same time, CERAD also developed a battery of assessments for the diagnosis of AD, with the aim to provide a standard and reliable method that will facilitate comparison. This included protocols for clinical profile, neuropsychology, neuropathology, family history assessment, and a behaviour rating scale for dementia (Fillenbaum et al. 2008).

Alzheimer's Association and National Institute on Aging recently proposed changes to the guidelines based on the advancement in technology and the capability to identify and differentiate AD from other diseases early on in the disease course. These technologies include functional neuroimaging techniques like PET (positron emission tomography) imaging, SPECT (single-photon emission computed tomography), and MRI (magnetic resonance imaging).

A few staging systems are available for rating dementia severity from normal aging through to dementia. They include mini-mental state examination (MMSE), clinical dementia rating (CDR), and global deterioration scale (GDS). Correlations between CDR and GDS, with MMSE were high, but there was slight discrepancy especially in the early stages involving mild cognitive impairment (Choi et al. 2003, Perneczky et al. 2006).

1.3 Neuroinflammation in Alzheimer's Disease

Inflammation is a prominent feature of AD, with the association of activated microglia with Aβ plaques providing strong evidence. Inflammation in AD is chronic- it starts even before the onset of AD symptoms, and is thought to be a driving force in acceleration of neuronal demise (Walker et al. 2006). Levels of inflammation correlate with synaptic loss and presence of NFT and Aβ plaques (Lue et al. 1996).

The classic view of inflammation in AD is that of a self-perpetuating cycle- microglia can become activated by triggers including toxic aggregates of protein, neuronal death, oxidative stress, head trauma, systemic infections and damage signals etc, resulting in an overproduction of pro-inflammatory and neurotoxic factors, leading to neuronal dysfunction and death (Fernandez et al. 2008, Maccioni et al. 2010).

Treatment with NSAIDs has also been shown to reduce/delay the development of AD, which further supports the theory that inflammation plays an important role in the pathogenesis of the disease (Etminan 2003).

1.3.1 Relationship between microglia and Aβ

Microglia are associated very closely with extracellular Aβ plaques, both neuritic and diffuse (Sheng et al. 1997b). Aβ plaques attract microglia, which can be seen from their graded concentration with respect to their proximity from the plaques. In response to Aβ plaques, both post mortem samples and experimental murine microglia have been shown to increase expression of pro-inflammatory cytokines e.g.

surrounding neurons (Akiyama et al. 2000, Lue et al. 2001, Yan et al. 2003, Colton et al. 2006).

In vitro studies using both human and rodent microglia show activation by fibrillar and oligomeric Aβ to release pro-inflammatory cytokines, chemokines, matrix metalloproteinases (MMP) and ROS (Maezawa et al. 2011, Li et al. 2013). This was not accompanied by an increase in phagocytic markers (Walker et al. 2006). In fact, evidence points to the suppression of phagocytic capabilities of microglia with increasing pro-inflammatory stimulation (Koenigsknecht-Talboo and Landreth 2005). Conversely, it was found that overexpression of IL-6, a pro-inflammatory cytokine found in enhanced levels in AD patients, increased microglia's uptake of Aβ *in vitro*, and attenuated Aβ deposition in AD mice models (Chakrabarty et al. 2010).

Microglia can interact with Aβ plaques through many receptors including CD14, toll-like receptors (TLR)- TLR2, TLR4, and TLR6 (Reed-Geaghan et al. 2009), pattern recognition receptors (Salminen et al. 2009), and a multi-component receptor complex made up of CD36, scavenger receptor A, CD47 and $\alpha_6\beta_1$ intergrin (Bamberger et al.

2003). Binding of fibrillar Aβ to CD36 have been shown to induce a classical activation state, releasing cytokines, chemokines, ROS, and stimulating cell migration (El Khoury et al. 2003).

Although microglia express receptors for Aβ, microglia still cannot clear Aβ plaques efficiently (Koenigsknecht and Landreth 2004). No immunoreactive Aβ has been detected within microglia *in vivo*, which might be due to immediate processing and degradation, or their inability at phagocytosing these Aβ plaques (Malm et al. 2008). The immune system in AD patients has been found to be defective in the clearance of Aβ plaques compared to controls. Monocytes from controls were able to phagocytose plaques from AD patients, while those from AD clearly demonstrated defective clearance ability, including downregulated TLR expression (Fiala et al. 2007). Macrophages from AD patients were also more susceptible to apoptosis upon exposure to soluble, protofibrillar, and fibrillar Aβ (Zaghi et al. 2009). One culprit that might prevent macrophages from recognizing Aβ plaques, as well as NFT, is serum amyloid P, which also prevents efficient proteolysis (Tennent, Lovat and Pepys 1995). The insoluble nature and persistence of these plaques are thought to cause an ongoing chronic inflammation in AD.

Naturally occurring anti-Aβ antibodies also exist in the blood and CSF of both AD and controls. They are found to be neuroprotective (Britschgi et al. 2009, Deleidi and Maetzler 2012). The levels of these antibodies are reduced in the sera of AD patients compared to healthy individuals (Weksler et al. 2002).

In a study with hAPP transgenic mice, ganciclovir application caused nearly complete microglia ablation, but this did not affect plaque formation and maintenance, nor plaque associated neural dystrophy (Weksler et al. 2002). This provides further evidence for microglia being inept at fibrillar Aβ clearance; and despite their association with a pro-inflammatory and neurotoxic reaction, microglia might not be involved in Aβ-driven neurotoxicity.

1.3.2 Relationship between microglia and neurofibrillary pathology

Using *Ricinus communis* agglutinin-1 to label microglia, Sheffield et al. found a correlation between the density of microglia with the frequency and distribution of NFT (Sheffield, Marquis and Berman 2000). Tangle maturation stages from granular perikaryal tau reactivity to neuronal inclusions and finally ghost tangles, also show positive association with the frequency of IL-1α positive microglia (Sheng, Mrak and Griffin 1997a). The idea that microglia are driving or sustaining NFT formation has been proposed (Mrak 2012).

In line with the tau hypothesis of AD, it has been postulated that pro-inflammatory cytokines released by microglia cause signaling cascades in neurons- both TNFα and IL-6 act to induce cell cycle entry without proliferation. This causes dysfunction of the enzymes involved in tau phosphorylations, e.g. kinases and phosphates, leading to hyperphosphorylation and aggregation of tau into toxic oligomers and fibrillar tangles (Morales, Farias and Maccioni 2010). The toxicity brought about by pathological tau aggregates brings about additional neuronal degeneration and death that further activates microglia, resulting in a feedback loop (Maccioni et al. 2010).

1.3.3 Alternative activation in AD

Although Aβ is known to elicit a pro-inflammatory reaction from classically activated microglia, its binding to Aβ receptors on microglia can also induce an alternative activation state, resulting in a heterogenous mix of pro and anti-inflammatory behavior (Colton 2009). Colton et al. found higher expression levels of alternative activation genes e.g. arginase 1 and chitinase 3-like 1, higher levels of TNFα, but similar levels of NOS2 and IL-1β in AD compared to age-matched controls (Colton et al. 2006). In a mouse model of AD, microglia around Aβ plaques expressed high levels of Ym-1, the mouse homolog for chitinase 3-like 1 in humans that serves as an alternative activation marker (Jimenez et al., 2008).

The reasoning behind co-existence of microglia's multiple activation states can be attributed to chronic inflammation in AD. Classical activation of microglia serves to remove and limit the amount of Aβ plaques. As mentioned in the earlier section, this is not done efficiently, and Aβ's persistence in the extracellular environment would probably result in an excessive pro-inflammatory reaction. In order to prevent too much damage done to the surrounding neurons, microglia also need to assume a partial immunosuppressive and reparative phenotype (Colton 2009).

It has been shown that the four major cytokines associated with alternative activation of microglia- IL-4, IL-10, IL-13, and TGFβ enhanced microglial uptake of fibrillar Aβ. While pro-inflammatory factors like TNFα, IL-1β, CD40L and IFNγ suppress its phagocytosis, this can be restored by anti-inflammatory cytokines (Koenigsknecht-Talboo and Landreth 2005). TGFβ and IL-10 have been found at high levels in AD patients compared with controls (Parachikova et al. 2007). However, the presence of TGFβ might not be beneficial in AD patients. It was found that genetic blockade of TGFβ drastically reduced the amount of Aβ plaques and helped improve behavioral and spatial working memory in mice models of AD (Town et al. 2008).

1.3.4 Treatment – Immunization in AD

Immunization in both humans and mice models of AD have shown successful removal of Aβ plaques, but with rather different outcomes. Immunization, both passive and active, significantly reduces the amount of plaques and has been found to increase cognitive performance in mice (Schenk, Hagen and Seubert 2004).

Clinical trials were carried out using AN-1792 + QS-21- $A\beta_{1-42}$ and saponin adjuvant respectively. While phase 1 trials were tolerated well by patients, a larger-scaled phase II clinical trial with 300 treated individuals and 72 on placebo had to be halted due to development of aseptic meningoencephalitis in 18 vaccinated subjects (Patton et al. 2006). While no significant improvement in cognitive function was found using psychometric tests, their z-score composite across the neuropsychological test battery favored antibody-responsive patients. In some of these antibody responders, the level of tau in the CSF was found to be significantly lower (Gilman et al. 2005).

Immunization with AN-1792 enabled clearance of neuritic plaques, especially in parts of the neocortex, while remaining plaques were associated with microglia (Nicoll et al. 2003). However, the amount of diffuse plaques and vascular amyloid deposits remain unchanged. Soluble levels of Aβ were also increased in both grey and white matter (Patton et al. 2006). This poses a risk as soluble amyloid are thought to be toxic, and for this reason were aggregated into inert fibrils in the first place (Lee et al. 2004). Soluble Aβ peptides can cause vasoconstriction, impair cerebral blood flow and inhibit angiogenesis (Iadecola 2003, Paris et al. 2004).

ELISA techniques revealed no reduction in total Aβ levels in the brain, an indication that the rate of soluble Aβ removal from the brain is slow and ineffective. An increase in vascular amyloid deposition correlates with a decrease in the amount of neuritic plaque, and also causes further compromise of the BBB and impairing brain perfusion. The severity of CAA in vaccinated patients raises the possibility that Aβ was retained in the vasculature during egress from the brain, which might be caused by pre-existing obstruction of interstitial fluid drainage (Patton et al. 2006).

1.3.5 Effect of NSAIDs on AD

NSAIDs are used to provide analgesic and antipyretic effects. They act to inhibit COX2, and activate the PPAR-γ pathway, which has an anti-inflammatory effect (Hirsch and Hunot 2009). Some NSAIDs also directly scavenge NO radicals from neurons, thus exerting a neuroprotective effect (Asanuma et al. 2001). A study by Koenigsknecht-Talboo et al. 2005 used cell cultures to demonstrate that NSAIDs relieve the suppression of phagocytosis of fibrillar Aβ caused by pro-inflammatory cytokines. There

is conflicting evidence about their ability to reduce the risk of developing AD, or halt its progression. NSAIDs have been shown to decrease the risk of developing AD (Etminan 2003, Vlad et al. 2008).

1.4 Parkinson Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after AD, and the most common progressive neurodegenerative disease in the category of motor function disorders, with an onset around 65 years of age. Approximately 1% of the population above the age of 60 is afflicted by PD (Abou-Sleiman, Muqit and Wood 2006, Probst, Bloch and Tolnay 2008), and there are around six million sufferers of PD worldwide (Litteljohn et al. 2010). While final stages of this multicentric disease often involve cognitive impairment, early and mid stages of PD are plagued by debilitating motor symptoms.

Patients suffering from PD exhibit four cardinal symptoms known as TRAP- tremor, rigidity, akinesia and postural instability. Other motor symptoms include bradykinesia, freezing, and flexed posture (Jankovic 2008). They are not spared from non-motor dysfunctions, with the disease also affecting autonomic and cognitive functions (Langston 2006), many of which are observed even before the decline of motor functions e.g. hyposmia and other olfactory disturbances, sleep disorders, hypotension and psychological disturbances like anxiety and depression (Litteljohn et al. 2010).

PD is diagnosed clinically by an asymmetrical onset of bradykinesia, rigidity, with resting tremor, and a sufficiently good response to dopaminergic (DA) treatments e.g. with levodopa (L-dopa), a DA precursor or DA agonists.

1.4.1 Epidemiological aspects of PD

PD affects ~0.3% of the world's population. The number of PD patients is expected to double by 2050 (Schapira 2009). The incidence and prevalence of PD drastically increases with age after 60 years (Van Den Eeden et al. 2003), affecting up to 4% of those above 80 years of age (de Lau and Breteler 2006), but seem to declines in the oldest old group (90 years of age) (Driver et al. 2009). Approximately 5-10% of PD patients are young onset i.e. they become symptomatic before 40 years old (Vanitallie 2008). The life expectancy and anticipated age at death for PD patients are lower compared to the general population, but the effect is even greater for patients with PD onset before 40 years of age (Ishihara et al. 2007). Both incidence and prevalence are higher in men than women (Van Den Eeden et al. 2003).

Premorbid depression is observed more frequently in patients with PD than controls (Ishihara and Brayne 2006). Obesity (Hu et al. 2006), higher cholesterol levels (de Lau and Breteler 2006), and type 2 diabetes mellitus are risk factors for PD. Patients with diabetes are 1.85 times more likely to develop PD (Hu et al. 2007b). One hypothesis for

the increased risk of PD might be attributed to chronic metabolic stress (Vanitallie 2008).

Dietary and lifestyle also present as risk factors to the development of PD. Smoking has an inverse association with PD. A study by Thacker et al. 2007 found that smoking could decrease risk of PD by up to 60%, depending on the intensity and duration of smoking, and number of years before onset of PD. Cigarettes contain nicotine, which protects against nigrostriatal damage (Quik, Perez and Bordia 2012). Nicotine is capable of inhibiting α-syn fibril formation and stabilizes soluble oligomeric forms (Hong, Fink and Uversky 2009).

Intake of coffee and tea is also associated with a decreased risk of PD (Hu et al. 2007a). On the other hand, intake of dairy milk increases the risk of PD (Chen et al. 2007). Milk's effect on lowering uric acid levels might be one of the reasons for this observation, as uric acid is an neuroprotective antioxidant that has been found to lower the risk of developing PD (De Vera et al. 2008).

1.4.2 History of PD

James Parkinson first gave a clinically based description of this disease in 1817, in "An essay on the shaking palsy". He goes on to define "shaking palsy" separately from "paralysis agitans". Shaking palsy is a combination of resting tremor, lessened muscular power, abnormal truncal posture, and festinent, propulsive gait. He also illustrated six case reports. Charcot subsequently named the disease after him in the 19th century (Kempster, Hurwitz and Lees 2007).

The cause of motor symptoms observed in PD was discovered in 1919 when it was found that patients suffered from cell loss in the substantia nigra. In 1957, the function of dopamine as a neurotransmitter was unveiled. Discovery of the severe loss of dopamine in the striatum of PD patients by Ehringer and Hornykiewicz in 1960 led to a treatment for the symptoms of PD. Trials administering L-dopa via intravenous therapy and oral intake were carried out simultaneously by Birkmayer and Barbeau respectively (Hornykiewicz 2006).

1.4.3 Neurotransmitter Deficits in PD

Extrapyrimidal deficits are evident in PD. These manifest when there is a loss of DA neurons in the SN pars compacta (SNpc) that project into the striatum, causing denervation via a loss of DA terminals, particularly in the dorsal and caudal putamen, leading to a depletion of dopamine (Lotharius and Brundin 2002). It is thought that dopamine depletion is responsible for the TRAP syndrome observed in PD patients (Farooqui and Farooqui 2011). There is a presymptomatic phase of about 6 years

between the start of DA neuron loss and the onset of visible motor symptoms, by which time there is a loss of ~50% of DA neurons in the SNpc, and ~70% reduction in dopamine levels in the basal ganglia (Schapira and Obeso 2006).

To date, the most effective symptomatic treatment for PD is L-dopa, as its application allows almost completely alleviation of motor dysfunction. Unfortunately, chronic use can cause severe motor and psychiatric side effects, such as dyskinesia, dopamine dysregulation syndrome, and hallucinations (Teismann and Schulz 2004). Clinicians tend to use this drug only as a last resort.

Many other regions of the brain are also affected, for instance the prefrontal cortex, LC, hippocampus, and striatum, causing a system-wide multi-neurotransmitter dysfunction that also affects autonomic, cognitive and psychological functions (Wolters and Braak 2006, Kostic et al. 2010, Litteljohn et al. 2010). These neurotransmitter systems include mesocortical dopaminergic (ventral tegmental area), noradrenergic (LC), serotoninergic (dorsal raphe nuclei), cholinergic (NBM), and histaminergic (Probst et al. 2008, Schapira 2009). Regions outside the brain e.g. the olfactory bulb and mesenteric system, are also affected in PD (Hirsch 2009).

1.4.4 Clinical aspects of PD

There are a number of diagnostic tests for PD, although none of them are definitive. The only confirmation lies in an autopsy evaluation of the presence of Lewy Body pathology. UK Parkinson's Disease Society Brain Bank and the National Institute of Neurological Disorders and Stroke have both developed diagnostic criteria for PD (Jankovic 2008).

There are also a number of rating scales for measurement of impairment and disability. The Hoehn Yahr scale is a 5-point system that assesses disease progression, severity and disability, ranging from stage 0 (no symptoms) to stage 5 (severe disability). The Unified Parkinson's Disease rating scale (UPDRS) is a 4 part multimodal system for assessing impairment and disability, and is most well established. It includes evaluation of motor examination, complications from therapy, activities of daily living, mentation, behaviour and mood (Ramaker et al. 2002).

1.4.4.1 Motor deficits

As mentioned in previously, clinical features of tremor, rigidity, akinesia and postural instability are characteristic of PD. In this section, they will be elaborated in greater detail.

Bradykinesia is an easily recognizable trait that is most characteristic of PD. It affects movement planning, initiation and execution, and manifests as slowness in movement

and reaction time, and performing repetitive sequential and simultaneous movements (Berardelli et al. 2001). Bradykinesia is associated with DA deficiency in areas including the striatum, specifically the putamen, globus pallidus and accumbens-caudate complex (Lozza, Marie and Baron 2002), and the motor cortex (Turner et al. 2003, Jankovic 2008).

Rest tremor is the most common feature of PD, affecting ~70% of PD patients at disease onset, and can be mistaken for essential tremor. It has a frequency of 4-6 Hz, starts off unilateral, rarely involves the lower limbs, and is characteristically displayed by a supination-pronation, or "pill-rolling" tremor involving the thumb and forefinger. Tremor can be confined to one limb or one side for many years, before progressing to the proximal joints of the same limb, and involving the other upper limb or ipsilateral lower limb (Factor 2002). It was found that PD patients without tremor had a sparing of the A8 neurons in the midbrain (Jankovic 2008). Tremor dominant PD has the slowest rate of neurodegenerative decline (Schapira 2008). Within SNpc, the ventrolateral part is severely affected in akinetic-rigid dominant PD, but is less affected in tremor dominant form. This is reversed for the medial part of SNpc (Probst et al. 2008).

Rigidity is caused by increased muscle tone, resulting in resistance to passive movement, and can be sustained or intermittent and ratchetty, especially when coupled with tremor. Rigidity is usually asymmetrical during initial stages of PD, but progresses to affect the whole body to cause a "flexed posture" (Factor 2002). While disruption in the activity of the motor cortex and basal ganglia is thought to cause bradykinesia, the basis for rigidity and tremor is linked to DA cell loss in the nigrostriatal system (Jankovic 2008, Schulz-Schaeffer 2010).

Postural instability and gait disturbances (PIGD), caused by a loss of postural reflexes, sets in during the later stages of PD, usually 9 years after its onset. This latency period distinguishes PD from other parkinsonism disorders, e.g. ~1 year for progressive supranuclear palsy and ~3 years for multiple system atrophy (Wenning et al. 1999). Impairment of postural reflexes is a major cause of frequent falls and bone fractures (Williams, Watt and Lees 2006).

1.4.4.2 Non-motor Symptoms

Non-motor symptoms (NMS) are prevalent, yet underrecognized among sufferers of PD. Efforts have been made to increase understanding in this field, including a systemic review by Chaudhuri et al. in 2006. The prevalence of NMS increases from 21% at the initial diagnosis, to 88% after 7 years of follow up (Bonnet et al. 2012). While NMS can precede the onset of motor symptoms, they coexist with, and even surpass the disabling features of motor dysfunction during late stages of the disease. The cause

of NMS is not just isolated to depletion of dopamine, but involves a network of malfunctioning neurotransmitter systems. These symptoms include sleep disorders, sensory, autonomic and gastrointestinal symptoms, and neuropsychiatric dysfunction, which will be covered in the following chapters (Chaudhuri et al. 2006).

1.4.4.3 Dementia

A study by the Sydney Multicentre study of PD found that approximately 80% of PD patients exhibited cognitive decline, with half of them meeting the criteria for dementia after 15 years (Hely et al. 2005). Dementia is able to predict mortality risk in PD patients. The mortality risk for PD with dementia (PDD) cases is almost double that of non-demented PD cases (Louis et al. 1997, Levy et al. 2002).

Several risk factors in PD have been found to cause a predilection for dementia. These include older age at disease onset and a longer duration of the disease (Bonnet et al. 2012). Patients with symptoms of rigidity and PIGD usually suffer from a rapid cognitive decline. PIGD-dominant subtype, but not tremor-dominant subtype, is found to have a much higher risk of developing dementia (Alves et al. 2006). Other cognitive symptoms like posterior cortically based deficits (Williams-Gray et al. 2007),visual hallucinations (Aarsland et al. 2003), and apathy (Pedersen et al. 2009) were also found to associate with an increased risk of dementia.

Dementia in PD differs from AD in several aspects. Attention deficit, executive dysfunction, visuo-perceptual and visuo-spatial processing is more pronounced in PDD than AD patients, while language functions, visual recognition and verbal memory are impaired to a lesser extent than AD. These differences are most prominent during the early and mid stages of dementia, while there is a large overlap of impairment across the cognitive domains in late stages (Emre et al. 2007).

The pathophysiology of dementia in PD can be partly attributed to monoaminergic and cholinergic deficit, causing a “subcortical dementia” that differs from the dementia of AD. The demise of neurons in the basal forebrain causes a loss of cholinergic input into the cortex (Whitehouse et al. 1983). Lewy Bodies (LB) were found to be extensive in the cortex of PDD patients as opposed to non-demented PD patients (Hurtig et al. 2000), and density of LB and Lewy neurites (LN) in the limbic regions-including CA2-3 of the hippocampus, the transentorhinal cortex and amygdala were statistically higher in PDD than PD patients (Churchyard and Lees 1997).

1.4.4.4 Anxiety and Depression

Anxiety and depression are common non-motor symptoms in PD. Both disorders indicate an increased risk of developing PD, and are thought to be an early

manifestation of the disease (Bonnet et al. 2012). This corresponds with the Braak staging system for PD, which specifies the degradation of neurons in the dorsal raphe nuclei (DRN) and dorsal motor vagus nerve (DMV) before that of DA neurons in the SN. Anxiety and depression do not correlate with cognitive decline or severity of motor dysfunction (Eskow Jaunarajs et al. 2011).

Anxiety is found in ~20% of PD patients (Nuti et al. 2004). PD patients are twice as susceptible to anxiety compared to the rest of the population. Anxiety can further aggravate motor and cognitive dysfunction already present in PD. Subtypes include generalized anxiety, panic disorder, social and other phobias (Bonnet et al. 2012).

Depression is most impactful on patients' quality of life (Schrag 2006), and can affect up to 45% of PD patients (Lemke 2008). Depression can precede motor symptoms, but can occur any time during the disease course. It consists of major and minor depressive disorder and dysthymia. Diagnosing depression comes as a challenge as its symptoms might be a psychological consequence of PD. However, PD patients with depression do not usually exhibit guilt, feelings of failure, nor suicidal tendencies (Costa et al. 2012).

The neurotransmitter systems thought to be involved in depression and anxiety include noradrenaline in the LC, dopamine in mesocortical and mesolimbic pathways, and serotonin in the DRN. PET imaging shows diminished noradrenergic activity in the LC and thalamus of both depressed and/or anxious patients (Remy et al. 2005). Neuronal loss and gliosis in the LC was 7 times greater in PD with depression than those without depression (Frisina, Haroutunian and Libow 2009). PET imaging also demonstrated dopamine deficiency in the putamen of depressed PD patients (Remy et al. 2005). PET studies on serotonergic function by assessing availability of serotonin transporter 5-HTT found higher 5-HTT binding in the amygdala, hypothalamus, caudal raphe nuclei, and posterior cingulate cortex, indicating lower serotonin levels (Politis et al. 2010).

1.4.4.5 Psychosis

Definition of psychosis includes hallucinations and/or delusions in patients with a clear sensorium. It can only be assessed by questioning the patient. Late onset PD, DA therapies, older age, REM sleep disorders and cognitive impairment are some examples that can cause an increased risk of psychosis. Incidence and prevalence of hallucinations are eight out of a hundred, and 46% respectively (Fenelon et al. 2000, Forsaa et al. 2010). Psychosis usually occurs in PD patients due to PD medications, and this factor can be used to differentiate between PD and dementia with lewy bodies (DLB) during diagnosis, as visual hallucinations are a core feature of DLB, regardless of treatment (McKeith et al. 2005).

Hallucinations can be differentiated into two types. Benign hallucinations are mostly well tolerated and typically do not require treatment. Most hallucinations are visual, while auditory, tactile or olfactory ones are less common. The other group exhibits complex psychotic symptoms that involve elaborated hallucinations and persecutory delusions that are usually disabling (Marsh et al. 2004, Fenelon and Alves 2010).

Most hallucinations are induced by PD drugs, and improvement can be seen with reduction of drug intake. These drugs include L-dopa, dopamine agonists, monoamine oxidase inhibitors, and anticholinergics. The DA medications might act to overstimulate dopamine receptors in the frontal and limbic regions, interfere with GABAergic neurons and upset the balance of dopamine and serotonin levels (Wolters 1999, Wolters 2001).

1.4.5 Neuropathology of PD

The histopathological hallmark of PD are alpha-synuclein (α-syn) inclusions throughout the central and peripheral nervous system, and are found in the cell bodies and axons of neurons as LB and LN respectively.

Many aberrant neurochemical pathways have been implicated in PD, e.g. inflammation, dysfunction of mitochondria, oxidative stress and increased production of ROS and NO, excitotoxicity, protein modification e.g. phosphorylation, and degradation e.g. ubiquitin-proteosome system, and toxicity of misfolded protein aggregates (Riederer et al. 2008, Hirsch and Hunot 2009, Farooqui and Farooqui 2011).

1.4.6 Neuronal loss in PD

Neuronal loss is prominent in the SN, and the loss of pigmented DA neurons is obvious even to the naked eye. However loss of neurons in other areas of the brain is found to be variable (Halliday et al. 2011). Neuronal loss is associated with selective brain regions where LB and LN are present, e.g. the SN, LC, DRN, NBM and DMV (Dickson et al. 2009).

1.4.7 Lewy inclusions and α-synuclein

LB are round eosinophilic inclusions (Figure 6) found in the soma of neurons of PD patients, and are required for definitive diagnosis of PD. Similar inclusions can also be found in neurites, and are called LN. LB are also found in the brains of non-PD, non-demented elderly (Parkkinen et al. 2001, Parkkinen et al. 2005). There are two types of LB, classical LB displaying a dense hyaline core and a pale periphery under light microscope, and cortical LB that are less well defined, lacking a distinct core and halo, and are less dense (Spillantini 1999). Pale bodies often accompany LB within the same

neuron, raising the possibility that they might be precursors to LB (Dale et al. 1992). The main component of LB is α-syn filaments measuring 200-600nm in length and 5-10nm in diameter with a β-sheet structure. Mutations and multiplications of the α-syn gene (SNCA) that cause autosomal dominant PD lead to discovery of its abnormal deposition in sporadic PD (Polymeropoulos et al. 1997). Other constituents of LB include ubiquitin (Kuzuhara et al. 1988), heat-shock proteins (Auluck et al. 2002), synphilin-1 (Eyal et al. 2006) and tyrosine hydroxylase (Riederer et al. 2008).

α-syn is transcribed from the SNCA gene found on chromosome 4q21, which consists of six exons, five of which are transcribed. There are three isoforms of α-syn, with the most focus placed on the longest (140 a.a.), most common isoform in the human brain (Xia et al. 2001). This particular isoform consists of three regions- an N-terminus with lipid binding properties, a hydrophobic non-amyloid component region in the center that is prone to aggregation, and an unfolded C-terminus that interacts with other proteins and has a chaperone function (Halliday and McCann 2008). The N terminal and central region have 5-7 repeats of the consensus sequence KTKEGV separated by repeats of 5-8 amino acids, and resemble apolipoprotein-like-A2 helix. The N terminal is susceptible to genetic mutations (Spillantini and Goedert 2000, Halliday and McCann 2008).

In normal physiological conditions, α-syn is found in neuronal presynaptic terminals in close proximity to synaptic vesicles, half of which are associated with synaptic membranes, and the other half found in the cytosol. In the cytosol, α-syn maintains a soluble random coil formation, but changes its secondary structure to 83% α-helix upon binding to phospholipids. This binding domain is found in its amino-terminal (Davidson et al. 1998). α-syn is probably involved in the cycling of synaptic vesicle between the reserve pool via interaction with synapsin 1. When α-syn was depleted by antisense oligonucleotides in cultured neurons, reduction of synaptic vesicles in the distal reserve pool was observed (Murphy et al. 2000). α-syn is also found in non-neuronal cells like astrocytes and oligodendrocytes (Mori et al. 2002) and vascular endothelial and smooth muscle cells (Tamo et al. 2002).

Oligomers of α-syn can aggregate to form β-pleated sheets called protofibrils, which then further aggregate into fibrils. A30P mutation retards the formation of fibrils from protofibrils, which first drew focus to the toxicity of protofibrils (Conway et al. 2000). Protofibrils were also observed in the brains of familial PD with SNCA triplication (Miller et al. 2004). These protofibrils are capable of forming pores and permeabilizing vesicles. While formation of protofibrils are further enhanced by A30P and A53T mutations, only A53T promotes fibril formation (Volles and Lansbury 2002). Ala30Pro

mutation eliminates monomeric α-syn ability to bind vesicles through creation of a structural kink, while Ala53Thr reduces its ability to interact with planar lipid membranes (Lotharius and Brundin 2002).

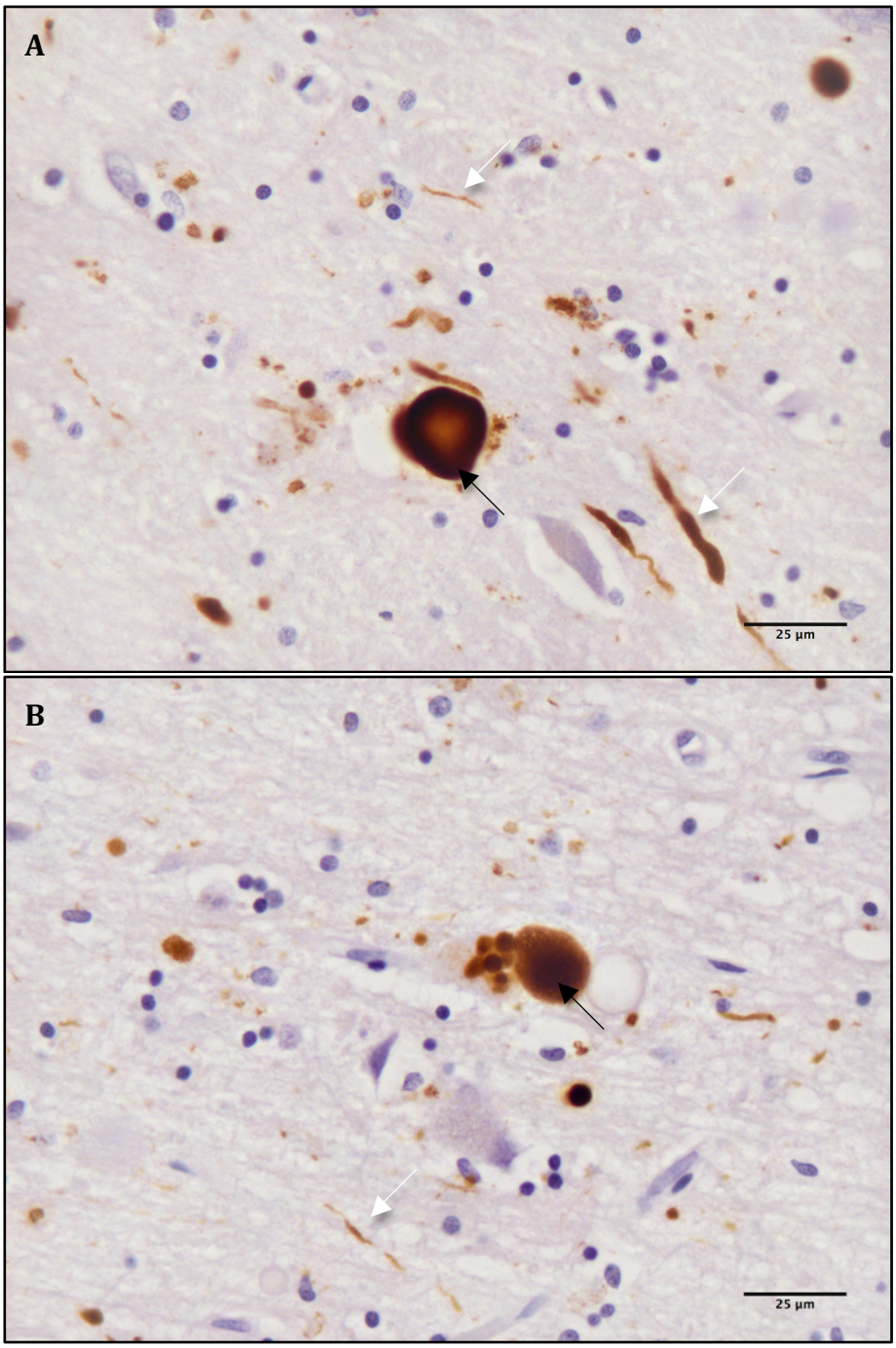
LB are thought to be neuroprotective, while the α-syn protofibrils are cytotoxic. Hence, the process of aggregating protofibrils into inert fibrils, and finally sequestering them into LB, would aid the cell in limiting damage from toxicity (Tompkins and Hill 1997). However, it is only a matter of time that LB will hinder cellular functioning with the increasing amount of space it takes up within the cell (Lotharius and Brundin 2002). α-syn found in LB is post-translationally modified, e.g. phosphorylation (Ser 87 and 129), nitration, truncation of carboxy terminal and ubiquitination. These modifications are thought to increase α-syn toxicity and susceptibility to aggregation (Tofaris and Spillantini 2005, Beyer and Ariza 2013).

Vulnerability of SN DA neurons has been attributed to oxidative stress, increased iron levels (Hirsch 2009), dopamine metabolism and H_2O_2 production. Auto-oxidation of dopamine produces dopamine-quinone molecules, which increase the toxicity of protofibrils by inhibiting the formation of fibrils through the formation of covalent adducts with α-syn protofibrils (Conway et al. 2000). However, these vulnerabilities cannot explain the degeneration of non-DA neurons e.g. serotoninergic, catacholinergic, which are also prominent in PD.

One hypothesis for the transmission of α-syn pathology is via a trans-synaptic spread. Patients who received foetal mesencephalic neurons as striatal transplant developed LB-like inclusion 11-13 years later in the grafted neurons (Kordower et al. 2008). This phenomenon was also observed in an α-syn transgenic mice model (Desplats et al. 2009). Extracellular α-syn is taken up by neurons via endocytosis, which then seeds aggregates by recruiting endogenous α-syn (Hansen et al. 2011, Volpicelli-Daley et al. 2011).

No correlation was found between LB and clinical disease symptoms or cell loss, suggesting that PD spreads in a format not indicated by the presence of LB (Schulz-Schaeffer 2010).

Figure 6. Lewy pathology in PD



(A & B) Lewy bodies (arrows) and Lewy neurites (arrowheads) in the nucleus basalis of Meynert of a PD case. Magnification x40

1.4.8 Ubiquitin-proteosome system

The ubiquitin-proteosome system (UPS) is essential for clearance of unwanted or damaged proteins; as such its dysfunction in PD has several consequences. The impaired degradation of proteins results in disruption of cellular processes that depend on the proper functioning of UPS, including the accumulation of α-syn (Lotharius and Brundin 2002). Defects in the 26/20S proteosome have been found in the SN of sporadic PD patients, which might cause protein accumulation, formation of LB and subsequent DA neuron cell death (McNaught and Jenner 2001). Binding of α-syn oligomers and filaments to this proteosome markedly inhibited its activity (Lindersson et al. 2004).

Mutations in genes PARK2 and PARK5 are implicated in the ubiquitin-processing pathway. PARK2 encodes for parkin, a component of the E3 ubiquitin-ligating enzyme (Zhang et al. 2000), while PARK5 encodes for ubiquitin carboxy-terminal hydrolase L1, a deubiquitinating enzyme that recycles ubiquitin (Lotharius and Brundin 2002). Patients who are autosomal recessive for parkin mutation have an early onset and do not develop LB.

1.4.9 Staging of Lewy related α-synuclein pathology

Lewy pathology spread in a predictable topographical pattern in most cases, from the brainstem to limbic structures, and finally affecting the cerebral cortices. Lewy pathology first appears in the olfactory and medullary regions, and also in peripheral autonomic nervous system, including the gastrointestinal tract and enteric plexus, and autonomic nuclei in the spinal cord (Probst et al. 2008). Widespread cortical LB are found in end-stage PD, and these patients are afflicted with cognitive impairments (Tsuboi, Uchikado and Dickson 2007). Two most commonly utilized staging systems are the Braak's staging and DLB consortium of LB pathology (Braak et al. 2003, McKeith et al. 2005).

In 2003, Braak and colleagues developed a staging system using α-syn as a marker. Braak stages start from the DMV and olfactory bulb during stage 1, and moves up into the lower raphe nuclei, magnocellular reticular formation, and the LC in stage 2. Both these stages are asymptomatic in terms of motor dysfunction, but exhibit autonomic dysfunction. Stage 3 sees the infamous SN being affected, together with the onset of motor symptoms. The basal forebrain and amygdala also develop Lewy pathology. The pathology extends to the cortex in stage 4, affecting the temporal mesocortex and CA2 of the hippocampus. By stage 5, Lewy inclusions are observed in the cingulate gyrus, insular cortex, and the temporal neocortex. The final stage of PD comes with Lewy

inclusions in the secondary and even primary fields of the neocortex (Braak et al. 2006).

An oversight of these two systems is the failure to include a subset of PD cases that have α-syn pathology that initially bypasses the brainstem and begins in the limbic system, specifically the amygdala. Hence, Leverenze et al has sought to resolve this problem by adding an amydala-predominant category (Leverenz et al. 2008). In 2009, BrainNet Europe (BNE) published a new staging system that combines both Braak and McKeith staging systems and incorporates the amygdala-predominant stage, in addition to brainstem, limbic, and neocortical-predominant types (Alafuzoff et al. 2009).

While these staging systems suggest a stepwise progression of α-syn pathology in PD, incidental Lewy body disease tells a different story. Both central and peripheral nervous systems are concurrently affected by Lewy pathology even when there are no symptoms for PD, indicating the possibility that a multicentric spread of α-syn pathology might also be taking place (Dickson et al. 2008). Some cases of PD were also observed to have Lewy pathology in the neocortex before the brainstem and limbic system were involved (Beach et al. 2009).

1.4.10 Causes of PD

1.4.10.1 Genetic causes of PD

Approximately 5-10% of PD cases are due to genetically inherited mutations, while the rest are idiopathic (Farooqui and Farooqui 2011). Genetic mutations give insight to aberrant proteins and pathways underlying the pathology of PD, and can be helpful in further defining the cause of PD. The PD-related genes are named PARK1-16, of which some of the better-known ones will be elaborated.

Genes that cause autosomal dominant PD include PARK1/SNCA on chromosome 4q21 that codes for α-syn, PARK3 on chromosome 2p13 of which gene product is still unknown, PARK5 found on chromosome 4q21 that codes for ubiquitin-C terminal hydrolase-L1 (UCH-L1), and PARK8 on chromosome 12 that codes for leucine-rich repeat kinase. Genes related to autosomal recessive PD include PARK2 on chromosome 6q25 coding for Parkin, PARK6 on 1p35-36 coding for PTEN-induced putative kinase 1 (PINK1), PARK7 and PARK9 on 1p36 that codes for DJ-1 and ATP13A2 protein respectively (Klein and Westenberger 2012).

Mutations in Parkin, an E3 ubiquitin ligase, UCH-L1, and ATP13A2- a lysosomal ATPase, all indicate a faulty protein degradation pathway (Ramirez et al. 2006, Wirdefeldt et al. 2011). Mutations in PINK1, a mitochondrial kinase, and DJ-1 have been associated with mitochondrial dysfunction, and protecting against oxidative stress (Mitsumoto and Nakagawa 2001, Valente et al. 2004).

1.4.10.2 Toxins

Both pesticides and metals have been linked to a higher risk of parkinsonism. However, it has been deemed by several groups that there is insufficient evidence to link pesticides exposure to PD (Li et al. 2005, Brown et al. 2006, Dick 2006). No association was found between metals and PD as well (Firestone et al. 2010, Wirdefeldt et al. 2011).

The most studied toxin models that result in parkinsonism include 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA). MPTP causes severe akinetic rigid syndrome when accidentally administered by a group of drug addicts, consistent with end-stage PD in a matter of days (Langston et al. 1983). It affects DA neurons of SNpc, but unlike in PD, those in the LC are spared (Forno et al. 1986). No LB-like inclusions have been observed in human or animal models either (Teismann et al. 2003). Administration of 6-OHDA causes extensive depletions of dopamine and noradrenaline. Application of 6-OHDA to the SN immediately obliterates the DA neurons in the region, while application to the striatum results in a slower progressive degeneration in the SNpc (Teismann and Schulz 2004).

Pesticides like paraquat and rotenone are chemically similar to MPTP (Litteljohn et al. 2010) and can also cause parkinsonism. These pesticides produce a neuropathological profile that resembles idiopathic PD more than MPTP i.e. they cause LB-like neuronal inclusions of α-syn (Manning-Bog et al. 2002, Cannon et al. 2009). They also exert toxicity through microglia, by triggering the release of pro-inflammatory cytokines and causing oxidative damage through the activation of NADPH oxidase (Gao et al. 2003, Wu et al. 2005).

1.5 Neuroinflammation in Parkinson's Disease

Inflammation is thought to contribute significantly to the pathogenesis of PD. This is most evident in the SN of PD patients, where there is pronounced microglia activation, coupled with massive DA neuronal cell loss (Hirsch et al. 2003, Croisier et al. 2005). The midbrain of the rat contains 4.5 times more microglia compared to other brain regions (Kim et al. 2000). SNpc is predisposed to neurodegeneration in PD. This might be due to having a high density of microglia, as well as DA neurons' susceptibility to oxidative stress (Qian and Flood 2008). Using PET scanning for activated microglia with marker [^{11}C](R)-PK11195, Ouchi et al. found that microglial activation positively associates with DA terminal loss in the midbrain (Ouchi et al. 2005).

An increase in oxidative stress mediators like COX-2 and iNOS, cytokines e.g. TNFα, IL-1β, IL-2, IL-4, IL-6, TGFβ and IFNγ, and a decrease in neurotrophic factors like brain derived neurotrophic factor (BDNF) and NGF are observed in the nigrostriatal regions and in the cerebrospinal fluid of PD patients (Hunot et al. 1996, Mogi et al. 1996, Knott, Stern and Wilkin 2000, Nagatsu et al. 2000, Liu 2006). COX-2 increases production of dopamine-quinone, an oxidative product that is harmful to mitochondria (Teismann et al. 2003). The release of MMP-3 by apoptotic neurons further induces microglia to release TNFα, IL-6 and IL-1β that activates the NF-kB pathway and exacerbate neuronal death (Kim et al. 2005). Increased expression of TNF-R1, B-cell lymphoma 2 (bcl-2), and caspases 1 and 3 were also seen in the neurons of nigrostriatal regions (Mogi et al. 2000, Nagatsu et al. 2000).

It is crucial to know that microglia activation is not restricted to the SN and striatum, but is widespread throughout the brain in PD. Activated microglia are also found in subcortical regions including the hippocampus, pons, striatum, pallidum and thalamus; as well as cortical regions including cingulate and precentral gyri, and frontal and temporal lobes. As revealed by PET imaging, microglia are activated even during early stages of PD, but do not correlate with disease progression and severity. Hence, the demise of DA neurons in the SN and striatum cannot be attributed to inflammation alone (Imamura et al. 2003, Gerhard et al. 2006).

Along the lines of inflammation, NSAIDs are thought to exert a protective effect against PD based on their anti-inflammatory actions. Their effects have been largely debated. A meta-analysis showed that NSAIDS did not offer any neuroprotection, although ibuprofen had a slight effect of lowering the risk of PD (Samii et al. 2009).

1.5.1 Inflammation caused by α-synuclein

α-syn is known to cause florid neuroinflammatory responses that trigger neuronal death (Tansey and Goldberg 2010). In a mouse model of PD, overexpression of human α-syn alone, without significant neurodegeneration, was sufficient to activate microglia, triggering a pro-inflammatory reaction and the gradual demise of DA neurons in the SN (Theodore et al. 2008). Inflammation in mice models engineered to express human or mutant α-syn resulted in aggregation of α-syn and neuronal death (Gao et al. 2008).

There has been no evidence of intracellular aggregates of α-syn attracting nor triggering microglia activation (Rozemuller et al. 2000). Microglial activation defined by the expression of MHCII in the SN correlates positively with the amount of intracellular and extracellular α-syn deposits, but do not show any association with clinical severity or disease progression (Croisier et al. 2005).

On the other hand, extracellular aggregates of α-syn are able to activate microglia. This happens when damaged DA neurons release α-syn into the extracellular environment. As demonstrated using murine cell cultures, neurotoxicity of α-syn is mediated by its phagocytosis and subsequent activation of microglia, leading to an increase in expression of NAPDH oxidase and production of superoxide and ROS (Zhang et al. 2005).

It has been suggested that microglia might be dysfunctional in PD, affecting their capacity for phagocytosis, which causes a buildup of excess of synuclein (Halliday and Stevens 2011). For example, activation of microglia with LPS in cell cultures slows the degradation of phagocytosed α-syn, resulting in accumulation of α-syn in cytoplasm of microglia (Lee et al. 2008).

1.5.2 Inflammation caused by neuromelanin

Extraneuronal neuromelanin (NM) deposits increase with pathological stages of PD, together with a gradual loss of melanized neurons (Braak et al. 2003). Phagocytes from the SN are also observed with NM. These NM pigments are derived from degenerated DA neurons. As one of the main functions of microglia is debris clearance, this freely accessible NM is most likely to attract and activate microglia (Croisier et al. 2005).

NM is a pigment that exerts its neuroprotective role by chelating iron, and can be found in the DA neurons of the SN and LC (Sulzer et al. 2000, Farooqui and Farooqui 2011). The higher levels of iron in PD might saturate the iron-binding sites of NM, overwhelming its protective capacity. The interaction between hydrogen peroxide (H_2O_2) and ferrous iron can generate hydroxyl radicals via the Fenton reaction. Iron can also disrupt the ubiquitine-proteosome system in mitochondria, leading to a failure to eliminate and regulate the levels of α-syn (Gerlach, Riederer and Double 2008).

Injection of human NM into rat SN caused a strong, pervasive microglia reaction together with a significant loss of DA neurons (Zecca et al. 2008). NM is able to induce chemotaxis and production of TNFα, IL-6, hydrogen peroxide, superoxide, and NO in murine microglia cultures via activation of NF-kB (Wilms et al. 2003, Zhang et al. 2011). An excess of NM in the extracellular matrix might ultimately lead to chronic inflammation (Ouchi et al. 2009).

1.5.3 Oxidative Stress

ROS is produced naturally as a by-product of metabolism. Oxidative stress occurs when there is insufficient scavenging of ROS and it overwhelms the antioxidant system, causing oxidative damage to proteins, lipids, and DNA. Microglia are able to detect and respond to neurodegeneration caused by oxidative stress, adopting a pro-inflammatory profile and releasing even more ROS, RNS and pro-inflammatory cytokines (Valko, Morris and Cronin 2005, Farooqui and Farooqui 2011).

ROS are produced upon the breakdown of dopamine. This can happen spontaneously in the presence of iron, resulting in the production of toxic dopamine-quinone, superoxide anions, and H_2O_2. Dopamine can be broken down via catalysis by monoamine oxidase, which also generates H_2O_2. While H_2O_2 is not harmful by itself, it can be catalysed by iron into cytotoxic hydroxyl radicals via the Fenton reaction (Lotharius and Brundin 2002). Another enzyme that produces ROS is COX-2, which is increased in DA neurons of the SN of PD patients and in mice models of MPTP (Teismann et al. 2003). It is obvious that several factors predispose DA neurons to oxidative stress- elevation of iron due to its affinity with NM (Sofic et al. 1991), reduced levels of antioxidants e.g. peroxidase and glutathione due to a sustained oxidative stress burden (Sian et al. 1994), and oxidation of mitochondrial complex 1 that increases the risk of neuronal apoptosis (Perier et al. 2005, Keeney et al. 2006). Indeed, a higher frequency of lipid, protein, and DNA oxidation were observed in the SN of PD patients.

Aside from DA neurons, other monoaminergic neurons that are affected in PD, e.g. noradrenaline and serotonin, are also prone to damage due to a high oxidative potential (Lotharius and Brundin 2002).

1.5.4 Trophic support in PD

Trophic factors have been considered a possible avenue for the protection of degenerating neurons, and a way to attenuate progression of PD. Some of them are produced by microglia, such as BDNF and glial-derived neurotrophic factor (GDNF).

BDNF are expressed at lower levels in the nigral melanised neurons of PD compared to control cases (Parain et al. 1999), and higher levels in the microglia surrounding fragmented nigral neurons (Knott et al. 2002). The lower number of BDNF-positive melanised neurons in PD might indicate that BDNF does not aid in neuronal survival, or that the lack of BDNF in nigral neurons results in a higher rate of cell death. Upregulation of BDNF in glia cells might be an attempt at providing neurotrophic support to the dying neurons (Knott et al. 2002). The latter is most likely the case as BDNF has been shown to increase survival rates of both mice and human fetal AD neurons using *in vitro* cell cultures (Zhou, Bradford and Stern 1994).

GDNF also exerts neurotrophic effects. Similar to BDNF, it has been shown to support the survival of postnatal nigral DA neurons in cell cultures (Burke, Antonelli and Sulzer 1998). Both BDNF and GDNF are able to induce DA fiber sprouting in a mice model of striatal injury (Batchelor et al. 1999). Intracerebroventricular injections of GDNF given to a 65-year-old PD patient had no effect on disease progression, nor was a reduction in loss of nigrostriatal neurons observed (Kordower et al. 1999). However, a later trial on five patients where GDNF was administered by continuous intraparenchymal infusion into the posterior putamen over 2 years resulted in an improvement in UPDRS scaling of the motor and activities of daily living, and health-related quality of life measure (Patel et al. 2005).

1.6 Traumatic brain injury

Traumatic brain injury (TBI) is an alteration of brain function or pathology brought about by an external force (Menon et al. 2010). Causes of TBI include falls, motor vehicle accidents, assaults, sports-related events, and blast injury (Nolan 2005, Ling et al. 2009, Mangat 2012). TBI is a major global health issue, with more than 10 million people affected every year (Hyder et al. 2007). This is especially relevant to the ageing population who are prone to falls and have the highest mortality rate linked to TBI (Rosenfeld et al. 2012).

TBI can range from mild to severe, and this can be measured using the Glasgow Coma Scale, a neurological scale developed by Teasdale and Jennett from the University of Glasgow in 1974, and which is the most commonly used scale today. It is able to provide a reliable assessment of the conscious state of a patient between 3-8 for severe TBI, 9-12 for moderate TBI, and 13-15 for mild TBI (Smith, Meaney and Shull 2003). While severe TBI usually results in death, survivors of TBI can go on to develop motor, cognitive and social disabilities (Moretti et al. 2012).

1.6.1 Pathophysiology of TBI

TBI refers to the original insult, but also evolves with time to cause further tissue damage. Injury can be classified as primary and secondary. The initial assault, also known as primary injury, directly damages the neuronal tissue and glial cells. Primary injuries can be further divided into focal and diffuse lesions based on pathology. Secondary injury arises as a normal physiological response to primary injury.

The type of impact determines the mechanism of primary injuries. Mechanistic classification includes inertial loading injuries, impact, penetrating or blast injuries (Smith 2013). Acceleration-deceleration/ inertial injuries comprise of two types- linear acceleration, which is relatively well-tolerated, and rotational acceleration which is particularly damaging to the human brain (Cantu 1996). Impact injuries can be a result of motor vehicle crashes, falls, and assaults, and are the most common form of TBI. Penetrating injuries, including bullet wounds, are caused by a breach of the skull and entry into the brain with a sharp or blunt object. Blast injuries are a combination of both blunt and penetrating forces (Nolan 2005).

Secondary injuries cause patients' conditions to deteriorate even further. They should be anticipated and prevented by caregivers. These injuries can be caused by dysregulation of CSF resulting in increased intracranial pressure (ICP) (Hiler et al. 2006), spreading depolarizations that result in a worse clinical outcome (Hartings et al. 2011), ischaemia, hypoxia, haemorrhage, cerebral oedema, and systemic changes in haemodynamics and temperature (Nolan 2005).

Molecular processes can also contribute to secondary damage e.g. mitochondrial injury resulting in metabolic impairment (Coles et al. 2004), uncontrolled inflammation and oxidative stress (Deng-Bryant et al. 2008), ionic imbalance and excitotoxicity caused by excessive glutamate (Rosenfeld et al. 2012). The neuronal membrane defects triggered by primary injuries cause mechanical damage to voltage sensitive sodium channels, increasing the amount of sodium in the neuron. Through the reversal of the sodium-calcium exchanger, and depolarization of voltage gated calcium channels, calcium level in the cell is increased (Smith, Hicks and Povlishock 2013). Calpain, a calcium activated protease, degrades the inactivation gate of sodium channels, causing a feed-forward process of increasing sodium influx, leading to a further rise in neuronal calcium levels (von Reyn et al. 2009). This in turn intensifies the release of glutamate, which binds to N-methyl-D-aspartate receptors to cause further depolarization and calcium influx (Barkhoudarian, Hovda and Giza 2011). Deregulation of calcium also causes microtubule disassembly and mitochondrial damage (Smith et al. 2013).

1.6.2 Focal brain injury

Focal brain injuries are localized and arise from impact forces. They include subdural and extradural haematomas (SDH and EDH), cerebral contusions with intracerebral haemorrhages (ICH) and traumatic subarachnoid haemorrhages (SAH) (Mangat 2012). As focal injuries arise from severe direct impacts, they are usually found in cases of severe TBI (Blennow, Hardy and Zetterberg 2012).

EDH are located outside the dura mater and are caused by an arterial tear. SDH develop below the dura mater and are venous in nature. A contusion is a bruise to the brain tissue with multiple microhaemorrhages, caused by compression of the brain against the skull at the point of impact, and 180^{o} from the point of impact. The frontal and temporal lobes are especially susceptible to contusions. Brain compression causes cerebral oedema, which may increase ICP, leading to secondary injury. ICH results from hypertension or gunshot wounds, and SAH from head injury or aneurysmal rupture (Nolan 2005).

1.6.3 Diffuse brain injury

Diffuse lesions differ from focal injuries in that they arise from inertial forces- shaking, shearing, in the absence of impact forces. Hence, it can occur in cases of mild TBI. Diffuse brain injuries can also result in secondary injury similar to that seen with focal injuries. Cerebral concussion and diffuse axonal injury (DAI) are the two main forms of diffuse brain injury (Nolan 2005). Long-term effects of diffuse brain injury can result in

prolonged microglia activation and increased susceptibility to AD. This will be covered in the following sections.

Cerebral concussion is a temporary neurological dysfunction caused by a force to the brain that can be transmitted directly or indirectly from other parts of the body. It causes functional disturbance in the absence of a structural injury, and can range from mild to moderate. Mild concussions may cause dizziness, nausea, headaches, and attention, concentration and memory problems. Moderate concussions can involve loss of consciousness (McCrory et al. 2009). Patients are susceptible to transient symptoms after the trauma; this is referred to as post-concussion syndrome (Hall and Chapman 2005).

1.6.3.1 Diffuse Axonal Injury

Rapid rotational motions distort the white matter and cause shearing of axons, resulting in DAI. DAI occurs in vulnerable regions in the brain- in deep and subcortical white matter especially in parasagittal white matter like the brainstem, corpus callosum, and internal capsule and thalamus (Smith et al. 2003, Smith et al. 2013). It disrupts cortical-subcortical pathways and causes extensive neurological dysfunction (Moretti et al. 2012). Mild TBI patients usually exhibit concentration and memory deficits, which might be a sign of DAI (Smith et al. 2003). Recently, a technique known as diffusion tensor imaging has been found to be effective in identifying DAI pathology (Mayer et al. 2010).

After shearing of axons, axonal transport becomes disrupted and axonal bulb and/ or varicosities are observed. Axonal varicosities occur when transport is still intact, and slight disruption occurs at multiple sites along the axon. As for axonal bulb, the distal segment of the axon is disconnected from the soma via Wallerian degeneration due to a complete interruption of axonal transport, while the proximal segment may die due to lack of connection from other neurons (Tang-Schomer et al. 2012, Smith et al. 2013). These pathology can be observed even years after the initial TBI incident- an indication that degeneration of axons is progressive (Chen et al. 2009). Due to a loss of connections, neuronal loss distal from the injury site might occur (Neumann 2003). It is thought that axonal pathology most likely contributes to the development of AD in TBI patients (Johnson, Stewart and Smith 2012).

APP is the benchmark for detection of DAI in standard neuropathological procedures. Normal levels of APP are not detected by immunohistochemistry. However after rupturing of axonal membranes, APP accumulates via anterograde axonal transport in damaged regions and becomes detectable (Gentleman et al. 1993).

1.6.4 Chronic Traumatic Encephalopathy

Chronic traumatic encephalopathy (CTE) is observed in boxers and can be associated with other types of contact sports e.g. wrestling, hockey, rugby, etc. as well as military personnel prone to blast injuries. The effects range from a decline in attention, memory and concentration, disorientation, dizziness and headaches, to deafness, dementia, Parkinsonism, and behavioral changes (McKee et al. 2009). Pathological characteristics of CTE include cerebellar, hippocampal, and cortical atrophy, degradation of SN, cavum septum pellucidum, DAI, gliosis, and deposits of Aβ, NFT, and Tar DNA-binding protein-43 (Corsellis, Bruton and Freeman-Browne 1973, Orrison et al. 2009, Levin and Smith 2013).

CTE is thought be a result of repeated mild TBI to the head. The initial concussion disturbs the cerebral physiology, and increases the susceptibility of the brain to further injury. Metabolic dysfunction and sodium channelopathy can both contribute to the increased vulnerability as well (Vagnozzi et al. 2008).

1.6.5 Long-term effects of TBI

The progressive nature of TBI, especially DAI, is thought to cause a predilection to the development of neurodegenerative diseases. Long-term structural deficits include white matter loss, size reduction of the frontal and temporal cortices, corpus callosum and hippocampal volume (Tasker et al. 2005, Wilde et al. 2005). Other pathological changes are also observed e.g. inflammation, wallerian degeneration, dendritic alterations, neurotransmitter imbalances, and impairment of metabolism (Johnson et al. 2012, Moretti et al. 2012).

TBI is one of the strongest risk factors for the development of AD (Mortimer et al. 1985, Fleminger et al. 2003). TBI could induce neuroplasticity for damage repair, while simultaneously draining the resources for neuroplasticity required later in life to counteract aging (Moretti et al. 2012). Aβ plaques and NFT are found in survivors of TBI of more than a year (Johnson et al. 2012). Even without the development of AD, concussions have resulted in a compromise of episodic memory and frontal lobe function (Moretti et al. 2012).

1.7 Neuropathology of TBI

1.7.1 Aβ

Extensive Aβ plaques can be found in victims of acute TBI and CTE. They are formed within hours of the injury and are found even in young individuals. Diffuse Aβ plaques are found in 30% of TBI patients, and are similar to those found in AD and Down's syndrome patients (Roberts et al. 1994, Gentleman et al. 1997). However, neuritic plaques were absent in cases of CTE (Smith 2013).

APP is upregulated in response to TBI and neuronal damage due to its neurotrophic functions, including synaptogenesis and a role in axonal sprouting (Blennow et al. 2012). Administration of soluble APP in a rat model of TBI reduced neuronal and axonal injury, apoptosis, and improved functional outcome (Thornton et al. 2006). In DAI, the accumulation of APP in damaged axons can serve as a source for Aβ plaque formation. APP, caspases, presenilin-1 and β-site APP cleaving (BACE) enzymes are all brought to close proximity in the axonal bulb (Chen et al. 2004, Chen et al. 2009). Whether the accumulation of Aβ and plaque formation leads to AD has been contested, as Chen et al. 2009 found virtually no plaques in long-term survivors (Tran et al. 2011).

1.7.2 Tau

NFT, NT, and glial tangles are found in survivors of moderate to severe TBI and CTE. The tau in these deposits is phosphorylated and ubiquitinated at the same amino acids as those found in AD. This raises the possibility that the same pathological pathways are affected in both AD and TBI, thus predisposing TBI patients to develop AD (Schmidt et al. 2001, Smith 2013). However, tau pathology in TBI is found in different locations from that in AD- tau pathology is found in both deep and superficial layers of the cortex in AD. In TBI cases, it is found in the superficial cortical layers of the frontal and temporal cortices, and with an apparent perivascular distribution. They also existed in irregular patches, and were most concentrated in sulci depths. The irregularity of their deposition can be attributed to the random direction of shearing forces that occur during TBI (McKee et al. 2009).

When γ-secretase inhibitors were used to treat cortical impact traumatic axonal injury (TAI) in mice models of AD, Aβ accumulation was attenuated, but tau pathology was not. This suggests that NFT formation is independent, and not downstream of Aβ plaque formation (Tran et al. 2011). Tau phosphorylation and accumulation in TBI can be explained by the activation of c-Jun N-terminal kinase (JNK), which is deregulated in

TBI. Inhibition of JNK reduced the accumulation of total tau and phosphorylated tau in injured axons of a mice model of TAI (Tran, Sanchez and Brody 2012).

1.7.3 Inflammation

During the acute phase of TBI, compromise of the BBB would allow leukocytes, T cells, natural killer cells, and macrophages to infiltrate the brain from the periphery. These cells release free radicals, complements, chemokines and pro-inflammatory cytokines with a goal of limiting tissue damage and initiate repair (Ziebell and Morganti-Kossmann 2010).

Microglia are the key mediators of the inflammatory response to TBI. They migrate towards lesions, and utilize their ramifications to form a protective barrier between the injured and healthy tissue. This is mediated by the release of ATP from damaged tissue, which activates microglia and induces chemotaxis (Davalos et al. 2005). Microglia also release a plethora of pro-inflammatory cytokines and neurotoxic substances like ROS and RNS (Ziebell and Morganti-Kossmann 2010). While the number of peripheral lymphocytes in the brain decreases rapidly, microglia and macrophages remain activated, even at areas remote from the lesion, for up to 18 years post-injury. PET imaging of activated microglia using the PK ligand also showed chronic inflammation up to 17 years after TBI, and florid activation were observed in brain regions remote from focal lesions, especially in subcortical structures like the thalamus and putamen (Ramlackhansingh et al. 2011). Hence, persistent neuroinflammation is thought to contribute to the long-term effects of TBI (Gentleman et al. 2004, Johnson et al. 2013).

Long-term survivors of TBI were observed to have amoeboid microglia that were immunoreactive for CR3/43 and CD68 throughout various regions of the brain, especially in the corpus callosum. These microglia were not associated with lesions and are linked to DAI. In these areas with dense microglia activation, luxol fast blue staining revealed a reduction in myelin density and disruption of white matter, demonstrating a progressive degeneration of the white matter. As seen with the long-term effects of TBI, inflammation might contribute substantially to the development of AD (Johnson et al. 2013, Smith 2013).

Hypothesis

Microglia phenotype, an indication of classical and alternative activation states, differs in AD, PD and TBI.

Aims

Inflammation is a common denominator in many neurodegenerative diseases. Microglia in AD and PD are associated with classical activation and pro-inflammatory factors. This led to the proposition that microglia are driving the pathogenesis of these diseases, ultimately causing synaptic and neuronal loss. The current perception of the role of microglia in neurodegenerative diseases is an injurious one. However, recent evidence revealed an increase in the mRNA of alternative activation markers in AD.

This study is based on the theory that microglia exist in a heterogeneous state in chronic neurodegenerative diseases. The purpose of this study is to explore the phenotypic profile of microglia and find evidence of alternatively activated microglia in AD and PD in order to disprove the notion that the sole outcome of microglial activation is the destruction of neurons.

Pathological similarities exist between AD, PD, DLB and TBI- evidence of α-syn has been found in AD, especially in the amygdala, while AD pathology also exist in PD and DLB cases. In fact, distinguishing between the contributions of α-syn, tau and Aβ pathology towards the clinical symptoms of DLB remains an issue. A theory that AD, PD, and DLB exist as part of a continuum has been proposed. These pathologies might instigate divergent microglial behaviour, resulting in a range of diseases with different progression and symptomatology. Investigation into microglia's association with different pathological hallmarks will be carried out.

Chronic inflammation after TBI is thought to predispose patients to an increased risk of AD later in life. Studying microglia in TBI cases may provide an insight into the contribution of inflammation towards the development of cognitive impairment.

Aims of the thesis:

1. Characterize the phenotype of microglia in AD using markers of classical and alternative activation.

2. Evaluate the consistency and reliability of microglial quantification methods

3. Investigate the spatial and temporal distribution of alternatively activated microglia, and their associations with other pathologies and clinical symptoms in AD and PD.

4. Compare microglial phenotype in TBI (acute) with AD and PD (chronic)

Chapter 2: Materials and Methods

2.1 Tissue specimens

Post-mortem brain specimens were obtained from the Corsellis archival collection (AD and control samples) and the Parkinson's UK Tissue Bank (UKPDSTB) at Imperial College London (PD, DLB and control samples).

31 AD cases of Braak stages 5 and 6 obtained from the Corsellis Archival Collection were used in this study. The patient cohort comprised of 21 females and 10 males, age range 65-88, mean age at death 76. The information on age at death, gender, and cause of death are listed in Table 2. Formalin-fixed, paraffin embedded samples from frontal cortex, hippocampus, and occipital cortex were obtained from all AD cases.

27 cases of LBD were obtained from the UKPDSTB- 10 cases from Braak stage 6, and 6 cases each from stages 4 and 5, and 5 cases from stage 3 were obtained from the UKPDSTB. This was made up of PD and DLB cases. The patient cohort comprised of 9 females and 18 males, age range 57-96, mean age of death 74. The age at death, gender, and cause of death for these cases are listed in Table 5. Formalin-fixed, paraffin-embedded samples from the frontal cortex, cingulate cortex, striatum, basal forebrain, hippocampus, midbrain, pons, and medulla were obtained from all LBD cases. Frozen sections from the frontal and cingulate cortices were obtained from 10 cases of LBD.

16 cases with no neurological or systemic disease were also selected as controls. These were classified as NPD (no psychiatric disease), and were obtained from both the Corsellis collection and the UKPDSTB. The patient cohort comprised of 8 females and 8 males, age range 59-81, mean age at death 70. Information on these cases is listed in Table 6. There were no significant differences in age at death between the AD, LBD and control group.

2.1.1 Clinical assessments of cases

Cases diagnosed with senile dementia by their respective physicians were obtained from the Corsellis archival collection. They were then neuropathologically assessed for both tau and Aβ pathology to ascertain that they were afflicted with AD.

Clinical records from the UKPDSTB were compiled retrospectively by Dr Ronald K.B. Pearce. Information on the gender, age of onset, age at death, and duration of disease; mode of motor onset i.e. tremor, akinetic-rigid syndrome, gait and balance impairment; as well as the presence of neuropsychiatric complications including dementia, anxiety, depression, visual hallucinations, and psychosis were also noted under the guidance of

Dr Pearce. Two or more of the cardinal signs- rigidity, bradykinesia, resting tremor and postural instability, and a good response to levodopa determined presence of PD. Development of dementia one year after onset of motor signs were classified as PDD, while DLB cases developed cognitive impairment within one year of, or preceding motor signs.

2.1.2 Neuropathological assessment of cases

Confirmation of patients' AD status was determined by assessing AT8 immunohistochemical (IHC) staining based on the staging protocol from BNE (Alafuzoff et al. 2008a), and the presence of Aβ plaques using 4G8 antibody. Those with Braak stages 5/6 were chosen for this study.

Neuropathological diagnosis of PD and screening for confounding pathology were based on haematoxylin and eosin staining, as well as IHC staining for Aβ, tau, and α-syn on 13 diagnostic blocks. Neuropathological assessment was carried out by four neuropathologists- Professor Steve M. Gentleman, Dr Federico Roncaroli, Dr Illaria Bravi and Professor Manuel B. Graeber. Using BNE's staging protocol for α-synuclein pathology (Alafuzoff et al. 2009), 10 cases from Braak stage 6, and 6 cases each from stages 4 and 5, and 5 cases from stage 3 were selected for this study. There were no significant differences in age at death between the AD, PD and control group ($p=0.001$).

2.1.3 Sectioning and processing of formalin-fixed, paraffin-embedded tissue

Formalin-fixed, paraffin-embedded blocks were refrigerated prior to sectioning for an easier cut. The blocks were mounted on the microtome and sectioned at 7 μm. The sections were then transferred to a 40^{O}C water bath and floated out for ~2 minutes or until smooth, before mounting onto Superfrosted slides. The slides were placed in an 37^{o}C oven overnight for drying and stored at room temperature before use.

Before the staining process, paraffin was removed from the slides via two xylene washes. They were then rehydrated by soaking in 4 industrial methylated spirits (IMS) washes of different concentrations, in the order – 100%, 100%, 90% and 70% for 5 minutes each. Sections were blocked for endogenous peroxidase activity by incubating in 1% hydrogen peroxide for 30 minutes, followed by rinsing in phosphate-buffered saline (PBS). If required, antigen unmasking is carried out at this point.

Formaldehyde fixation creates hydroxylmethyl groups that cross-links with adjacent proteins via the formation of methylene bridges, hence masking antigens from detection by steric interference. Antigen retrieval destroys the cross-links and re-exposes the antigen/epitope for antibody recognition and binding (Bogen, Vani and Sompuram

2009). Power Block reagent (BioGenex, UK) was used before application of the primary antibody to reduce non-specific binding and background staining.

2.2 Antibodies

Monoclonal antibodies are generated by a single B-cell clone and hence recognize the same epitope on the antigen. After immunization against the antigen, B-lymphocytes from the animals e.g. mouse, rabbit, or rat, are retrieved from the spleen and fused with an immortalized myeloma cell line, also called a hybridoma. A stable clone with a high antibody production capacity is picked and isolated from the hybridoma cell line. Large amounts of antibody can be generated from the hybridoma cell cultures using bioreactors.

While monoclonal antibodies are more consistent and less likely to cross-react than polyclonal ones, the multi-clonality of polyclonal antibodies makes them less vulnerable to destruction of epitopes during tissue processing. Polyclonal antibodies are generated from different B-cell clones of an animal, and thus comprise of a heterogeneous concoction of antibodies with different specificities recognizing a range of epitopes from the same antigen. They can be produced in different animals, the most common being rabbits due to a lower frequency of human antibodies against rabbit proteins and easier maintenance.

All primary antibodies employed in IHC, double immunofluorescence (DIF), Western blotting (WB) are shown their respective results chapters. Both monoclonal and polyclonal antibodies labeling the same antigens were utilized for the purpose of DIF. Secondary antibodies used in DIF and WB are listed in table 1.

Table 1: List of secondary antibodies used in the study

Alexa Fluor 488	Alexa Fluor 568
Donkey anti-goat IgG	Donkey anti-goat IgG
-	Donkey anti-rabbit IgG
Donkey anti-mouse IgG	-
Goat anti-rabbit IgG	Goat anti-rabbit IgG
Goat anti-mouse IgG	Goat anti-mouse IgG
Goat anti-mouse IgG_1, IgM	Goat anti-mouse IgG_1
Goat anti-mouse IgG_3	-
-	Goat anti-mouse IgG_{2a}

Non-Alexa Fluor conjugated antibodies	Conjugation
Goat anti-Chicken IgY	Northern lights 493
Rabbit anti-Goat IgG	Biotinylated

2.3 Methodology of immunohistochemistry

The Super Sensitive Polymer-HRP (horse-radish peroxidase) Detection system (BioGenex, UK) was used in most IHC experiments in this study. The detection system is suitable for both rabbit and mouse antibodies. For goat-derived antibodies, Vectastain Elite ABC detection system (Vector labs, UK) was used instead. For all solutions mentioned in this chapter, please refer to the appendix for their ingredients.

The primary antibody was diluted in PBS to its appropriate concentration, applied to the tissue and left overnight at $4^{O}C$ in a moist incubation chamber. After rinsing any unbound primary antibody (all rinses were done with 3 x PBS for 5 minutes each), the Super Enhancer was added for 20 minutes before rinsing again. Application of the Poly-HRP reagent followed and was left on for 30 minutes. A final rinse was done before staining was carried out using a solution of chromogen 3,3'-diaminobenzidine (DAB). Sections were incubated for 3 minutes and rinsed in distilled water. This was followed by counter-staining with haematoxylin for 10 seconds before transferring to running tap water.

Steps for using Vectastain Elite ABC kit are similar to that of the Super Sensitive Polymer-HRP system. The primary antibody was diluted to its appropriate concentration in PBS and the animal serum corresponding to the host species of the secondary antibody. The addition of the serum serves to prevent non-specific binding from the secondary antibody. The secondary antibody was used in place of the Super Enhancer and left on for an hour before rinsing. Vectastain ABC kit was used in place of Poly-

HRP reagent, and applied for another hour before rinsing. DAB and counterstaining steps remain the same.

The sections were dehydrated by soaking in 3 IMS washes of different concentrations, in the order of 70%, 90% and 100%, for 5 minutes each, before transferring to xylene. Cover slips were adhered to tissue sections with the help of DPX, a xylene-based mountant, and dried in the fume hood overnight. All slides were examined with an Olympus Vanox AHBT3 light microscope, pictures were captured with an attached Qimaging micropublisher 3.3 RTV camera and analysed using Image Pro Plus 7.0.

Control slides were included in each run of IHC procedure where possible. Negative control slides were processed the exact same way as test slides with omission of primary antibody; they should remain unstained in the absence of non-specific binding. Positive control slides were performed using known positive tissues; if no immunoreactivity was observed, the results from the same run were rendered invalid.

2.4 Methodology of double immunofluorescence

Two primary antibodies were selected based on their animal source and immunogloubulin type or subtype. Both primary antibodies to be analysed were prepared by diluting an appropriate amount of antibody with a diluent containing PBS and the animal serums corresponding to the host species in which the secondary antibodies was generated from. The appropriately diluted antibodies were then applied simultaneously to the tissue and left overnight at $4^{O}C$ in moist incubation chambers, before rinsing in 3 x PBS.

Secondary antibodies conjugated to fluorophores of different emission spectra were diluted to their respective concentrations in PBS and added to the sections for an hour before rinsing in 3 x PBS. During this time, all sections were kept in the dark to prevent photobleaching. 0.3% Sudan Black was applied for 10 minutes and rinsed under running water for 5 minutes. This was done to eliminate autofluorescence. Finally, sections were mounted with ProLong Gold Anti-fade reagent with DAPI (Invitrogen, UK) and cover-slipped. 4',-6-diamidino-2-phenylindole, also known as DAPI, is used as a counterstain and forms a complex with double-stranded DNA, especially at A-T clusters, to produce a fluorescence signal. All fluorescent slides were examined with Nikon Eclipse 50i microscope, images were captured with an attached Nikon DS-2MBW camera and analysed using NIS-Elements F 3.0.

2.5 Analysis of immunohistochemical staining

Sensitive, reliable, and easily replicable methods are required for quantification of microglia. While flow cytometry and western blotting can reliably separate and quantify microglial cells, they would require unfixed, frozen tissue sections. They would also be unable explore the associations of immunoreactive microglia with other components in the tissue. Immunohistochemically stained sections are commonly used for this purpose, and a variety of markers can be used to label and distinguish microglia heterogeneity. Methods for assessing IHC microglia staining include observer based subjective estimations, semi-quantitative assessment based on gray scale intensity, percentage area measurements, manual counting, and stereology (Reinacher et al. 1999, Blackbeard et al. 2007).

Although some papers have compared and verified these methods, they can still be hard to replicate due to several factors. With regards to stereology, the assumption that systemic randomized sampling will give a proper representation of an evenly distributed stain cannot be applied in a situation where the distribution of staining is inhomogeneous, as this can result in systemic errors. Other sources of systemic error include uneven compression of tissue along the z-axis, especially with use of paraffin as an embedding material. One also needs to have access to the entire brain region, which is usually not possible when an invaluable source is utilized i.e. human tissue (Schmitz and Hof 2005).

There are several considerations with regards to assessing microglia quantity and activation using IHC stained sections. Most studies have performed microglia quantification in rodent tissue. The size difference between rodent and human tissue sections makes it hard to properly assess immunoreactivity across a large area of interest. Distribution of microglia with the use of different markers can range from an even distribution to a patchy one. While evenly distributed microglia cells are relatively easy to quantify, as a small area would serve as a good representative for the entire section, quantifying an erratic microglia distribution over a large area would prove a lot more problematic. The following chapters will attempt to address this issue. Finally, the shape of microglial cells can also cause difficulties with pixel measurements. The ramifications of a microglial cell can occasionally be too fine to be picked up imaging programs. An amoeboid microglia cell would not present with the same problems as the fine processes of a resting or semi-activated microglial cell. It is essential to train an observer for consistency in determining the cut-off point between the staining of microglial processes and background.

Semi-quantitative methods can be performed rapidly in large-scale studies, but judgmental errors can arise while distinguishing between classes of abundance,

especially when dealing with a new scale. Training for intra-rater and inter-rater consistency can minimize these errors (Armstrong 2003).

In this thesis, two semi-quantitative methods and Western blotting were used to assess the upregulation of microglial markers. Semi-quantitative methods were done blinded to case numbers. Microglial cells were rated by percentage area (%area) of detected cells based on the region with the highest density of microglial cells (subjectively assessed), and subjective assessment of microglial abundance based on scores 0-3, representing absent, mild, moderate and severe respectively (referred to as microglia load). More details on how they were assessed will be covered in chapters 4 and 5. Before ratings were performed on all cases, 20 of them were picked and rated four times or more in a span of 3 months, each time in a randomized order. The measurements were performed blinded to previous values. This served as training to reduce intra-rater variability. Cronbach's alpha was used to assess the internal consistency- only after its value exceeded 0.95 was the training considered sufficient.

2.6 Protein extraction and quantification

2.6.1 Protein extraction

Grey matter from the frontal and cingulate cortices was extracted from frozen tissues where possible. The tissues were weighed and added to volume of PBS 8 times the mass of the tissue (with Roche complete protease inhibitor cocktail). They were then homogenized with Peqlab minilys homogenizer at 3000 rpm for 1 minute. The supernatant (containing the water-soluble proteins of the interstitial fluid and cytoplasm) was removed and stored at -80^{O}C for use in enzyme-linked immunosorbant assays (ELISAs).

Lysis buffer (4 times the volume of tissue) was then added to the pellet, mixed, and left on ice for 20 minutes before centrifugation at 9000 rpm for 10 minutes. The supernatant derived from this procedure contained membrane-associated proteins for use in gel electrophoresis, and was stored at -80^{O}C.

2.6.2 Protein quantification

Quick Start Bradford Protein Assay (Bio-Rad, UK) was added to 5 μl of supernatant from the protein extraction step and 20ul of bovine serum albumin (Sigma, UK) protein standards (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1mg/ml) to make up a total of 1ml per sample. These were added in triplicates to a 96-well plate, and read using a spectrophotometer (Molecular Devices VersaMax ELISA Microplate Reader) using SoftMax Pro software.

A protein standard curve was derived from the average of the bovine serum albumin (BSA) readings. The concentrations of protein in the samples were derived from the curve and multiplied by 4.

2.7 Western Blotting

2.7.1 Gel preparation

For running a protein of size 130kDa, 8% running gel of 0.75mm thickness was used. Running gel mixture was added to the glass mould up to the indicator, leaving space for the stacking gel. Isopropanol was added to get rid of air bubbles. After letting the gel polymerize for an hour, the isopropanol was removed and the stacking gel was poured on top of the running gel. A comb was placed to create wells, before allowing the gel to polymerize for another 20 minutes.

2.7.2 SDS-PAGE gel electrophoresis

10 μl of sample buffer was added to the samples (volumes containing 50 μg of protein), and boiled for 10 minutes. In the meantime Mini-PROTEAN Tetra 4-gel vertical electrophoresis system (Bio-Rad, UK) was set up, gels were fitted into the cell, and 1 litre of running buffer was added to the cell. After boiling, the samples were allowed to cool before being loaded into the gel. 5 μl of molecular weight marker (SeeBlue Pre-stained standard from Invitrogen) was also added to the first lane of each gel.

The gels were ran at 120mV for 1 hour 30 minutes, or till the samples have reached the end of the gel.

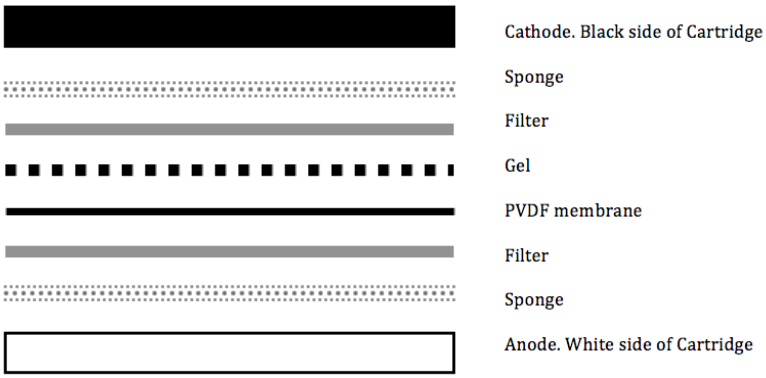
2.7.3 Transfer and Detection

The Mini Trans-Blot cell (Bio-Rad, UK) was used for western blotting. PDVF membranes (Millipore, UK) were wetted with methanol. The gel was removed from the electrophoresis cell. The PDVF membrane and gel were then sandwiched in filter papers and foam pads that were pre-soaked in transfer buffer, and held together by a cartridge. (Figure 7) Care was taken to eliminate any air bubbles.

These were placed into the tank together with an ice block and 1 litre of transfer buffer, and ran at 400mA constant amp for an hour. The blot was then removed and blocked in 5% nonfat milk in Tris-Buffered Saline with Tween 20 (TBST) for another hour. A wash step was next, involving 3 x TBST washes for 10 minutes each. The primary antibody was diluted to its appropriate concentration in TBST with 5% nonfat milk and added to the blot for an overnight incubation at $4^{O}C$ on a shaker. Another wash step was done, before incubation with HRP-conjugated secondary antibody diluted in TBST with 5% nonfat milk for an hour on the shaker, and a final wash step.

The membranes were developed using electrochemiluminescence (ECL) reagents and Hyperfilm ECL autoradiography film in an automated developer from Konica (SRX 101A).

Figure 7: Blot transfer arrangement



2.7.4 β-actin normalization

The membranes were incubated in stripping solution (Reblot Plus Strong Antibody Stripping Solution 10x from Millipore, UK) for 20 minutes at room temperature, before washing once with TBST. Blocking was done with 5% nonfat milk in TBST for an hour, before washing in 3 x TBST for 10 minutes each. Steps for probing with antibodies and membrane development were repeated, this time replacing the primary antibody with β-actin antibody (mouse monoclonal, Abcam, UK), and its secondary antibody HRP-conjugated goat anti-mouse IgG (Sigma, UK).

The films were scanned, band intensities were analysed with ImageJ (NIH) and normalized to the bands of β-actin.

2.8 Enzyme-linked immunosorbant assay

50μl of coating antibody was added to each well of a 96-well plate and left overnight. After emptying the plate and doing three washes with their respective wash buffers, the block buffer was added and left on for 1 hour. This was followed by another three washes.

Two-fold serial dilutions of protein standards were performed seven times and added in triplicates to the wells. In the last row, only diluent was added to serve as a blank. Equalized samples were also added in triplicates to the plate. The amount of samples and standards added to each well varied with the antibody kits used. The plates were incubated for 2 hours at room temperature on a shaker, and washed five times. The detection antibody was added next and incubated for another 2 hours before five washes.

Two color development systems were used for detection- HRP/TMB and HRP/ABTS. 1N hydrochloric acid and 1% SDS were used as stop solutions respectively. 3,3',5,5'-tetramethylbenzidine, also known as TMB, yields a blue color upon reaction with HRP. It oxidizes at a faster rate and results in a quicker color development. 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt, or ABTS, yields a green color instead, and takes more time to develop compared to TMB substrate.

After applying the stop solutions, plates were read with a spectrophotometer (Molecular Devices VersaMax ELISA Microplate Reader) using SoftMax Pro software. The antigen concentration in the samples were derived from the protein standard curve, and correlated back to the dilution factor used during protein equalization to give the original antigen concentrations.

Chapter 3: Evaluating microglia phenotypes in AD

3.1 Introduction

The heterogeneity of microglia activation has been demonstrated in AD patients and animal models of AD. Both classical and alternative activation markers are expressed by microglia in AD (Colton et al. 2006). However, there is relatively little evidence of alternative activation in PD. Additionally, most of these markers have only been characterized in peripheral macrophages. Studying these markers in microglia is essential for identifying their functions and detecting their response to pathology. Co-localization of these markers may prove useful in defining the phenotypic subtypes of microglia.

A summary of the functions and activation states conferred by specific microglia markers used in this chapter will be covered based on current literature. They are major histocompatibility class II antigen (MHCII), CD68, ionized calcium binding adaptor molecule 1 (Iba1), mannose receptor, C type 1 (MRC1), IL-1α, Arg1, Arg2, CD163, CD14 and CD36.

3.1.1 Non-specific markers

3.1.1.1 MHCII

The functions of MHCII include antigen presentation and activation of CD4 T helper cells. MHCII are expressed on the surface of lymphocytes and other antigen presenting cells. It is upregulated by the actions of IFNγ, IL-4 and IL-13, but drastically downregulated by IL-10, hence low expression is observed in acquired deactivation state. In cell cultures, human adult microglia have a high baseline of MHCII expression. However it is downregulated when exposed to granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4. This downregulation is accompanied by an increase in production of IL-10. This is in stark contrast to blood monocytes, which took on a pro-inflammatory profile, and strongly increased MHCII expression (Gordon 2003).

MHCII was once thought to indicate a pro-inflammatory state, but is now known to be expressed by both classically and alternatively activated macrophages (Colton 2009). When double stained with Iba1, MHCII labeled a smaller group of microglia, suggesting that it is more discerning for activated microglia than Iba1. MHCII immunoreactive microglia are associated with amyloid and NFT pathology in AD. Their immunoreactivity increases along with microglial morphological changes indicating activation (Lue et al.

2001). MHCII expression in primates also increases along with age (Sheffield and Berman 1998).

3.1.1.2 CD68

CD68 is an 110kDa intracellular late endosomal glycoprotein that can be found in the cells of mononuclear phagocyte lineage (Rabinowitz and Gordon 1991). It labels microglial lysosomes associated with phagocytosis, hence is often used to depict evidence of phagocytic activity (da Silva and Gordon 1999). Minocycline, a tetracycline derivative antibiotic that is known to downregulate inflammation, does not affect lipopolysaccharide (LPS)-induced CD68 immunoreactivity (Malm et al. 2008).

Macrosialin, the murine counterpart of CD68, is heavily glycosylated. This confers several advantages, including resistance to proteolysis especially in a lysosomal environment, and a higher affinity of the terminal sialic acid residues for pathogens including viruses, bacteria and tumor cells (Rabinowitz and Gordon 1991).

3.1.1.3 Iba1

Iba1 is a 17kDa protein that performs as a marker highly specific for microglia/macrophages (Imai et al. 1996). It also stains other cells belonging to the monocytic lineage (Ito et al. 1998). It contains two EF hand motifs that bind to calcium, and is involved in membrane ruffling and phagocytosis through the actions of actin – bundling and reorganization. Iba1 expression is upregulated in activated microglia (Ohsawa et al. 2004).

3.1.1.4 MRC1

MRC1 is a type 1 transmembrane C-type lectin that recognizes branched mannose-containing carbohydrate structures and thus acts as a pattern recognition receptor for pathogens including bacteria and viruses (Allavena et al. 2004). Its expression is also linked to MHCII through the increase of antigen internalization, thus facilitating antigen presentation (Taylor, Gordon and Martinez-Pomares 2005a).

MRC1 also recognizes endogenous ligands and participates in the clearance of self-antigens (McGreal, Miller and Gordon 2005), induces secretion of cytokines and facilitates endocytosis and phagocytosis. This is initiated by binding of MRC1 to its ligand, causing cross-linking of the receptors and subsequent phagocytosis of the ligand (Galea et al. 2005). This in turn stimulates an anti-inflammatory phenotype (Chieppa et al. 2003). Endocytosis through MRC1 by primary human macrophages is enhanced by IL-4 and IL-13 but downregulated by IL-10 and IFNγ (Montaner et al. 1999).

3.1.2 Classical activation markers

3.1.2.1 IL-1α

IL-1α is a 17kDa pro-inflammatory cytokine with a diverse range of functions, e.g. induction and amplification of the inflammatory response, activation of T cells, fibrosis and angiogenesis (Mrak and Griffin 2001). It is constitutively expressed at a low level in a healthy adult brain, but highly expressed by microglia in AD, Down syndrome patients, head injury, multiple sclerosis (MS), and HIV (Griffin and Mrak 2002). IL-1 immunoreactive microglia are found in close proximity to Aβ plaques in AD patients, but not in controls, suggesting that IL-1 might be a contributing factor to the pathogenic nature of plaques in AD. As IL-1 promotes neuronal synthesis and βAPP processing, overexpression of IL-1 causes neuronal dysfunction, excessive growth of dystrophic neurites, and increased deposition of Aβ plaques (Griffin et al. 1998).

3.1.2.2 TNFα

TNFα is a pro-inflammatory cytokine that orchestrates a self-propelling inflammatory reaction by inducing microglial activation and further production of pro-inflammatory cytokines (Qin et al. 2007). It is involved in the acute-phase reaction, stimulates chemoattraction of inflammatory cells, and mediates systemic reactions e.g. lowered blood pressure and septic shock. The effects of TNFα can be conflicting- on one hand, high levels of TNFα have been found to cause cytotoxic effects in demyelinating CNS diseases, on the other hand presence of TNFα in animal models of ischemia and excitotoxicity has a protective effect on neuronal survival (Gosselin and Rivest 2007).

TNFα production is suppressed by IL-4 and IL-13. Treatment of cultured murine microglia with IL-4 or IL-13 drastically reduces TNFα mRNA expression (Colton et al. 2006). Exposure to apoptotic cells, IL-10, or TGFβ also decreases the levels of TNFα (Lodge and Sriram 1996, Minghetti et al. 2005). TNFα is also able to suppress cell proliferation, as seen in TNFα receptor 1-KO mice- increased neuroblast production in the subventricular zone (SVZ) was observed after a stroke (Iosif et al. 2008). However, administering TNFα antibody decreased survival rates of the newly formed neurons in the SVZ, suggesting a role for TNFα receptor 2 in neuroprotection (Ekdahl, Kokaia and Lindvall 2009).

Age is also a contributing factor to an increased microglial pro-inflammatory cytokine profile. This is shown in microglia of older transgenic AD mice, which had an increased TNFα expression, when compared to young mice (Jimenez et al. 2008). Neurotoxicity towards DA neurons caused by administering MPTP in TNF receptor KO mice was

attenuated (O'Callaghan, Sriram and Miller 2008). Higher levels of TNFα have also been detected in the CSF and brain parenchyma of PD patients (Reale et al. 2009).

3.1.3 Alternative activation markers

3.1.3.1 Arginase 1 and 2

There are two isoforms of arginase. Arginase 1 (Arg1) is the cytosolic form mostly found in the liver, while arginase 2 (Arg2) is the mitochondrial form that is also found in many other cell types include macrophages (Witte and Barbul 2003). Arg1 is inducible and expressed by both neurons and microglia, while Arg2 is constitutively expressed throughout the brain at low levels and does not participate in the innate immune response. These findings are obtained from murine models, and may be different in the case of humans (Xia et al. 2001, Colton and Wilcock 2010). Arg1 is thought to be expressed by alternatively activated macrophages. Treatment with IL-4 and IL-13 increases expression of Arg1. The addition of Aβ further increases expression of Arg1, an indication that Aβ may act to promote the alternative activation state of microglia (Colton et al. 2006).

Arginase competes with NO synthase for arginine, limiting the production of NO. Arginase also promotes polyamine synthesis and cell proliferation. TGFβ stimulates arginase while inhibiting the actions of iNOS, coordinating the actions of both enzymes to ensure effective wound healing. Ornithine, a precursor for the synthesis of proline, is also produced by arginase. Hence, arginase has been touted to play a role in wound healing and tissue reparation by indirectly affecting production of proline-rich proteins like collagen (Morris 2007). However, chronically high levels of TGFβ can go on to cause excessive matrix production, increased collagen levels in the vasculature and thickening of the blood vessel walls. This situation is observed in both mouse models of AD and in AD patients (Colton and Wilcock 2010).

3.1.3.2 CD36

CD36 is a class B scavenger receptor that is localized on the plasma membrane. It plays a role in lipid metabolism and is involved in the binding and transportation of long-chain fatty acids in adipocytes, heart and skeletal muscle (Febbraio, Hajjar and Silverstein 2001). It has been shown to aid in the clearance of apoptotic cells (Husemann et al. 2002, Stolzing and Grune 2004). Human macrophages deficient in CD36 secrete less TNFα and IL-1β with reduced NF-kB activation when stimulated with oxidized low-density lipoproteins, suggesting that the CD36 pathway might lead to

classical activation (Janabi et al. 2000, Husemann et al. 2002). It is expressed by both microglia and capillary endothelium in the brains of AD patients. CD36 also mediates adhesion to fibrillar Aβ, and induces ROS secretion upon binding (Coraci et al. 2002).

3.1.3.3 BDNF

BDNF is a 28kDa dimer that is expressed widely throughout the brain by both neuronal and non-neuronal cells, including DA neurons in the SN and ventral tegmental area, and to a lesser extent in the striatum (Zhang et al. 2007a). BDNF recruits microglia and induces proliferation and phagocytic activity. It also reduces MHCII expression (Neumann et al. 1998).

BDNF levels are reduced in the SN of PD patients compared to controls (Mogi et al. 1999). In AD patients, BDNF and trkB levels in the frontal and temporal cortices, basal forebrain, and pyramidal cells of the hippocampus and the granular cells of the dentate gyrus were also found to be lower than controls (Murer, Yan and Raisman-Vozari 2001).

BDNF stimulates survival of rat and human fetal DA neurons, as well as rat hippocampal neurons in cell cultures. It also mediates neuroprotection in rat hippocampal lesions and axotomy models (Zhou et al. 1994, Murer et al. 2001). It protects against the effects of MPP+ and 6-OHDA (Kirschner et al. 1996, Peterson and Nutt 2008). BDNF has a regulatory effect on dopamine release from DA neurons and increases DA uptake (Hyman et al. 1994, Blochl and Sirrenberg 1996). It also increases the average firing rate of SN, cortical and hippocampal neurons in rodents *in vitro* (Shen, Figurov and Lu 1997, Murer et al. 2001). Knockout of BDNF receptors trkB and trkC in mice resulted in lower number of SN DA neurons, and caused accumulation of α-syn in the remaining neurons (von Bohlen und Halbach, Minichiello and Unsicker 2005). It exerts neuroprotection through enhancing calcium sequestration *in vitro* (Cheng and Mattson 1994).

3.1.3.4 IL-10

IL-10 is a major regulator of the immune response, and is expressed by lymphocytes and monocytes, including microglia. It is involved in inhibiting the development of T Helper 1 effector cells (Chabot et al. 1999), blocking MHCII expression (O'Keefe, Nguyen and Benveniste 1999), and suppressing pro-inflammatory cytokines expression (Moore et al. 2001). It also has a protective effect against cell death caused by glutamate excitotoxicity (Bachis et al. 2001), hypoxia (Grilli et al. 2000), and LPS (Molina-Holgado, Grencis and Rothwell 2001). This protective effect against LPS-mediated neurotoxicity is executed via the suppression of NADPH oxidase activity

(Qian et al. 2006). IL-10 also coordinates its immuno-regulatory activity via heme-oxygenase 1 (Lee and Chau 2002).

Immunization of transgenic AD mice models with an altered myelin-derived peptide caused a shift in inflammatory profile, including a reduction in TNFα, increase in IL-10, and diminished Aβ plaques and soluble Aβ (Koronyo-Hamaoui et al. 2009). Increased levels of IL-10 mRNA have also been found in AD patients (Colton and Wilcock 2010).

3.1.3.5 CD163 and CD14

CD163 and CD14 both have functions relating to phagocytosis. CD163 acts as a scavenger receptor for haptoglobin-haemoglobin complexes (Hp-Hb). CD14 mediates the recognition of bacterial pathogens, apoptotic cells and fibrillar Aβ. However, while CD163 and CD14 induce an alternative activation state in microglia, CD14 can also induce a classical activation state. More details of these two markers will be covered in the later chapters.

3.2 Aims

The aim of this study was to characterize the phenotype of microglia in AD based on markers associated with both classical activation and alternative activation states. Based on the observed immunoreactivity and distribution of the microglia in AD, markers of interest were picked for analysis in all three neurological disorders- AD, Lewy Body disease (LBD), and TBI.

3.3 Materials and methods

3.3.1 Case selection

All AD cases were obtained from the Corsellis Archival Collection. Paraffin-embedded blocks from the frontal and occipital cortices, and hippocampus, were obtained from a total of 101 cases that were clinically diagnosed with senile dementia (ICD-10: F00). The aim was to find AD cases of Braak stages 5 or 6. Cases were also excluded based on their cause of death- systemic inflammation or other forms of neurological disorder or injury were not used in this study.

These blocks were then sectioned at 7μm; neuropathological diagnosis was carried out by staining for 4G8 and AT8, which labels Aβ plaques and tau respectively. 4G8 against Aβ is able to stain a variety of Aβ plaques (fleecy, diffuse and cored), CAA, as well as intracellular Aβ- its detection of intracellular Aβ is assessed most consistently among a panel of antibodies against Aβ (Alafuzoff et al. 2008b). AT8 clone against tau specifically recognizes tau which are phosphorylated at serine residue 202 and threonine 205 (Goedert, Jakes and Vanmechelen 1995). AT8 has also been used as a diagnostic standard against phosphorylated tau in AD by both the Braak staging system and BrainNet Europe (Alafuzoff et al. 2006, Braak et al. 2006).

Staging of AD was done by assessing neurofibrillary changes using the protocol from BNE (Alafuzoff et al. 2008a). A neuropathological diagnosis of AD was given on the basis of presence of NFT and neuritic plaques. Tau pathology at the peristriate/striate region indicated Braak stage 5 and 6 respectively. 31 cases were eventually selected from the initial cohort - 21 females and 10 males, age range 65-88, mean age at death 76. (Table 2)

Table 2 - Age, gender and causes of death of AD cases

Alzheimer's Disease cases (Braak stages 5 or 6)			
Case no.	**Age at death**	**Gender**	**Cause of death**
1	70	F	Other heart disease classified elsewhere
2	65	F	Pneumonia
3	65	M	Pneumonia
4	71	F	Pulmonary embolism
5	71	F	Pneumonia
6	69	F	Pneumonia
7	67	F	Intracerebral haemorrhage
8	65	F	Pulmonary embolism
9	88	F	Pneumonia
10	74	M	Pneumonia
11	74	F	Pneumonia
12	71	F	Pneumonia
13	88	F	Pneumonia
14	75	F	Pneumonia
15	71	M	Pneumonia
16	76	F	Pneumonia
17	76	F	Pneumonia
18	76	M	Pneumonia
19	83	M	Pneumonia
20	80	F	Peritonitis
21	85	F	Heart failure
22	82	F	Heart failure
23	77	M	Pneumonia
24	78	F	Malignant neoplasm of bronchus and lung
25	85	M	Pneumonia
26	87	M	Pneumonia
27	81	F	Acute myocardial infarction
28	79	M	Intracranial injury
29	77	M	Pneumonia
30	79	F	Pneumonia
31	72	F	Pneumonia

3.3.2 Immunohistochemistry

IHC staining was done on sections from the frontal and occipital cortices, and the hippocampus region from 31 selected AD cases. A variety of antibodies against microglia markers was tried and tested using different pretreatments for optimum results. Antibodies that were successfully used in the IHC staining are listed in Table 3, while those that were not are listed in Table 4. Please refer to section 2.3 for the protocol on IHC procedure.

3.3.3 Pretreatments

Pretreatments used in this chapter include microwaving at 600W with citrate buffer at pH 6 (CA) or EDTA buffer at pH 8 (EDTA), incubation with proteinase K/ guanidine thiocyanate/ pepsin solution at 37^{O}C for 10 minutes (PK, GT, Pep respectively), and soaking in 80% formic acid for an hour (FA). The best pretreatment was determined for each antibody based on intensity of staining and the pretreatment recommended by their respective companies.

Table 3: List of antibodies used for characterization of microglial phenotype

Antibody	Clone/type	Epitope	Dilution	Pre-treatment	Source
MHCII	CR3/43, Mouse IgG1	β-chain of the αβ heterodimer of all products of the gene families DP, DQ and DR	1: 200	CA	Dako
Iba1	Rabbit Polyclonal	Synthetic peptide corresponding to C-terminus of Iba1	1:400	CA	Wako
MRC1	Rabbit Polyclonal	Macrophage mannose receptor 1 precursor recombinant protein epitope signature tag (PrEST)	1:1000	CA	Prestige, Sigma-Aldrich
IL-1α	Goat Polyclonal	C-terminus of mouse IL-1α	1:400	CA	Santa Cruz
CD68	PG-M1, Mouse IgG3	Fixative-resistant epitope on macrophage-restricted form of CD68	1: 500	CA	Dako
CD14	7, Mouse IgG2a	External domain of CD14	1:100	CA+ GT	Novocastra
CD163	10D6, Mouse IgG1	Prokaryotic recombinant protein corresponding to domains 1-4 of N-terminal region of CD163	1:200	CA	Novocastra
Tau	AT8, Mouse IgG1	Tau phosphorylated at Ser-202/Thr-205	1:800	None	Autogen Bioclear
Abeta	4G8, Mouse IgG2b	a.a. 17-24	1:2000	FA	Signet

Table 4: Additional antibodies and pretreatments tested for characterization

Antibody	Clone/type	Epitope	Titrations	Pretreatment	Control tissue	Source
Arg1	Rabbit Polyclonal	Arg-1 recombinant protein epitope signature tag	1:1000, 2000, 4000	FA, CA, PK	Kidney, AD	Prestige, Sigma-Aldrich
Arg2	Rabbit Polyclonal	Arg-2, mitochondrial precursor recombinant protein epitope signature tag	Neat, 1:150, 300, 600	FA, CA, PK	Kidney, AD	Prestige, Sigma-Aldrich
CD36	Rabbit Polyclonal	Synthetic peptide corresponding to a region within amino acids (a.a.) 302 and 395 of CD36	1:50, 100, 200, 500, 1000, 2000	None, CA, FA, EDTA	Appendix, tonsil, brain infarct	GeneTex
TNFα	Goat Polyclonal	E. coli-derived recombinant human TNF-α. Val177-Leu233	1:20, 50, 100, 200	None, CA, EDTA	Tonsil, AD	R&D systems
BDNF	Polyclonal Chicken IgY	S. frugiperda insect ovarian cell line Sf 21-derived recombinant human BDNF. His129-Arg247	Neat, 1:10, 20	None, CA, EDTA	Tonsil, AD	R&D systems
IL-10	Goat Polyclonal	S. frugiperda insect ovarian cell line Sf 21-derived recombinant human IL-10. Ser19-Asn178	1:10, 20, 50, 100, 200	None, CA, EDTA	Tonsil, AD	R&D systems
IL-1α	Rabbit Polyclonal	Recombinant human IL-1α	1:50, 100, 200, 400	CA, EDTA, Pep	Brain infarct, AD	Peprotech

3.4 Results

3.4.1 Negative results using IHC on paraffin-embedded, formalin-fixed tissue

Anti-Arg1 and Arg2 did not show immunoreactivity in AD brain tissue. Anti-Arg1 has been proven to work in paraffin-embedded, formalin-fixed lung tissue (Yan et al. 2010).

Anti-IL-1α (Peprotech) did not show immunoreactivity in AD and brain infarct tissue. However, other papers have previously shown it to work in paraffin-embedded, formalin-fixed temporal lobe sections from epilepsy patients (Aboud et al. 2012), and AD brain tissue (Liu et al. 2011a).

No immunoreactivity was observed for antibodies against cytokines TNFα and IL-10, and growth factor BDNF in AD and control brain tissues. This is most probably attributed to the labile nature of the antigens. However, other papers have previously shown for TNFα to work in paraffin-embedded, formalin-fixed mice ovarian tissue (Emmerson et al. 2009) and endoscopic biopsies specimens (Kuo et al. 2008). IL-10 was found to work in paraffin-embedded, formalin-fixed human melanomas and metastases (Yaguchi et al. 2012, Itakura et al. 2011), lymphomas (Ogden et al. 2005), and lung tissue (Garantziotis et al. 2006). ELISA was used to investigate their expression levels and this is elaborated in Chapter 5.

Antibodies used against Arg2, BDNF and CD36 also did not work well in the IHC experiments performed. Other laboratories have not tested them on paraffin-embeded, formalin-fixed tissues.

3.4.2 Markers for identifying microglial activation are uniform within AD

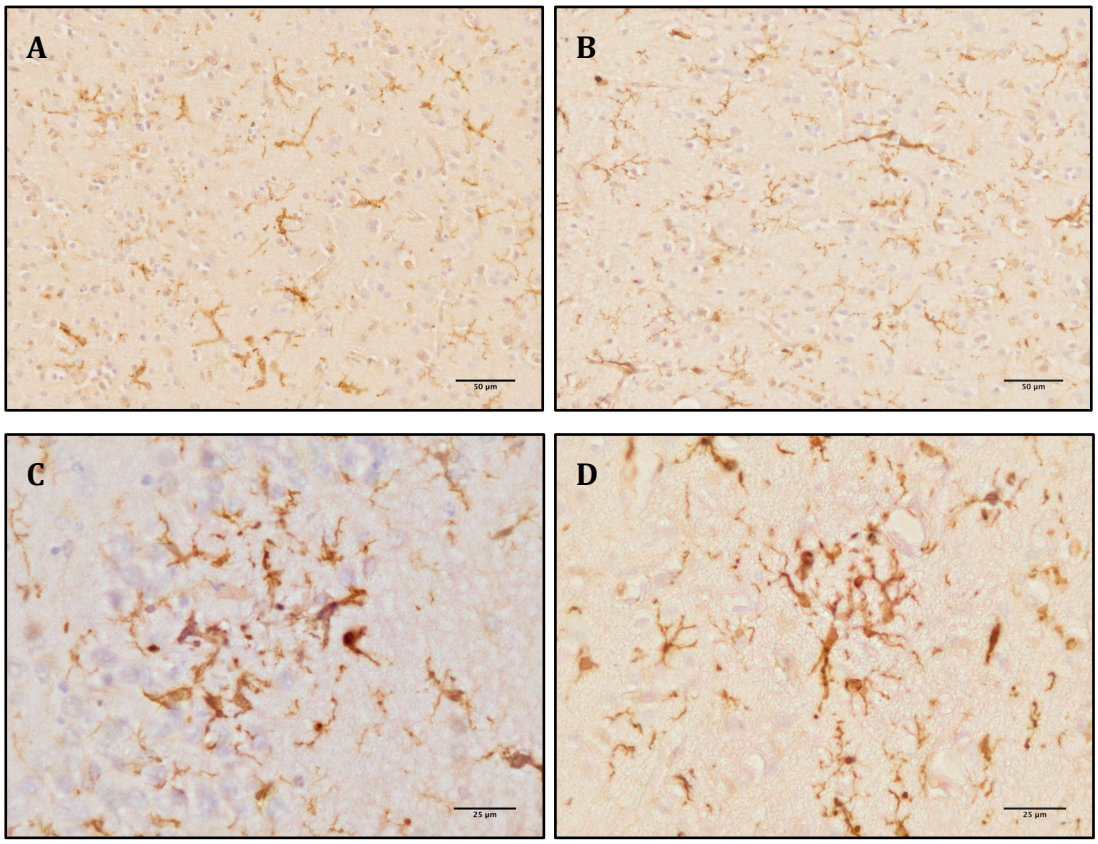
In order to identify microglial markers that would provide an insight into their varying activation states in the brain, MHCII, CD68, and Iba1 were utilized. While these three markers did not differentiate between classical or alternative activation, they gave information as to the degree of activation through intensity of staining and morphology.

Iba1 immunostaining was done on a selection of AD cases. 20 slides were chosen at random from a range of cases and brain regions (frontal and occipital cortices, and hippocampus). Semi-ramified microglia – microglia with slight hypertrophy of their soma, but have yet to become fully amoeboid, with noticeable fine processes, were observed in both white and grey matter in most of these cases. (Figure 8)

IHC was performed against MHCII on all AD and PD cases. Uniform immunoreactivity was observed throughout most brain regions in every case. Immunoreactivity was more prominent for MHCII than Iba1, suggesting that MHCII protein was upregulated more than Iba1 upon microglial activation. (Figure 9)

CD68 staining was carried out in three cases of AD as a proof-of-principle that microglial activation involved the upregulation of lysosomes and their ensuing phagocytic activity. CD68 immunoreactivity was seen in both amoeboid and ramified microglia. (Figure 10a, b) The staining was observed to be granular, ranging from large granules found in the soma to small particles found in both the soma and within microglial processes. Clusters of amoeboid microglia were also observed, almost certainly associated with Aβ plaques. (Figure 10c, d)

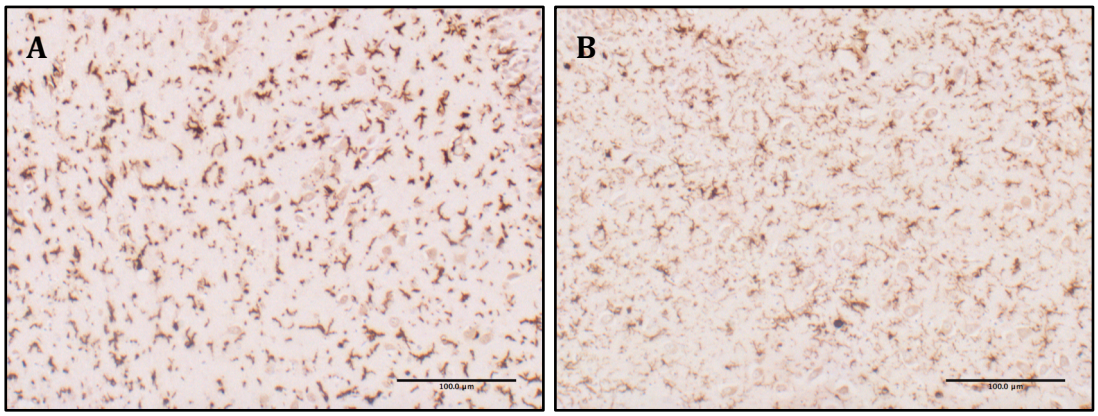
Figure 8: Iba1 as a pan-microglia marker



(A & B) Semi-ramified microglia reactive for Iba1 with a relatively even distribution in the occipital lobes of AD cases. Magnification x20

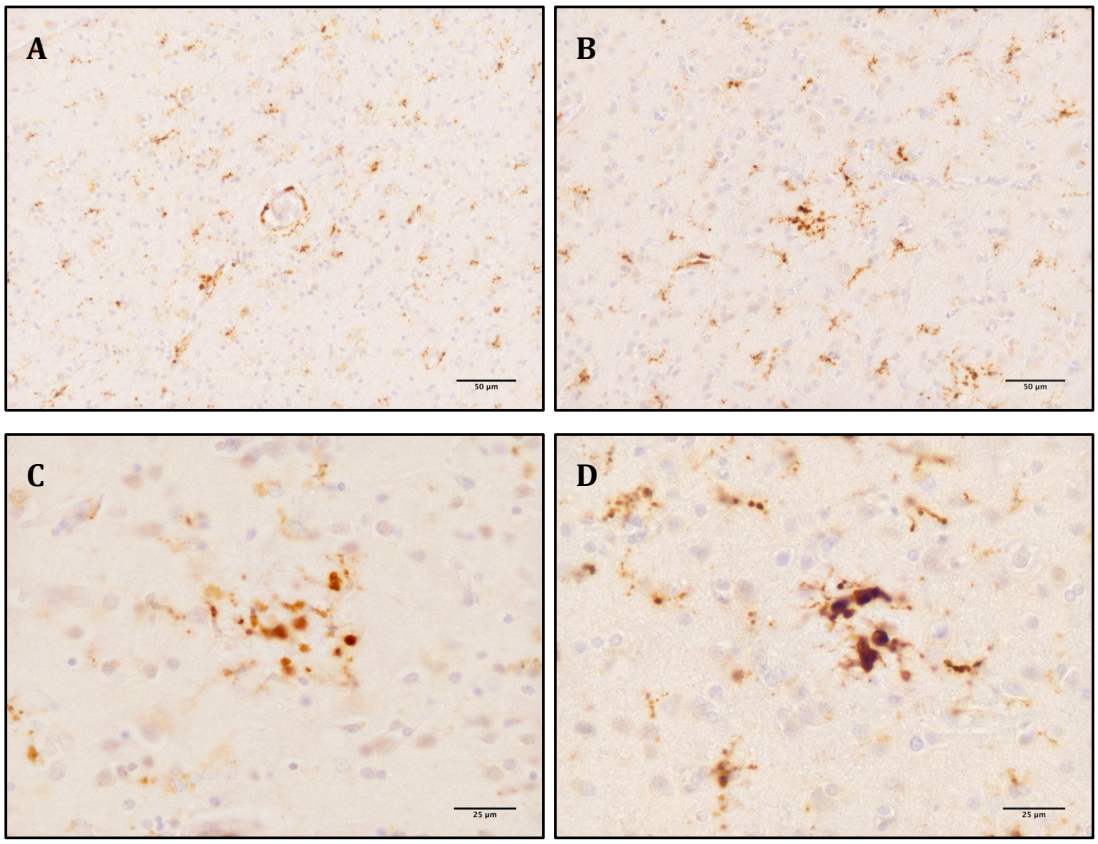
(C & D) Clusters of Iba1-positive microglia in the **(C)** granular cell layer of the dentate gyrus and **(D)** entorhinal cortex of AD cases. Magnification x40

Figure 9: MHCII immunoreactivity in diseased brains



(A & B) Florid microglial activation immunoreactive for MHCII in the hippocampi of PD cases. Many of these microglia appeared more amoeboid than ramified. Distinction between density of microglia and level of microglial activation becomes difficult due to the intense upregulation of MHCII. Magnification x20

Figure 10: CD68 immunoreactivity in AD cases



(A) CD68 is expressed by microglia and PVM in the occipital cortex. Most of the microglia appeared ramified but still contain granules of late endosomes/ lysosomes. Magnification x20

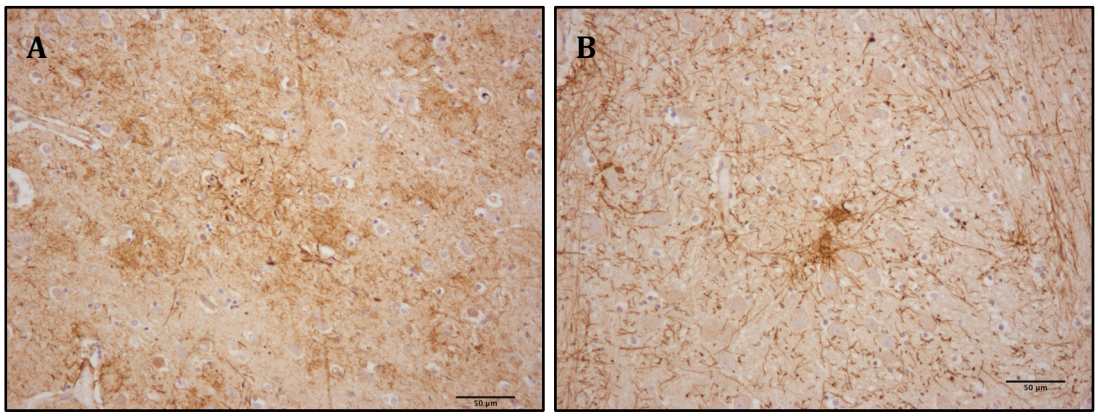
(B) A cluster of CD68 positive microglia in the occipital lobe that is most likely associated with an Aβ plaque. This implies that the microglial cells are active phagocytes. Magnification x20

(C & D) Clusters of CD68 positive microglia around Aβ plaques in the occipital lobes. Magnification x40

3.4.3 Astrocytic labeling by IL-1α

Immunostain using pro-inflammatory cytokine IL-1α antibody was performed on a random selection of AD and control cases. Staining was observed in astrocytes, instead of microglia. Protoplasmic and fibrous astrocytes were stained with IL-1α, in both AD cases and controls. (Figure 11a) The molecular layer of the cortex had a higher amount of astrocytic IL-1α stain. (Figure 11b) Clusters of astrocytes were also observed, and are clearly associated with Aβ plaques.

Figure 11: Immunostaining of astrocytes by IL-1α



(A) Astrocytes immunoreactive for IL-1α. Aggregation of astrocytes around multiple neuritic Aβ plaques in the frontal lobe of an AD case. Magnification x20

(B) Astrocytes found in the pons of a control case are also immunopositive for IL-1α. Protoplasmic and fibrous astrocytes are seen in the grey and white matter respectively. Magnification x20

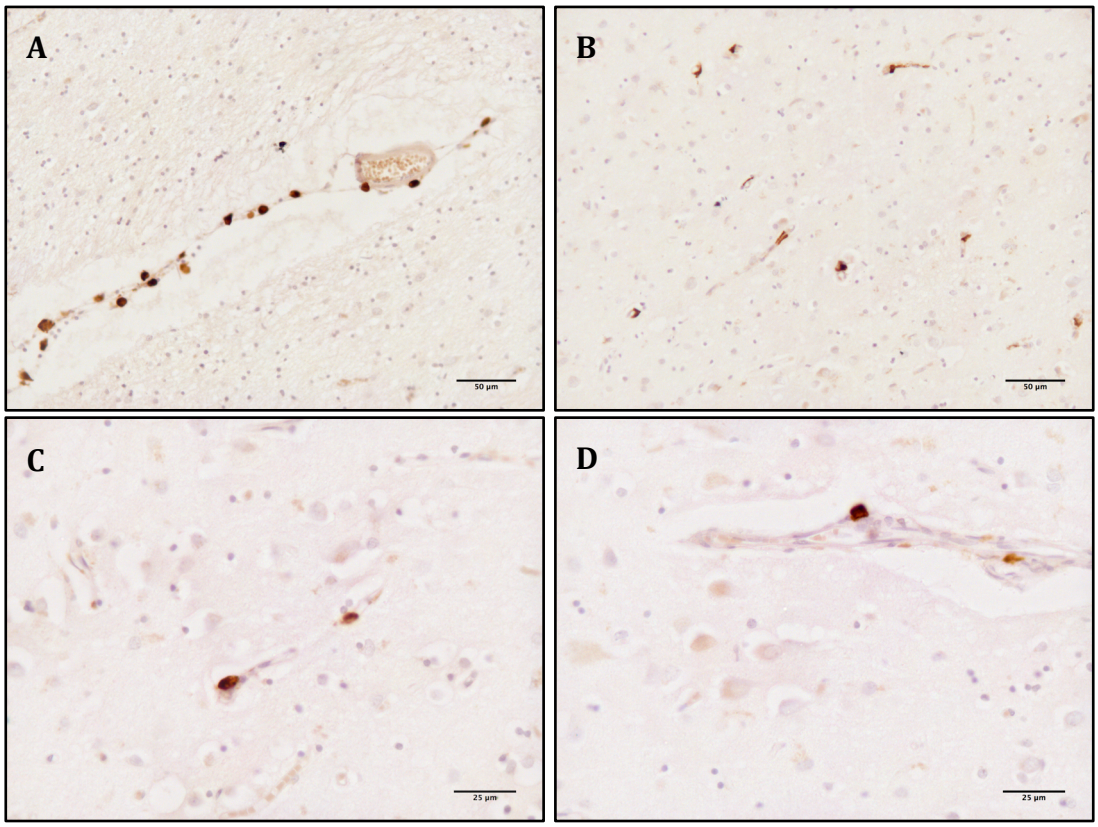
3.4.4 Markers exclusive to perivascular macrophages are observed in the diseased brain parenchyma

Studying the immunoreactivity of PVM markers in diseased brains is essential to gain insight into the influence of systemic inflammation and a possible breakdown in the blood-brain barrier (BBB) on microglial's activation states and phenotypes. PVM are constitutively phagocytic, and investigating the function of phagocytosis in AD is especially relevant due to extracellular pathology like Aβ plaques and CAA. All three antibodies- MRC1, CD14 and CD163 were tested on a random selection of AD and control cases.

MRC1 positivity in control and AD cases was restricted to PVM, meningeal (MM) and choroid plexus macrophages (CPM). (Figure 12) PVM were found around vessels in an elongated and flattened morphology, sandwiched between the end-feet of astrocytes forming the glial basement membranes and endothelial cells of the vessels. No MRC1 immunoreactivity was observed in parenchymal microglia in control cases or AD cases.

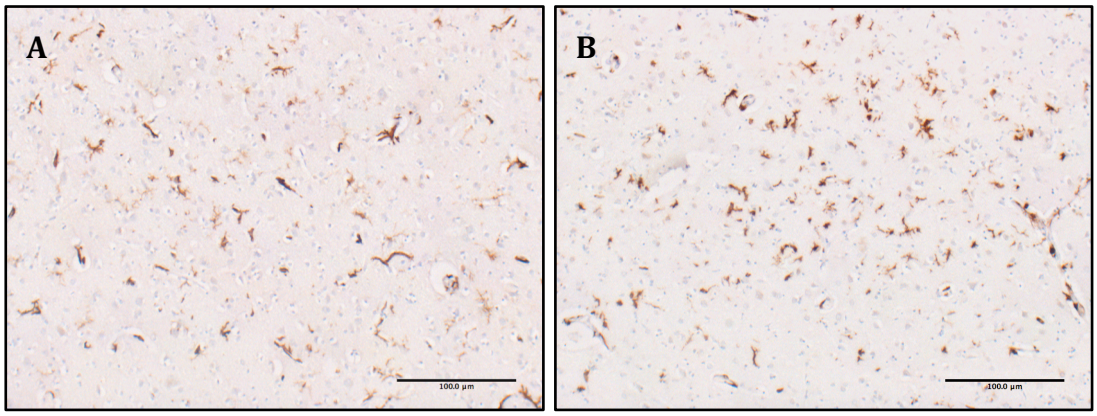
CD14 and CD163 positivity were mostly restricted to PVM, MM, and CPM in control cases. Staining for CD14 and CD163 in AD cases also labeled the three populations of macrophages, and revealed unexpected immunopositivity in parenchymal microglia. (Figure 13)

Figure 12: MRC1 immunoreactivity exclusive to brain macrophages



MRC1-immunopositive **(A)** MM and **(B, C, D)** PVM in the hippocampi of AD cases. Magnification x 20 **(A & B)** and x40 **(C & D)**

Figure 13: CD14 and CD163 positivity in brain macrophages and parenchymal microglia



PVM and microglia were immunoreactive for **(A)** CD14 and **(B)** CD163 in the occipital and frontal lobes of AD cases respectively. Magnification x10

3.5 Discussion

Characterizing the heterogeneous phenotypes of microglia using IHC requires the study of the several criteria, including the morphology, density, regional and localized distribution, and associations of each of these markers with one another and with pathology found in neurological diseases. Initial testing on a random selection of AD cases was done in order to identify markers that would immunostain sufficiently diverse in order to distinguish between separate microglial phenotypes.

Novel antibodies that to our knowledge, have yet to be explored thoroughly in AD and PD were tested in this study. However, many of them did not show any positivity in human formalin-fixed, paraffin-embedded tissue (refer to table 4). This might be due to the destruction of antigens through fixation or IHC processing and harsh organic solvents. Residual water molecules that might have been trapped in the tissue by paraffin embedment will gradually cause degradation of antigens (Xie et al. 2011). Antigenic binding sites are not altered in frozen tissue sections as no chemical fixatives are used, and thus no harsh processing is required e.g. paraffin removal and antigen retrieval. These antibodies can be tested on frozen sections for future investigation. Levels of cytokines TNFα and IL-10, and neurotrophic factor BDNF will be assessed in PD cases using Western blotting (Chapter 5).

Markers for nonspecific microglial activation were tested in this study. This included MHCII, CD68, and Iba1. MHCII is expressed by both classically activated and alternatively activated microglia (Taylor et al. 2005b). While it is not able to distinguish between activation states, MHCII can give an idea of the degree of activation. However, as observed in all AD and PD cases, a large majority of the cases consistently demonstrated extremely florid microglial activation, which excludes the use of this marker to differentiate between microglia phenotypes. This observation might be due to chronic microglial activation that was initiated early in the disease course (Croisier et al. 2005), agonal state of the patient (Smith et al. 2012), or systemic inflammation (Perry 2004). Iba1 is a pan microglia marker and similar to MHCII, does not differentiate between microglia activation states (Ito et al. 2001). The intensity and density of Iba1 immunoreactivity throughout the AD cases tested were rather consistent, excluding this marker for characterization purposes.

CD68 immunoreactivity was upregulated by ramified and amoeboid microglia. While amoeboid microglia have always been associated with phagocytic activity, in actual fact microglial morphology tells us little about their phenotype or function (Colton and Wilcock 2010, Kettenmann et al. 2011). IHC staining in AD showed that late endosomal particles exist even in microglial processes, hence microglia do not need to take on an

amoeboid shape to carry out phagocytosis. Another way to view semi-ramified microglia immunoreactive for CD68 is a compromise in their ability to phagocytose, which provides an explanation for the incomplete clearance of Aβ plaques in the brains of AD patients.

Another set of markers associated with microglia's alternative activation state and phagocytic function was tested. The function of phagocytosis is intricately linked to microglia's alternative activation/ acquired deactivation states (Colton 2009). PVM are extremely efficient phagocytes and are not restricted in their ability to engulf and digest cellular debris (Gate et al. 2010). To this end, PVM markers involved in scavenging and uptake of foreign, waste or pathogenic material were used in the study.

MRC1 is a mannose receptor involved in adhesion, pathogen recognition and clearance. Staining with MRC1, a PVM marker, remains restricted to perivascular spaces in AD. This is similar to other findings in which no other MRC1 expression was found in the parenchyma, despite acknowledged BBB damage e.g. in MS and models of excitotoxic damage and acute inflammation (Fabriek et al. 2005, Galea et al. 2005).

CD14 has previously been used to distinguish between PVM and microglia, but later was found to be expressed by microglia in AD, HIV and HIV encephalitis (HIVE) (Cosenza et al. 2002, Liu et al. 2005). Our findings indeed show that microglia in AD cases were immunopositive for CD14 (CD14+), and the regional distribution of these microglia was patchy and uneven. CD14 has yet to be investigated in PD, and had only been tested in 9 cases of AD in another study (Liu et al. 2005).

Previous investigation by our laboratory (investigator Lynn Christian) demonstrated CD163 immunoreactivity in microglia of TBI cases, providing incentive for testing this antibody in AD cases. CD163 is another PVM-specific marker (Fabriek et al. 2005, Borda et al. 2008) and its expression was restricted to PVM, MM and CPM in control cases. However we found CD163 immunopositive (CD163+) microglia in the parenchyma of AD cases. Foamy macrophages and microglia that are immunopositive for CD163 have been found in the parenchyma of HIVE (Roberts, Masliah and Fox 2004), MS, and head injury cases (unpublished work). Profiling CD163 immunoreactivity in AD and PD would be a novel study that can aid in the understanding of microglial alternative activation state and its phagocytic function.

Chapter 4: Characterization of CD14 immunoreactivity in AD and LBD

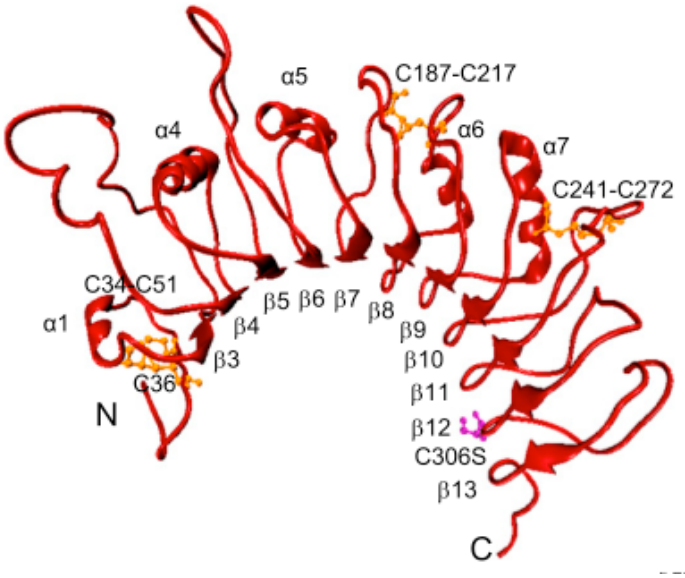
4.1 Introduction

4.1.1 Structure of CD14

CD14 is a 55kDa glycosylphosphatidyl inositol-anchored protein found on the cell plasma membrane (Poussin et al. 1998). CD14 also exists in a soluble form. It is expressed by monocytes, macrophages and granulocytes, but is not found in neurons (Gregory 2000, Milatovic et al. 2004).

CD14 takes on the shape of a bowed solenoid, made of up 11 turns, each containing leucine-rich repeats from amino acids 25-335. The convex side of the bent includes 5 α-helices; the concave side contains a β sheet structure comprising 11 β-strands. This forms a large hydrophobic pocket at the N-terminus that probably functions as a binding site (Kelley et al. 2013).

Figure 14: X-ray crystal structure of CD14 (human)



The structure is made up of human CD14 from a.a. 26-335. Locations of α-helices 1, 4, 5, 6 and 7, and β-strands 3-13 are indicated in the diagram. (Image taken from Kelley et al. 2013)

4.1.2 Functions of CD14

Functions of CD14 include mediating immune responses to bacterial and microbial organisms through the recognition of PAMP. Both soluble and membrane-bound CD14 are able to bind LPS, resulting in activation of myeloid and non-myeloid cells (Ulevitch and Tobias 1999). The activity associated with LPS interaction is mediated by the N-terminal of CD14, which is kept intact in both the soluble and membrane-bound forms (Kelley et al. 2013).

CD14 specifically interacts with TLR2 and TLR4 in response to bacterial pathogens to initiate downstream activation signaling, which brings about a pro-inflammatory response (Kielian 2006). It also plays a crucial role in the phagocytosis of apoptotic cells e.g. lymphocytes and erythrocytes (Schlegel et al. 1999). However, this initiates an anti-inflammatory response instead of a pro-inflammatory one. While the site of interaction on CD14 for the two respective targets are similar (Devitt et al. 1998), the difference in cellular response after binding of CD14 to either LPS or apoptotic cells is probably due to divergence of signaling pathways, either with the participation of a signaling partner e.g. LPS-binding protein, or additional accessory proteins (Gregory 2000).

4.1.3 Inflammatory roles of CD14

While CD14 levels are reduced by IL-4, LPS or IFNγ treatment (Becher, Fedorowicz and Antel 1996), they are increased with GM-CSF. Co-treatment with GM-CSF and IL-4 resulted in the lowering of CD14 expression by microglia and monocytes, even after being stimulated with LPS. A decrease in phagocytic activity was also observed (Lambert et al. 2008). Soluble CD14 is also able to bind to and downregulate T cell responses (Rey Nores et al. 1999).

4.1.4 Expression of CD14

CD14 is expressed by both fetal and adult microglia in cell cultures, but at a lower level compared to monocytes. In the normal brain, CD14 immunoreactivity is only observed in monocytes/macrophages, not in microglia (Ulvestad et al. 1994). Hence, CD14 was initially used as a marker to distinguish between PVM and microglia in macaques (Williams et al. 2001). However, CD14+ parenchymal microglia have been found in HIV and HIVE, as well as AD cases (Cosenza et al. 2002, Liu et al. 2005). They were not observed in DLB patients (Letiembre et al. 2009).

CD14 binds to fibrillar Aβ (Fassbender et al. 2004). CD14 is closely associated with fibrillar Aβ in AD cases, and CD14 greatly facilitates Aβ phagocytosis *in vitro* using

murine microglia, suggesting a role in the clearance of plaques *in vivo* (Liu et al. 2005). *In vitro* experiments also showed that when fibrillar Aβ is used as a stimulant, it leads to activation of NADPH oxidase and ROS production (Reed-Geaghan et al. 2009). CD14 parenchymal microglia are more pronounced around diffuse than neuritic plaques (Letiembre et al. 2009).

In a murine model of AD, CD14 knockout animals showed a reduced plaque burden, reduced number of microglia, and an alteration in microglia activation status with regards to Aβ plaques. This observation was not expected in the light of *in vitro* experiments where CD14 was essential for the phagocytosis of Aβ plaques. An increase in the mRNA of TNFα, IFNγ, and IL-10, and decrease in Fizz1 and Ym1 were also observed in these knockout animals. Specifically, IL-10 mRNA increase has been linked to reduced cerebral amyloidosis (Town et al. 2008, Reed-Geaghan et al. 2010).

4.2 Aims

The aim of this chapter was to investigate CD14 immunoreactive microglia and its spatial distribution in AD and PD. A possible temporal relationship between CD14 immunoreactivity and the developmental stages of PD using Braak's staging system was explored. Finally, the associations of CD14 immunoreactivity with clinical symptoms of PD and pathological hallmarks of AD and PD were studied.

4.3 Materials and Methods

4.3.1 Case selection

31 AD cases of Braak stages 5 and 6 obtained from the Corsellis Archival Collection were used in this study. (Refer to chapter 3 for selection criteria) Formalin-fixed, paraffin embedded samples from frontal cortex, hippocampus, and occipital cortex were used. The patient cohort comprised of 21 females and 10 males, age range 65-88, mean age at death 76. The information on age at death, gender, and cause of death are listed in Table 2.

Using BNE's staging protocol for α-syn pathology, 10 cases from Braak stage 6, and 6 cases each from stages 4 and 5, and 5 cases from stage 3 were obtained from the UKPDSTB, making up a total of 27 cases of LBD. This was made up of PD and DLB cases (McKeith et al. 2005, Alafuzoff et al. 2009). Formalin-fixed, paraffin-embedded samples from the frontal cortex, cingulate cortex, striatum, basal forebrain, hippocampus, midbrain, pons, and medulla were used. Frozen sections from the frontal and cingulate cortices were obtained from 10 cases of LBD. The patient cohort comprised of 9 females and 18 males, age range 57-96, mean age of death 74. The age at death, gender, and cause of death for these cases are listed in Table 5.

16 cases with no neurological or systemic disease were also selected as controls. These were classified as NPD (no psychiatric disease), and were obtained from both the Corsellis collection and the UKPDSTB. Formalin-fixed, paraffin-embedded samples from the frontal cortex, occipital cortex, cingulate cortex, striatum, basal forebrain, hippocampus, midbrain, pons, and medulla were used. The patient cohort comprised of 8 females and 8 males, age range 59-81, mean age at death 70. Information on these cases is listed in Table 6. There were no significant differences in age at death between the AD, LBD and control group.

Table 5: Age, gender, causes of death and BNE α-synuclein staging of cases with LBD

Parkinson's disease (without dementia)				
Case no.	**Age at death**	**Gender**	**α-syn staging (Braak)**	**Cause of death**
1	75	F	4	-
2	81	M	4	Thrombosis histology
3	80	F	4	"Old age" and Parkinson's disease
4	87	F	4	Gastrointestinal bleeding
5	49	M	5	-
6	75	M	5	Myocardial Infarction, Acute Renal Failure, Pneumonia
7	72	M	4	Cardio-respiratory arrest; aspiration pneumonia, Parkinson's disease
8	77	F	5	End stage cardiac failure; moderate to severe LV dysfunction; Aortic & mitral valve disease; Colonic bleed, UTI
9	79	M	5	Bronchopneumonia, Parkinson's disease & GI Bleed
10	66	M	4	-
11	61	M	6	Bronchopneumonia
12	86	M	6	Aspiration pneumonia and small bowel obstruction
13	96	F	3	-
14	74	M	6	Bronchopneumonia
15	73	F	3	Progressive PD and recurrent CVA
16	89	M	3	-
17	57	M	5	Gastric cancer, liver and bone metastases
Parkinson's Disease with Dementia (PDD)				
1	80	F	6	Hypostatic pneumonia X 1 week; Parkinson's disease; senile/presenile Dementia
2	72	M	6	-
3	66	M	6	Chest infection
4	80	M	3	Dementia in Parkinson's Disease: Lewy Body Dementia
5	84	F	6	-
6	78	F	6	Sepsis, pneumonia

Dementia with Lewy Bodies				
1	61	M	3	Fall; upper cervical spinal cord damage
2	70	M	5	Bronchopneumonia
3	69	M	6	Dementia with Lewy bodies
4	74	M	6	-

Table 6: Age, gender and causes of death of control cases

Control cases			
Case no.	**Age at death**	**Gender**	**Cause of death**
1	M	59	Polyarteritis nodosa and related conditions
2	F	59	Gastric ulcer
3	M	62	Acute myocardial infarction
4	M	63	Seropositive rheumatoid arthritis
5	F	63	Chronic renal failure
6	F	68	Crushing injury of thorax and traumatic amputation of part of thorax
7	F	69	Acute myocardial infarction
8	M	71	Chronic tubulo-interstitial nephritis
9	M	71	Haemopericardium, Ruptured acute myocardial infarction
10	F	72	Peptic ulcer
11	M	74	Acute myocardial infarction
12	M	75	Squamous cell carcinoma of the lung
13	F	76	Pulmonary embolism
14	M	78	Acute myocardial infarction
15	F	80	Breast carcinoma with spinal metastasis; carcinosarcoma uterus
16	F	81	Bronchial pneumonia, old age

4.3.2 Immunohistochemistry

CD14 immunostaining was performed on all AD and LBD cases. Dilutions and pretreatments used are listed in Table 7. Frontal and occipital cortices, and the hippocampus region from AD cases, and frontal and cingulate cortices, striatum, basal forebrain, hippocampus, midbrain, pons, and medulla from the LBD cases were stained.

Table 7: Primary antibodies used with CD14 in double immunofluorescence

Antibody	Clone/type	Epitope	Dilution	Pretreatment	Source
CD14	7, Mouse IgG2a	External domain of CD14	1:100	CA+ GT	Novocastra
CD14	EPR3652, Rabbit Monoclonal IgG	Synthetic peptide of human CD14	1:500	CA	Lifespan Biosciences
α-synuclein	42, Mouse IgG1	Rat α-syn a.a. 15-123	1:1000	FA	Becton-Dickinson
Tau	AT8, Mouse IgG1	Tau phosphorylated at Ser-202/Thr-205	1:800	None	Autogen Bioclear
Abeta	4G8, Mouse IgG2b	a.a. 17-24	1:2000	FA	Signet

4.3.3 Double Immunofluorescence

Investigation into the relationship between CD14+ microglia and pathological hallmarks of AD and PD was carried out by double immunofluorescence (DIF). Antibodies against pathogenic Aβ, tau and α-syn (monoclonal mouse anti-human) were used together with CD14 (monoclonal rabbit anti-human). Pretreatments used are listed in Table 7. Antibody dilutions used for DIF are twice as concentrated as those used for IHC. When both antibodies used required different antigen unmasking, the pretreatments were done consecutively before addition of the primary antibodies concoction.

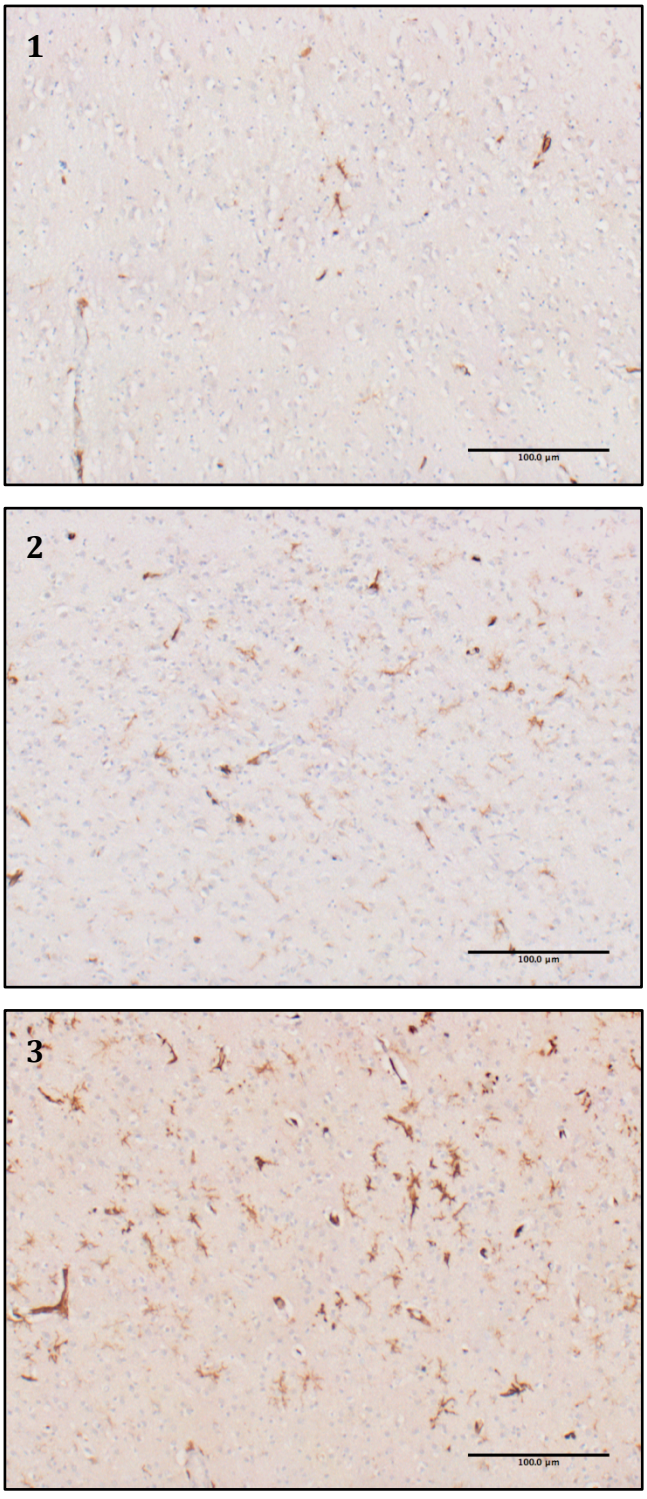
Antibody clone 42 against α-syn was chosen due to its specificity and strong immunoreactivity towards a range of structures with α-syn inclusions (Croisier et al. 2006).

4.3.4 Semi-quantitative assessment of CD14+ microglia

Rating of microglia's CD14 positivity was carried out using subjective assessment of microglial abundance on all cases of AD, LBD and controls. This was based on a scale of 0-3- absent, mild, moderate and severe (refer to Figure 15 for an illustrative representation of the scale). Assessment was carried out in the frontal, cingulate and occipital lobes, caudate, internal capsule, putamen, nucleus basalis of Meynert (NBM), CA1-4, subiculum, entorhinal cortex, SN, LC and dorsal motor nucleus of the vagus (DMV). The region with the highest concentration of immunoreactive microglia was chosen for the ratings. All assessments were done at x10 magnification.

Aside from the internal capsule, rating of microglia load was restricted to the grey matter in all brain region assessed. PVM were excluded during the assessment. Intra-rater reliability gave a Cronbach's alpha value of 0.977, indicating high internal consistency during assessment. Please refer to the appendix for CD14+ microglia ratings.

Figure 15: Figure legend of CD14+ microglia load



1- Extremely sparse ramified microglia throughout the brain region (≤ 3 per field)

2- A slight increase in the number of microglia (≤ 10 per field) throughout the brain region, microglia display shortened and clumpy processes

3- Dense concentrations of microglia with hypertrophy of cell bodies and retraction of processes, resulting in an apparent amoeboid morphology

All images were taken at magnification x10.

4.3.5 Statistical analysis

Statistical analysis was performed using SPSS version 20. Shapiro-Wilk test was used to assess normality for all comparisons. Kruskal-Wallis test followed by post hoc Dunn-Bonferroni's test for correction of multiple comparisons was used to assess the differences in ages at death in AD, LBD and control cases.

Comparison of semi-quantitative assessment of CD14+ microglia load between AD and LBD, and association between CD14+ microglia load and gender, neuropsychiatric complications, or motor onset type were assessed using Mann Whitney U test. Any association between CD14+ microglia load and Braak staging of PD or tau pathology was assessed using either Mann Whitney U test, or Kruskal-Wallis test, followed by post hoc Dunn-Bonferroni's test. Comparison across different brain regions within each disease was done using Friedman's two-way ANOVA, followed by post hoc Dunn-Bonferroni's adjustment. Spearman correlation and partial correlation were used to detect the relationship between age of disease onset, age at death, and duration of disease with CD14+ microglia load. The criteria for all statistical significance was set at $p<0.05$.

4.4 Results

4.4.1 Mild upregulation of CD14 immunoreactivity by parenchymal microglia in control cases

CD14 immunoreactivity was observed in PVM, MM, and CPM as expected (Williams et al. 2001). (Figure 16a) Most of the 16 control cases used in this study also demonstrated CD14 immunoreactivity in microglia. These microglia took on a semi-ramified morphology (Figure 16b), and had a patchy, irregular distribution. 4 cases did not demonstrate any parenchymal CD14 positivity (CD14+). This is in contrast to common understanding that microglia in control cases are negative for CD14 (Ulvestad et al. 1994).

4.4.2 PVM and parenchymal microglia are CD14 immunopositive in AD and LBD

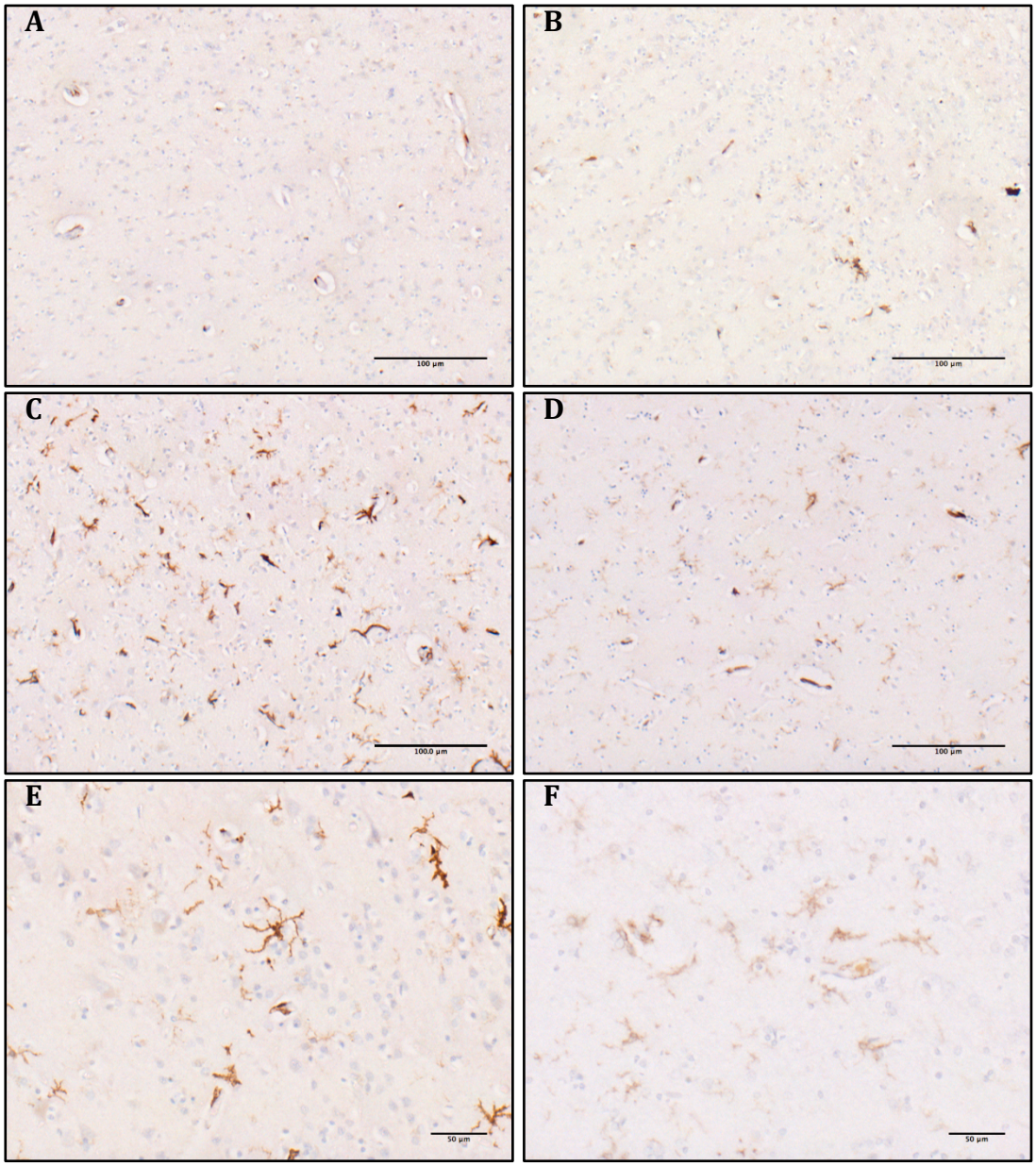
PVM, MM and CPM were positive for CD14 in AD and LBD cases. CD14+ microglia were observed in all AD cases, and 23 out of 27 of the LBD cases. (Figure 16c- f) 4 of the LBD cases that did not demonstrate any CD14+ microglia consist of two DLB cases both of Braak stage 6, and two PD cases of Braak stages 4 and 5. These microglia appeared semi-ramified with an uneven distribution, similar to those observed in control cases. In addition, perivascular and sub-arachnoid macrophages of AD and PD also tend to display increased CD14 immunoreactivity. Blood vessels in close association with CD14+ amoeboid macrophages/microglia were rarely observed. (Figure 17)

4.4.3 CD14 immunoreactivity is more extensive in AD than in LBD cases

There was an increase in CD14+ microglia load in the frontal cortex ($U= 182.00$, $p<0.001$), CA1 ($U= 126.50$, $p<0.005$), subiculum ($U= 148.00$, $p<0.005$) and entorhinal cortex ($U= 89.00$, $p<0.001$) in AD compared to LBD cases. (Figure 18) The comparisons were carried out using Mann Whiney U tests.

CD14 positivity in parenchymal microglia was compared within the available brain regions for each disease type using Friedman ANOVA, and brain regions with the area of highest CD14 load were noted. The frontal and occipital cortex of AD cases had the highest and second-highest CD14+ microglia load respectively ($p<0.001$), while in LBD cases, the highest area was the putamen, and a tie for the caudate and SN for the second highest CD14+ microglia load ($p<0.001$).

Figure 16: Immunoreactivity of CD14 in AD, PD, and control cases



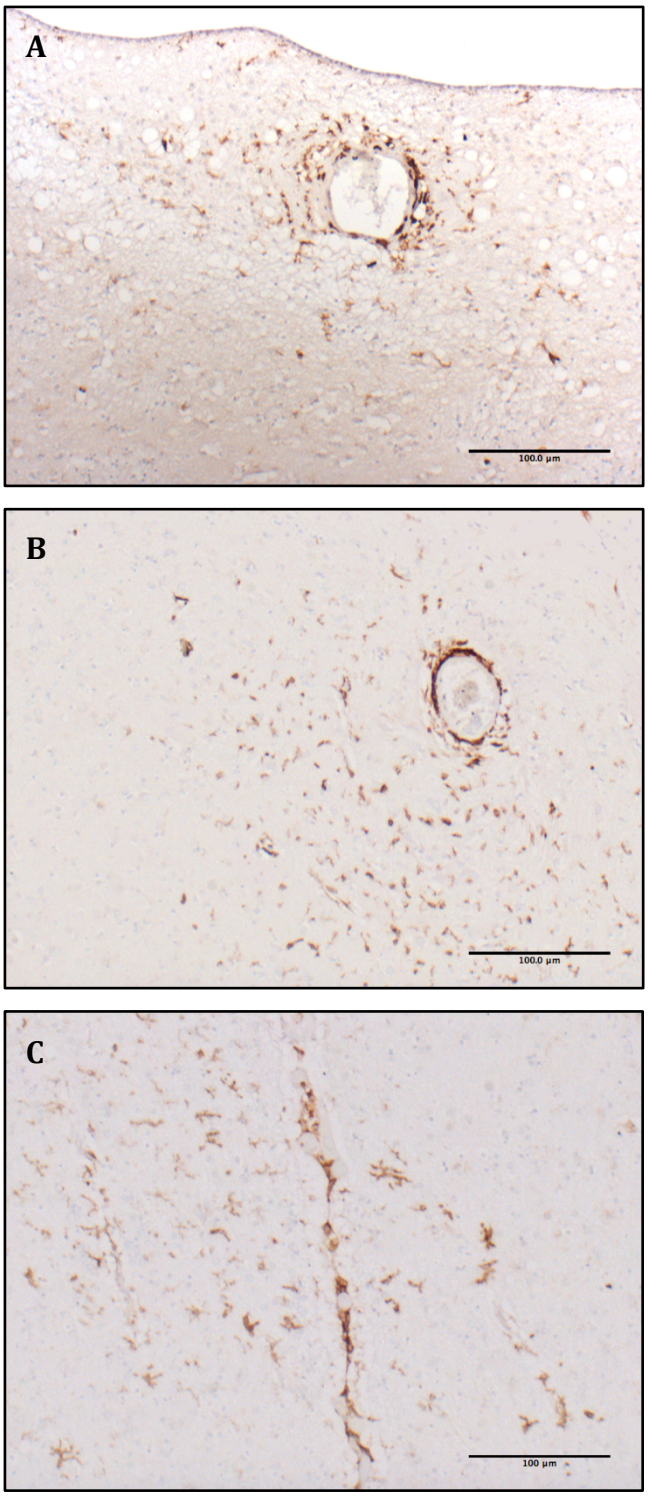
(A) PVM are immunoreactive for CD14 in the frontal cortex of a control case. Magnification x10

(B) CD14+ microglia in the occipital cortex of a control case. These are occasionally observed in control tissues and are usually semi-ramified. Magnification x10

(C & E) CD14+ microglia in the parenchymal of the occipital cortex of two AD cases. Morphologies of microglia in AD range from semi-ramified to amoeboid. Magnification at x10 and x20 respectively

(D & F) CD14+ microglia in the parenchyma of the **(D)** putamen and **(F)** frontal cortex of PD cases. The microglia immunoreactive for CD14 in PD cases appear to be lightly stained compared to AD cases, an indication perhaps that CD14 is upregulated to a greater extent in AD. This is most likely relevant to its function as a receptor for fibrillar Aβ. Magnification x10 and x20 respectively

Figure 17: Close association of CD14+ macrophages with blood vessels



CD14+ microglia/macrophages were observed in close proximity to blood vessels. Microglia density diminishes away from the vessels, suggesting there is an influx of macrophages/microglia from the periphery into the parenchyma. These were observed on rare occasions in cases of AD (A- roof of inferior horn of lateral ventricle & B- occipital) and PD (C- frontal). Magnification x10

Regions of AD and LBD cases with the highest CD14+ microglia load were compared using Mann Whitney U tests as well. Microglia load were higher in the frontal cortex of AD cases than in the putamen of LBD cases ($U=241.50$, $p<0.005$). They were also higher in the occipital cortex of AD cases than the SN or caudate of PD cases ($U=127.00$ for caudate, $p<0.001$. $U=109.50$ for SN, $p<0.001$). There was no difference between PD without dementia and PDD in any of the brain regions tested.

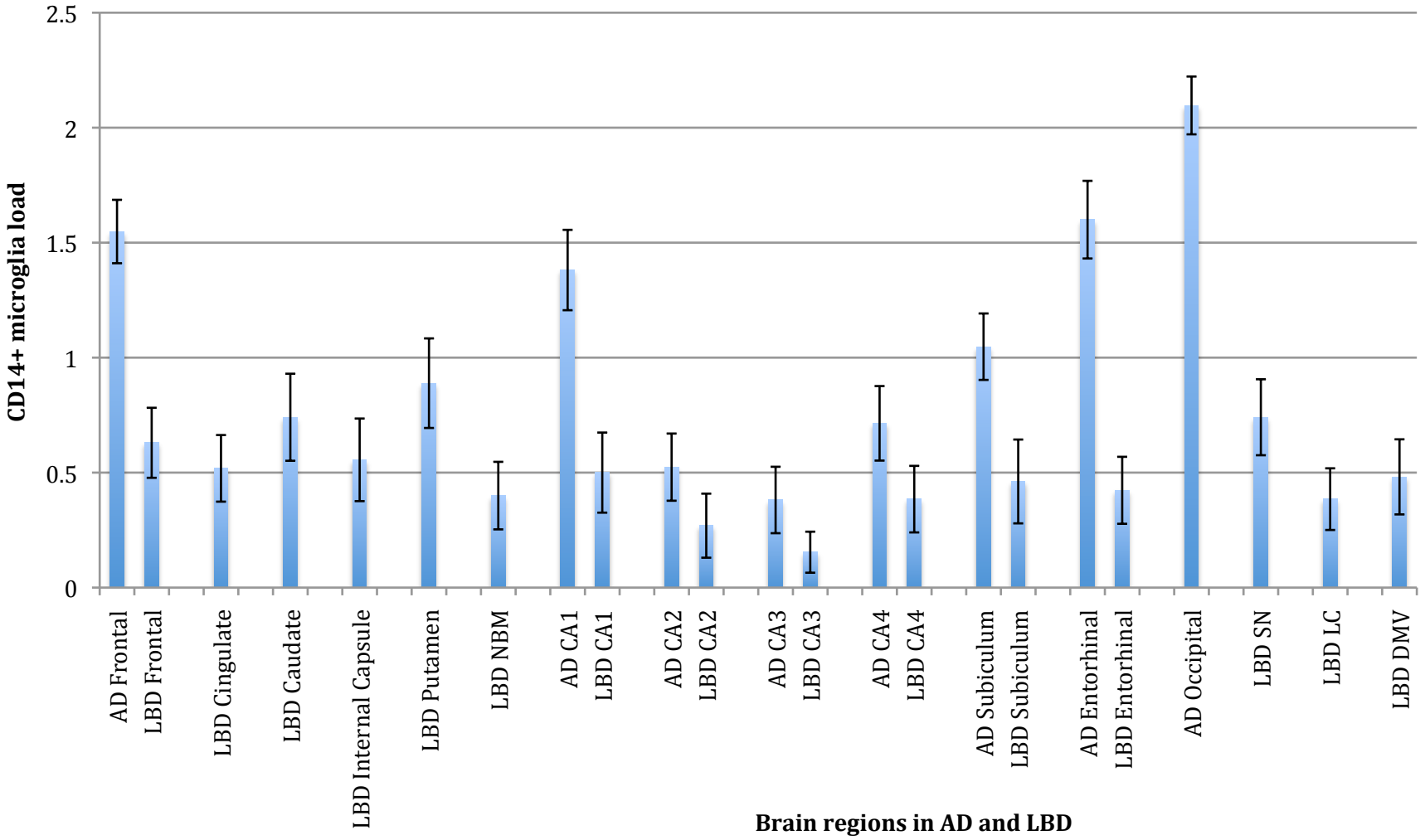
4.4.4 Age at death correlates positively with immunoreactivity of CD14 in AD

CD14+ microglia load in AD and PD cases were analysed for their associations with gender, age of onset, age at death and duration of disease. Using Spearman correlation, there was a statistically significant positive association between age at death and CD14 load in CA2 ($\rho=0.508$, $p<0.05$) and CA4 ($\rho=0.576$, $p<0.01$) of the hippocampus in AD. In PD and PDD cases, partial correlation did not find any association between gender, age of onset, age at death and duration of disease and CD14+ microglia load. When both AD and LBD cases were analysed together, the association between age at death and CD14+ microglia load in CA2 ($\rho= 0.422$, $p<0.005$) and CA4 ($\rho= 0.444$, $p<0.005$) remained significant. This relationship also became significant for CA3 ($\rho= 0.355$, $p<0.05$).

4.4.5 Correlation between CD14 load and clinical and motor symptoms

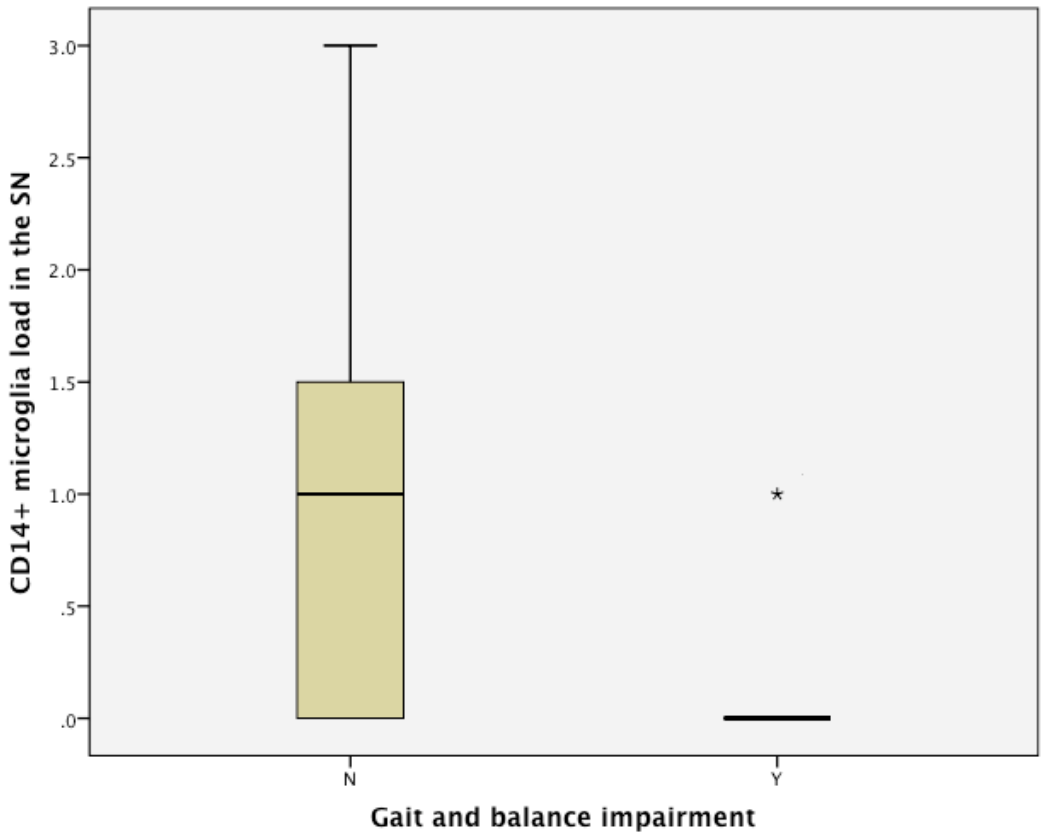
CD14+ parenchymal microglia load in all brain regions assessed in LBD did not show any association with neuropsychiatric dysfunctions (dementia, anxiety, depression, visual hallucinations, and psychosis), nor exhibit a temporal relationship with disease stages using Braak staging system (Alafuzoff et al. 2009). However, a lower CD14+ microglia load was found to correlate with gait and balance impairment as a motor onset symptom. ($U= 32.00$, $p<0.05$) (Figure 19)

Figure 18: Comparison of CD14+ microglia load in various brain regions of AD and LBD cases



CD14+ microglia load was assessed in all available brain regions in 31 AD cases and 27 LBD cases. Significant differences were observed from comparison of medians between the frontal lobe and hippocampal regions (CA1, subiculum and entorhinal cortex) of AD and LBD cases. Columns represent mean CD14+ microglia load in their respective brain regions in AD and LBD cases.

Figure 19: Boxplot showing CD14+ microglia load in the substantia nigra in relation to gait and balance impairment as a motor onset symptom in LBD patients



CD14+ microglia load was compared between LBD cases (n=27) with and without gait and balance impairment as the initial motor onset type. Out of all the brain regions tested, the only region that displayed significant difference in CD14+ microglial load between these two groups was the substantia nigra.

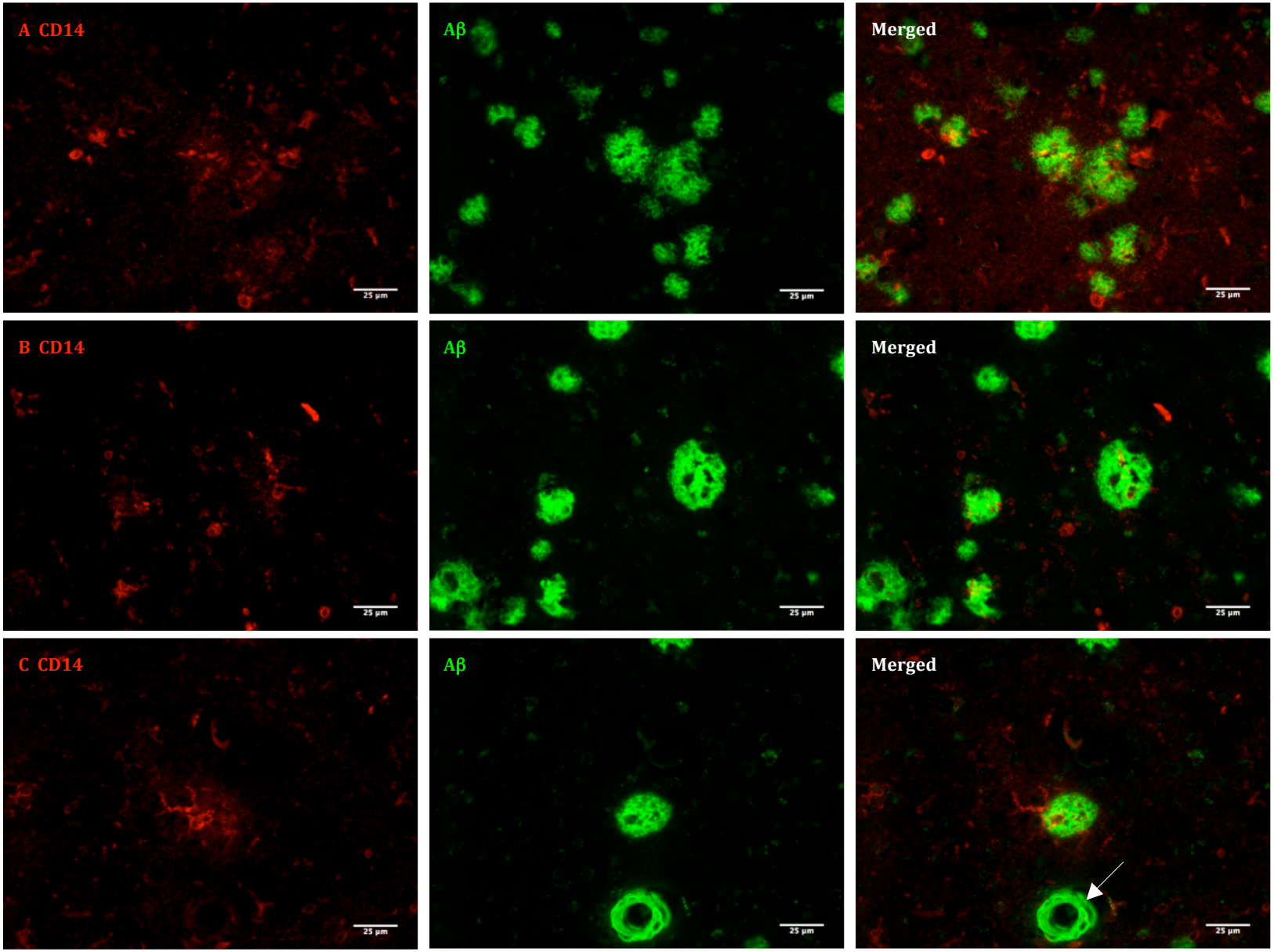
4.4.6 CD14+ microglia are associated with Aβ plaques and α-syn pathology, but not tau pathology

DIF demonstrated co-localisation of CD14+ microglia with Aβ plaques in AD cases. The co-localisation was seen with both diffuse and neuritic plaques, but not with CAA. (Figure 20) Many of these microglia have taken on a more amoeboid shape.

DIF was performed using anti-CD14 and anti-tau antibodies. NFT, NT and neuritic plaques were stained by AT8. (Figure 21) Similar to Lewy pathology, CD14+ microglia were also found within areas of intense tau pathology, but there was no obvious association between CD14+ microglia and NFT or NT.

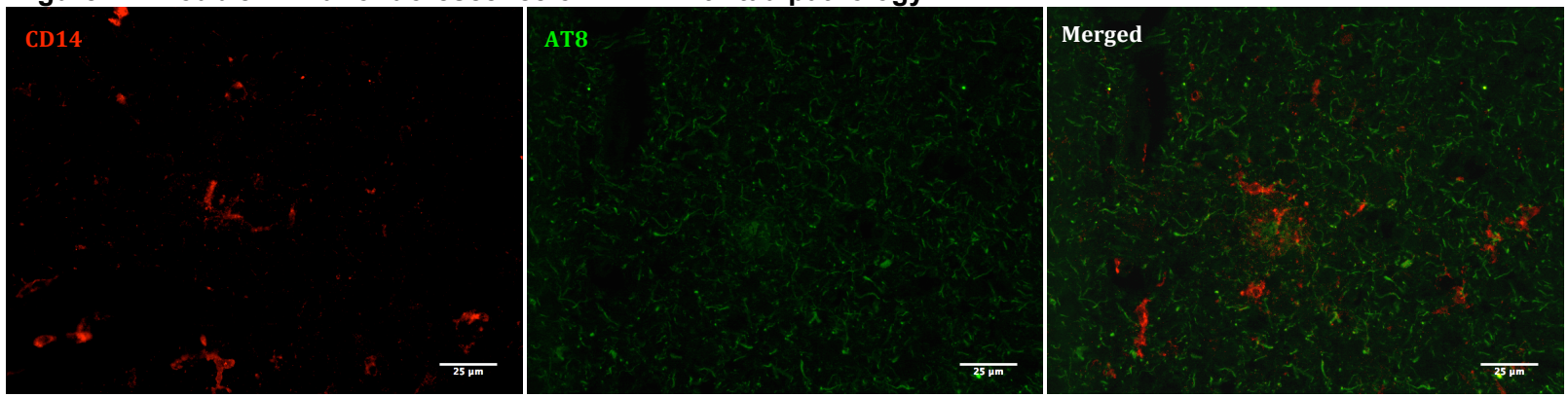
When DIF was performed with anti-CD14 and anti-α-syn, intracellular Lewy inclusions within CD14+ amoeboid microglia were observed. (Figure 22) CD14+ microglia were found at close proximity to other neurons containing Lewy inclusions e.g. LB or LN, but any associations cannot be confirmed with immunofluorescence microscopy alone.

Figure 20: Double immunofluorescence of CD14 with Aβ pathology



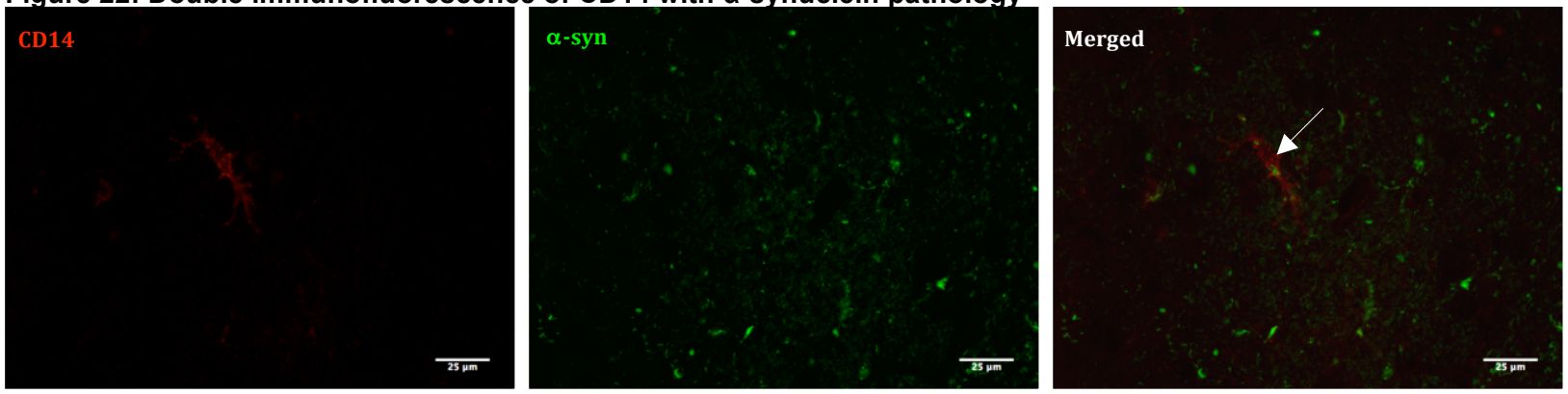
(A-C) DIF for CD14 (red) and Aβ (green) in the occipital cortex of AD cases. Amoeboid CD14+ microglia coincide with **(A & C)** diffuse and **(B)** neuritic Aβ plaques. A few microglia were seen around the amyloid laden vessel (arrow) in **(C)**. Magnification x40

Figure 21: Double immunofluorescence of CD14 with tau pathology



DIF for CD14 (red) and AT8 (green) in the occipital lobe of an AD case. CD14+ microglia are in close proximity with neuropil threads but no specific associations can be confirmed. Magnification x40

Figure 22: Double immunofluorescence of CD14 with α-synuclein pathology



DIF for CD14 (red) and α-syn (green) in the hippocampus of a PD case. Amoeboid CD14+ microglia contained α-syn inclusions (arrow). Magnification x40

4.5 Discussion

This chapter explored CD14 as a parenchymal microglial marker in AD, LBD and control cases. The results are in agreement with Letiembre et al. 2009 with respect to the presence of CD14+ microglia in AD. While other studies have demonstrated AD positivity, DLB were found negative for microglial CD14, and investigation has not been made in PD yet (Liu et al. 2005, Letiembre et al. 2009). This discrepancy might be due to the limited number of cases tested. Contrary to expectations, CD14 immunoreactivity in microglia was also observed in PD, DLB, and control cases, albeit to a lesser extent than in AD cases. Out of 4 DLB cases tested, two demonstrated CD14 positivity in microglia. CD14+ microglia load was most prominent in the striatum and SN of PD cases, coinciding with brain regions with the greatest loss of DA neurons.

Using DIF, CD14+ microglia were shown to associate with Aβ plaques. CD14+ microglia is upregulated in AD, and this is probably due to the ability it confers to microglia i.e. to enable them to recognize and internalize fibrillar Aβ. Their phagocytic function is further substantiated by their amoeboid morphology. A greater number of microglia were observed to cluster around diffuse plaques compared to neuritic plaques (Figure 20). This is also observed in the study done by Letiembre et al. 2009. A greater attraction towards diffuse plaques could be due to several factors. Diffuse plaques might be most malleable to breakdown and phagocytosis compared to other plaque types. CD14+ microglia might also aid in the maturation of plaques, from diffuse to end-stage "burnt-out" plaques (Dickson and Vickers 2001).

Qualitative assessment shows a huge discrepancy between the number of CD14+ microglia and the amount of Aβ plaques present in AD cases. The antibody against CD14 evidently highlights only a subset of microglia, compared to activated microglia stained against MHCII (Figure 9) or a pan-microglia stain against Iba1 (Figure 8). We hypothesize that only a certain population of microglia are equipped with the ability to phagocytose Aβ plaques; or due to the dystrophic nature of microglia in neurodegenerative diseases (Streit et al. 2009), most microglia are unable to effectively phagocytose Aβ plaques. This could explain the poor clearance of plaques in AD.

CD14 is constitutively expressed on monocytes and macrophages. IHC experiments consistently showed PVM, MM and CPM immunoreactive for CD14 in diseased and control cases. On rare occasions, clusters of CD14+ macrophages were seen surrounding blood vessels, probably signifying a compromise in the vessel wall and an influx of macrophages into the brain parenchyma. Although CD14 is not usually expressed by microglia, results from the study support the idea that CD14+ microglia are not derived from the periphery, but instead upregulate CD14 expression in

response for the increased need for phagocytosis. The lack of association between most vessels with CD14+ microglia, including vessels with CAA, substantiates this theory.

In LBD cases, CD14+ microglia were found to coincide with α-syn Lewy inclusions. These microglia had an amoeboid morphology, an indication of their phagocytic ability. However, most LB and LN did not associate with CD14+ microglia. These inclusions within CD14+ microglia might be derived from the ingestion of extracellular LB.

Microglia labeled with Iba1 has been shown to associate with NFT in a rat model of taupathy (Zilka et al. 2009), but this was not observed with CD14+ microglia, even though CD14+ microglia are in very close proximity to neurons containing NFT and NT. There is most likely an array of microglia subsets with diverse functions- different types of microglia are equipped to respond to an array of pathology. As NFT and NT are intracellular, the microglia interacting with affected neurons might respond by taking on a neurotrophic profile instead of a phagocytic one.

CD14+ microglia load increases along with age at death in the CA2, 3, and 4 sectors of AD and LBD cases. CA2 and CA4 are not implicated in AD unlike CA1 and CA3, where significant neuronal loss is observed (Padurariu et al. 2012). In PD, the hippocampal region is also affected, the most vulnerable site being CA2 (Pereira et al. 2013). The susceptibility of the hippocampus towards pathological changes might stimulate the upregulation of CD14 in microglia.

The correlation between gait and balance impairment as a motor onset symptom and lower amounts of CD14+ microglia in the SN might support the idea that these microglia are neuroprotective. PIGD-dominant PD patients exhibit a faster cognitive decline compared to tremor-dominant patients (Alves et al. 2006). Nigral NFT relate positively with gait impairment (Schneider et al. 2006), but no correlation between tau pathology in SN and CD14+ microglia was found in our study.

Chapter 5: Characterization of CD163 immunoreactivity in AD and LBD

5.1 Introduction

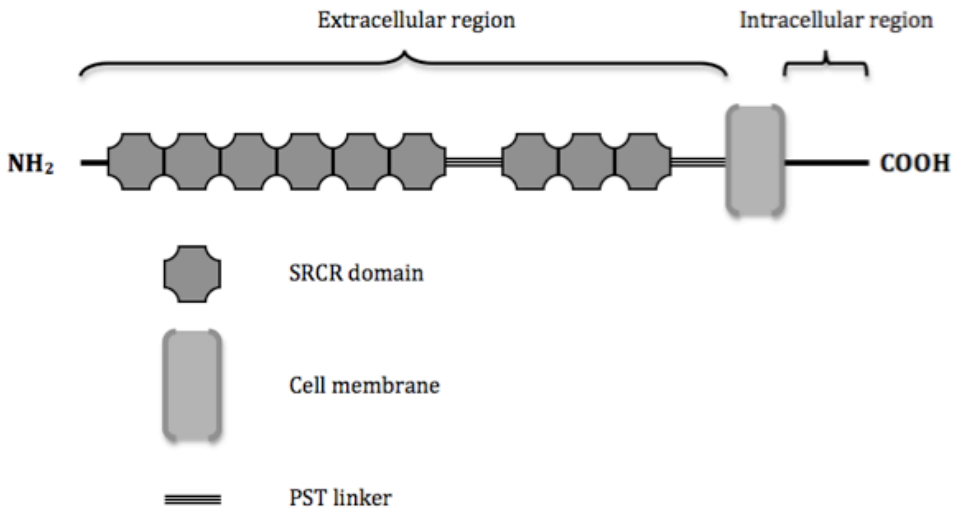
5.1.1 Structure of CD163

CD163, also known as M130, RM3/1, or p155 is a 130 kDa transmembrane glycoprotein found on monocytes and macrophages. It belongs to a subfamily of scavenger receptor cysteine-rich (SRCR) domain proteins, which are characterized by having a short cytoplasmic tail, a transmembrane segment and an extracellular part formed from 9-SRCR domains (Figure 23). 4 splice variants that differ in the cytoplasmic tail have been reported (Hogger et al. 1998, Van den Heuvel et al. 1999, Graversen, Madsen and Moestrup 2002).

The SRCR domain is made up of around 100 amino acids. CD163's SRCR domains belong to class B, which are found only in vertebrates and is encoded by a single exon. Its conformation in 3D probably resembles a six-stranded β-sheet cradling a single α-helix, similar to a class A SRCR domain. The interaction site is made of highly variable residues located on a long loop of amino acids between β-strands 5 and 6 of the SRCR domain, of which several come together to form an ideal ligand binding site (Graversen et al. 2002).

The splice variants of CD163 differ mainly in the lengths of their cytoplasmic tails, ranging from 49 amino acids (more abundant isoform) to 84 and 89 amino acids. The first 42 amino acids after the membrane-spanning segment containing consensus sequences for internalization and phosphorylation with protein kinase C and creatine kinase are retained among the isoforms (Graversen et al. 2002). Their varying subcellular localization, i.e. shorter tail variant is mainly localized to the cell surface, while longer tail variants are more often found in the trans-golgi network and endosomes, and might indicate different functionalities of the isoforms (Akila et al. 2012).

Figure 23: Schematic representation of the structure and domain organization of membrane-bound CD163



CD163 consists of a 1003 amino acid extracellular portion with 9 class B SRCR domains, with domains 6 and 7 separated by a 31 amino acid linker. This linker is composed of many proline, serine and threonine residues (PST). Another PST linker connects the last SRCR domain to the transmembrane segment, which is made up of 24 amino acids. The intracellular cytoplasmic domain ranges from 49 to 89 amino acids that contain consensus sequences for phosphorylation and internalization

5.1.2 Functions of CD163

CD163 functions as a hemoglobin (Hb) scavenger by binding and internalizing haptoglobin-hemoglobin (Hp-Hb) complexes from the blood (Kristiansen et al. 2001, Nguyen et al. 2005). After Hp-Hb complexes are endocytosed, the globin undergoes lysosomal proteolysis, while the heme gets broken down into iron and bilirbin; around 10% of all Hb is cleared by this method (Graversen et al. 2002).

CD163 might have a role in host defense, as it is able to recognize and bind both Gram-negative and Gram-positive bacteria. This causes production of pro-inflammatory cytokines in monocytes, which leads to elimination of bacterial infection, though phagocytosis was not observed (Fabriek et al. 2009).

5.1.3 Inflammatory roles of CD163

CD163 expression is induced/ enhanced by IL- 6, IL-10, macrophage colony stimulating factor (M-CSF), and glucocorticoids (Zwadlo-Klarwasser et al. 1990, Wenzel, Roth and Sorg 1996, Hogger et al. 1998, Van den Heuvel et al. 1999). The promoter region of the gene contains potential binding sites for glucocorticoid receptor and transcription factors for myeloid differentiaton (Ritter et al. 1999). Production of IL-6 and IL-10 actually occurs simultaneously with upregulation of CD163, and act as intermediates in boosting CD163 expression (Weaver et al. 2007). Though touted as an anti-inflammatory marker, it is not induced by IL-4 nor IL-13. It is downregulated by LPS, TNF-α, TGFβ and IFN-γ (Buechler et al. 2000, Sulahian et al. 2000, Pioli et al. 2004).

5.1.4 Expression of CD163

CD163 expression is observed on monocytes and macrophages (Zwadlo et al. 1987, Pulford et al. 1992, Lau, Chu and Weiss 2004, Nguyen et al. 2005, Fabriek et al. 2009). CD163 expression has been hypothesized to occur only on macrophages at certain stages of differentiation, or activation state (Lau et al. 2004). Augmented expression of CD163 on phagocytic macrophages e.g. in liver and spleen, as compared to monocytes, might point to the result of a maturation process (Pulford et al. 1992, Sanchez et al. 1999, Schaer, Alayash and Buehler 2007). It can be induced on microglia *in vitro* by stimulation with Hp-Hb (Borda et al. 2008).

CD163 expression has been observed in different diseases. In SIVE and HIVE cases, CD163 expression is highly upregulated in the microglia found within the grey matter. The reason for this observation was the presence of Hb-Hp complex in the tissue due to vascular compromise (Roberts et al. 2004, Borda et al. 2008). Both foamy macrophages and microglia were found to express CD163 in MS near to or within the

lesions. Some of the macrophages contained myelin debris instead of Hb-Hp complexes (Fabriek et al. 2005). In SIV, AD, systemic sepsis and AIDS, expression was restricted to PVM (Roberts et al. 2004, Borda et al. 2008).

5.2 Aims

The aim of this chapter was to investigate CD163 immunoreactive microglia and its spatial distribution in AD, PD and TBI. A possible temporal relationship between CD163 immunoreactivity and the developmental stages of PD using Braak's staging system was explored. A comparison of consistency and reliability between assessment methods of microglia load was carried out using semi-quantitative measurements on IHC stains and Western blot.

The associations between CD163 immunoreactivity and pathological hallmarks of AD and PD were studied. The association between level of CD163 immunopositivity and clinical symptoms of PD were also analysed. Finally, the relationships between level of CD163 immunopositivity and levels of TNFα, BDNF, and IL-10 were investigated using ELISA.

5.3 Materials and methods

5.3.1 Case selection

For IHC, AD, LBD, TBI, and control cases were used. All cases other than TBI were previously used in chapters 3 and 4. Please refer to chapter 3.2.1 and 4.2.1 for selection criteria. 41 cases of TBI were obtained from the tissue archive in the Department of Neuropathology, Southern General Hospital at the University of Glasgow. (Table 8) All cases were assessed by Professor Gentleman and exhibited TAI. A single slide from various regions of the cortex was obtained for all cases. In addition, cingulate cortices were obtained for 8 of these cases.

For Western blotting and ELISA, 18 samples consisting of frontal and cingulate cortices were obtained across 10 cases of LBD from the UKPDSTB. Grey matter was obtained from the frontal cortices. A mixture of grey and white matter was obtained from the cingulate cortices due to tissue availability and orientation. CD163+ microglia load in these brain regions were assessed previously and ranged from mild to severe.

Table 8: List of traumatic brain injury cases

Case no.	Age at death	Survival time (days)	Aβ plaques
1	45	13	1
2	46	10	1
3	24	4	0
4	20	7	0
5	20	5	0
6	47	0	0
7	0.58	0	0
8	56	3	0
9	20	2	0
10	26	3	0
11	52	4	0
12	18	2	0
13	22	13	0
14	47	1	0
15	65	1	1
16	20	5	0
17	46	10	0
18	21	1	0
19	17	7	0
20	29	12	0
21	26	1	0
22	37	2	0
23	9	2	0
24	27	1	0
25	9	5	0
26	25	0	0
27	62	1	1
28	32	3	0
29	28	1	1
30	16	2	1
31	14	0	0
32	16	2	0
33	5	9	0
34	62	11	0
35	32	5	0
36	17	4	0

37	21	8	0
38	75	3	0
39	56	4	1
40	43	11	0
41	59	4	1

5.3.2 Immunohistochemistry

CD163 immunostaining was performed on all AD, LBD, and TBI cases. Dilution and pretreatment used are listed in Table 9. Brain regions analysed for AD and LBD were similar to those used in Chapter 4.

5.3.3 Double Immunofluorescence

DIF was used to investigate the relationship between CD163+ microglia and pathological hallmarks of AD and PD. Antibodies against pathogenic Aβ, tau and α-syn (polyclonal rabbit anti-human) were used together with CD163. Staining against CD68 was carried out to ascertain the phagocytic ability of CD163+ microglia. Antibodies against MRC1, Iba1 and fibrinogen were examined together with CD163 to learn the source of CD163+ microglia.

Pretreatments used are listed in Table 9. Antibody dilutions used for DIF are twice as concentrated as those used for IHC (refer to table 9). When both antibodies used required different antigen unmasking, the pretreatments were done consecutively before addition of the primary antibodies concoction.

Table 9: Primary antibodies used with CD163 in double immunofluorescence

Antibody	Clone/ type	Epitope	Dilution	Pretreat-ment	Source
CD163	10D6, Mouse IgG1	Prokaryotic recombinant protein corresponding to domains 1-4 of N-terminal region of CD163	1:200	CA	Novocastra
α-synuclein	Rabbit Polyclonal	a.a. 111-131	1:2000	FA	Millipore
Tau	Rabbit Polyclonal	C terminal a.a. 243-441	1: 20000	None	Dako
Abeta	Rabbit Polyclonal	Synthetic peptide corresponding to a.a. 1-16 of Aβ peptides 38, 40, and 42	1:500	FA	Synaptic Systems
MRC1	Rabbit Polyclonal	Macrophage mannose receptor 1 precursor recombinant protein epitope signature tag (PrEST)	1:1000	CA	Prestige Antibodies
Iba1	Rabbit Polyclonal	Synthetic peptide corresponding to C-terminus of Iba1	1:400	CA	Wako
CD68	PG-M1, Mouse IgG3	Fixative-resistant epitope on macrophage-restricted form of CD68	1:500	CA	Dako
Fibrinogen	Rabbit Polyclonal	Fibrinogen isolated from human plasma	1:5000	CA	Dako

5.3.4 Semi-quantitative assessments of CD163+ microglia

CD163 immunoreactivity in parenchymal microglia was measured using two semi-quantitative approaches. The first was carried out using subjective assessment of microglia abundance based on a scale (CD163+ microglia load) identical to the one used in chapter 4. Please refer to figure 15 for a pictorial representation of the scale. CD163+ microglia in the cingulate cortex, entorhinal cortex, and LC of PD cases, and TBI cases were quantified using microglia load.

The second semi-quantitative assessment method involves percentage area (%area) measurement of detected cells in the region with the highest density of microglia cells. Assessment was carried out on all AD, LBD and control cases. CD163+ microglia from the frontal and occipital lobes, as well as the entorhinal cortex of AD and control cases were quantified using %area. Those in the frontal, cingulate and occipital lobes, caudate, internal capsule, putamen, NBM, CA1-4, subiculum, entorhinal cortex, SN, LC and DMV of PD and the remaining control cases were also quantified with %area.

For each brain region, the area with the highest amount of CD163+ parenchymal microglia was determined by eye and examined at x10 magnification with a field area of 0.153mm^2. On the occasion that the rater is unable to pick out the region with the highest microglia density, multiple regions in the same tissue section were measured. Similar to microglia load assessment, measurement of %area was restricted to grey matter for all brain regions assessed other than the internal capsule. Image Pro Plus software was used to assess the area occupied by parenchymal microglia within the image generated. The software measures the areas of immunoreaction using a process called segmentation, which allows the isolation of color components in the image based on a color threshold. This measures the number of pixels occupied by immunoreactive microglia. As DAB produces a brown precipitate, segmentation was performed using the blue component of the image as this enhances the brown of the immunolabelled microglial cells. Thresholding was performed individually for each slide as the intensity of immunostaining might differ with each experimental batch. In order to prevent CD163+ PVM from influencing the measurement of CD163+ microglial cells in the parenchyma, all PVM were manually deselected from images. Intra-rater reliability gave a Cronbach's alpha value of 0.973, indicating high internal consistency during assessment. Please refer to the appendix for CD163+ microglia ratings.

5.3.5 Western Blot

Refer to section 2.6 and 2.7 for protein extraction and quantification, complete protocol of Western blotting and β-actin normalization. Primary antibody rabbit polyclonal IgG against CD163 (Santa Cruz, USA) recognizing the C-terminal of CD163 amino acid. 1027-1156 was used at 1:200. Secondary antibody HRP-conjugated goat anti-rabbit IgG (Sigma, UK) was used at 1:5000.

5.3.6 ELISA

ELISA was carried out following the instructions from the respective antibody kits. TNFα and IL-10 ELISA Development kit from Peprotech (UK) and BDNF E_{max} Immunoassay system from Promega (UK) were used in this study. Please refer to section 2.8 for experimental details.

5.3.7 Statistical analysis

Statistical analysis was performed using SPSS version 20. Shapiro-Wilk test was used to assess normality for all comparisons. Comparison of CD163 %area between AD and LBD, and association between CD163 %area and gender, neuropsychiatric complications, or motor onset type was assessed using Mann Whitney U test. Any association between CD163+ microglia load and Braak staging of PD was assessed using either Mann Whitney U test, or Kruskal-Wallis test, followed by post hoc Dunn-Bonferroni's test. Comparison across different brain regions within AD and LBD was done using Friedman's two-way ANOVA, followed by post hoc Dunn-Bonferroni's adjustment. Spearman correlation and partial correlation were used to detect the relationship between CD163 %area and age of disease onset, age at death, and duration of disease for AD and LBD.

Pearson and Spearman correlations were used to correlate measurements of CD163 expression (Western blot) with %area, and %area with CD163+ microglia load (scale 0-3). Spearman correlation was used to study the correlation between CD163 %area and expression levels of TNFα, IL-10 and BDNF detected by ELISA. The criteria for all statistical significance was set at $p<0.05$.

Kruskal-Wallis tests followed by Dunn-Bonferroni correction, Jonckheere-Terpstra tests and partial correlations were used to study the association of CD163+ microglia load with the age at death and survival times of TBI cases. Presence of Aβ plaques was correlated with age at death, survival times, and CD163+ microglia load using Mann Whitney U tests.

5.4 Results

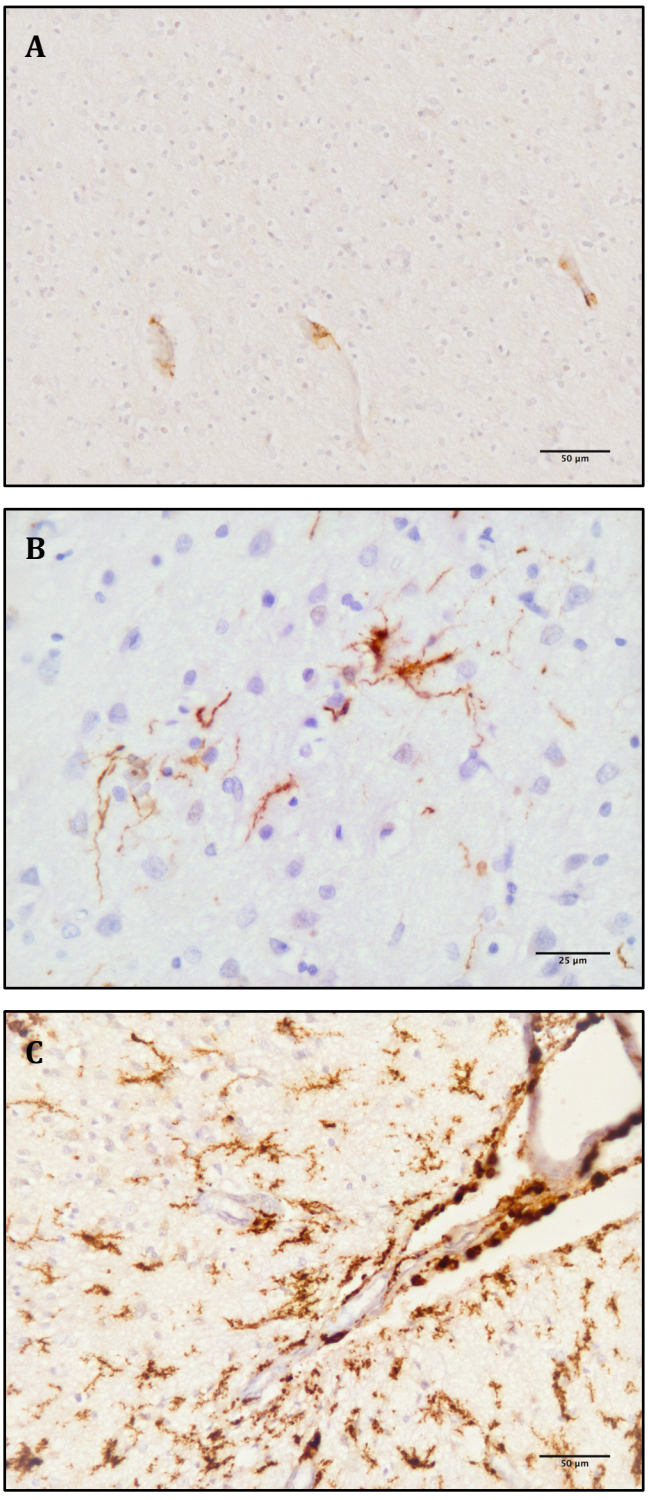
5.4.1 CD163 immunoreactivity is restricted to PVM in majority of control cases

In 12 out of 16 control cases, the only CD163 positivity seen was in PVM, MM and CPM. (Figure 24a) Little or no CD163 immunostaining was observed in parenchymal microglia. This observation is in agreement with the concept that CD163 is a marker specific for monocytes (Fabriek et al. 2005, Kim et al. 2006, Borda et al. 2008).

5.4.2 PVM and parenchymal microglia are CD163 immunopositive in AD and LBD

CD163+ microglia in the parenchyma was found in all cases of AD and LBD, in addition to CD163+ PVM, CPM and MM. These microglia typically took on a semi-ramified morphology (Figure 24b), were distributed in a patchy pattern and tended to be close to the meninges (Figure 24c). CD163 immunoreactivity in both AD and LBD cases ranged from mild to severe. (Figure 25) This finding is different from other reports (Roberts et al. 2004) whereby CD163+ microglia were mostly restricted to perivascular and sub-arachnoid spaces; once again, this observation might be due to the limited number of cases tested in previous studies.

Figure 24: IHC detection of CD163

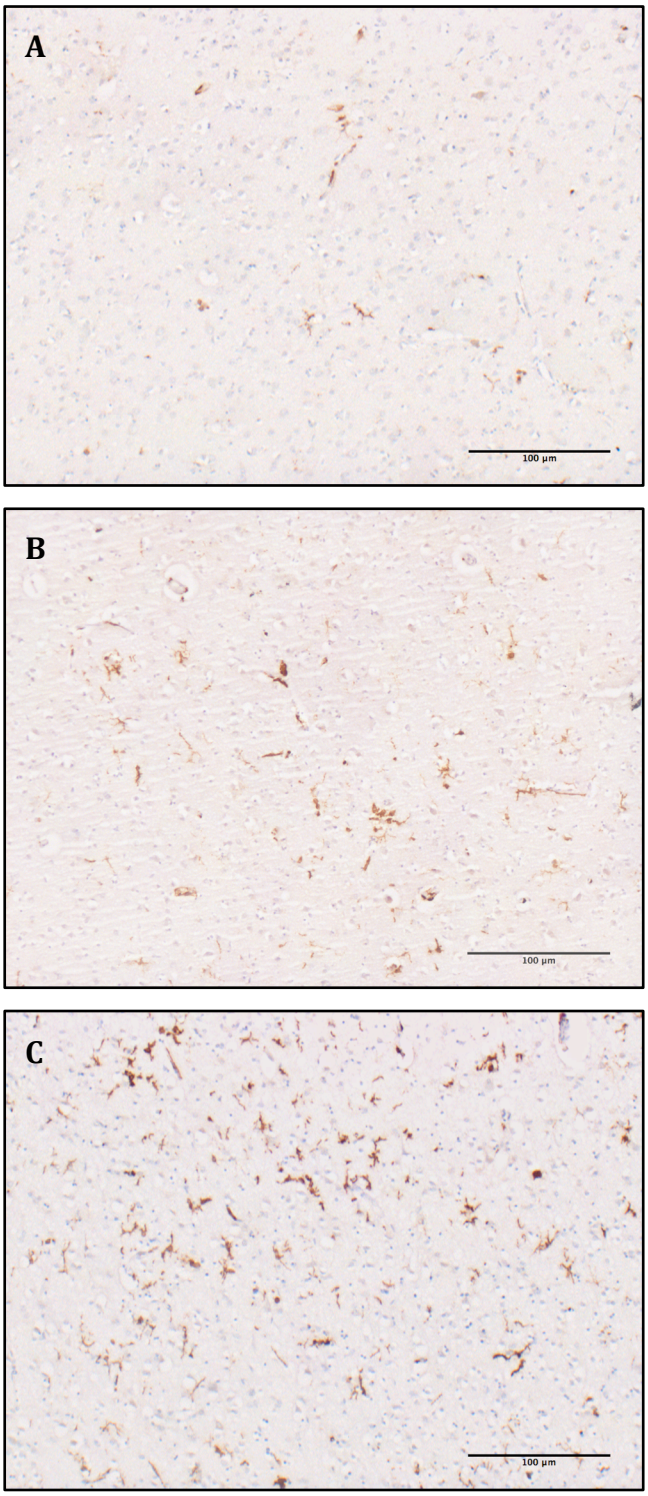


(A) PVM expressing CD163 in the frontal cortex of a control case. Magnification x20

(B) Parenchymal microglia expressing CD163 in the frontal cortex of an AD case. Magnification x 40

(C) Microglia expressing CD163 in close proximity to meningeal spaces in the occipital cortex of an AD case. Magnification x20

Figure 25: Range of CD163 immunoreactivity in microglia of AD cases



(A) mild upregulation in the frontal cortex **(B)** moderate upregulation in the occipital cortex **(C)** severe upregulation in the frontal cortex. Magnification x10

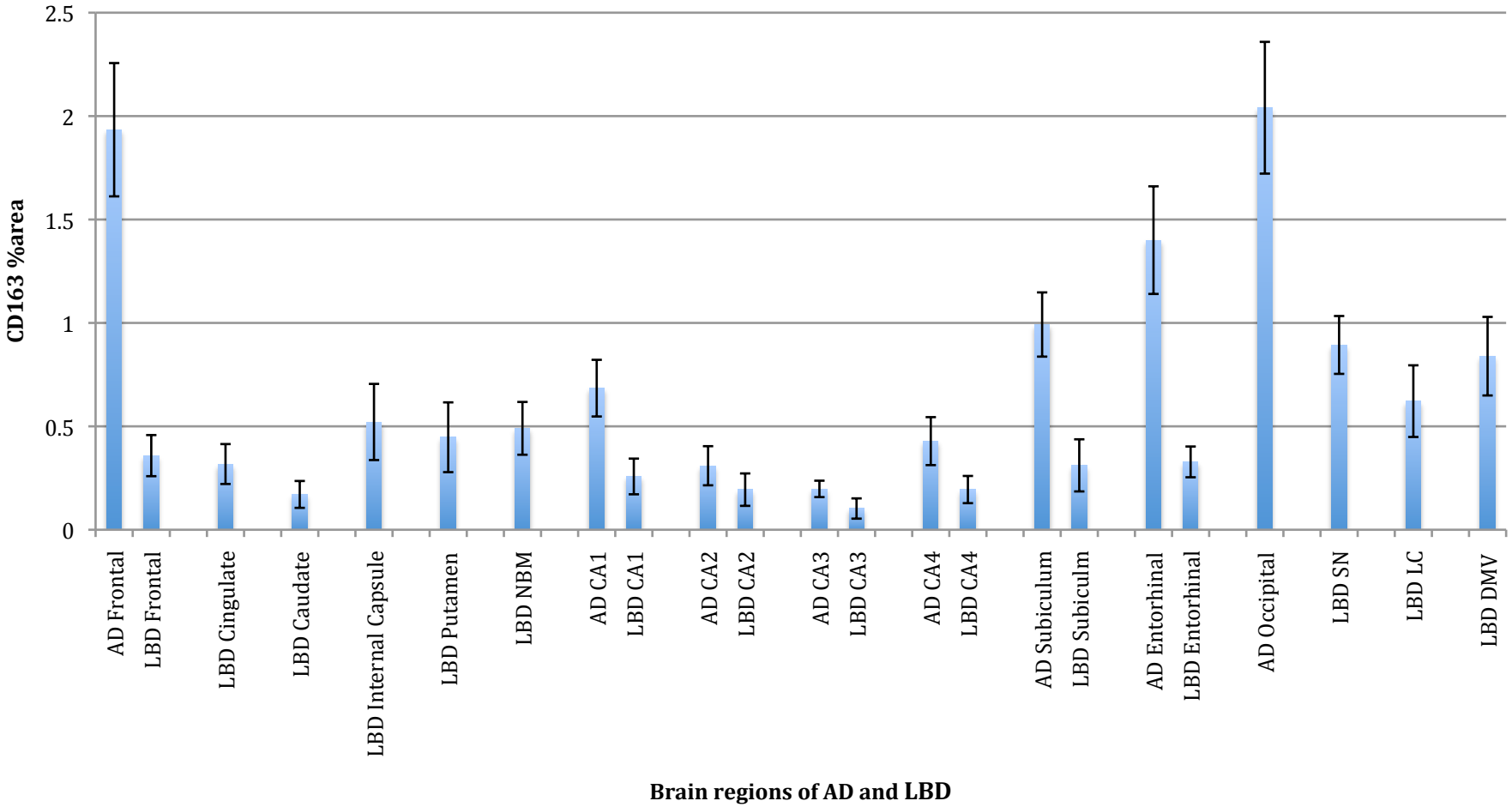
5.4.3 CD163 immunoreactivity is more extensive in AD than in LBD cases

There was a significant increase in CD163+ microglia load in the frontal cortex ($U= 105.00$, $p<0.001$), CA1 ($U= 135.00$, $p<0.005$), CA3 ($U= 190.50$, $p<0.05$), CA4 ($U= 177.00$, $p<0.05$), subiculum ($U= 94.00$, $p<0.001$) and entorhinal cortex ($U= 141.00$, $p<0.005$) of AD compared to LBD cases. (Figure 26) Qualitative assessment of PVM also showed them to be more numerous and prominently stained in AD than LBD.

%area of CD163 positivity in parenchymal microglia was compared within the available brain regions for each disease type using Friedman ANOVA and brain regions with the area of highest CD163 load were noted. The frontal and occipital cortex of AD cases had the highest and second-highest %area occupied by CD163+ microglia respectively ($p<0.001$), while in LBD cases this was observed in the SN and DMV respectively ($p<0.001$).

Regions of AD and LBD cases with the highest %area occupied by CD163+ microglia were compared using Mann Whitney U tests, with median (Mdn) given as a reference point. %area measurements were significantly higher in the frontal cortex (Mdn= 1.19%) of AD cases than SN (Mdn= 0.70%) in LBD cases ($U=254.00$, $p<0.01$). They were also significantly higher in the occipital cortex (Mdn= 1.62%) of AD cases than DMV (Mdn= 0.43%) of LBD cases ($U=194.00$, $p<0.001$). There was no difference between PD without dementia and PDD in any of the brain regions tested.

Figure 26: Comparison of percentage area of CD163+ microglia in various brain regions of AD and LBD cases



%area of CD163+ microglia was assessed in all available brain regions in 31 AD cases and 27 LBD cases. Significant differences were observed from comparison of the median between the frontal lobe and hippocampal regions (CA1, CA3, CA4, subiculum and entorhinal cortex) of AD and LBD cases. Columns represent mean %area of CD163+ microglia in their respective brain regions for AD and LBD cases.

5.4.4 Do CD163 parenchymal microglia originate from the periphery?

DIF was carried out with Iba1 and MRC1 to explore the similarities between CD163+ microglia and PVM. Iba1 is a pan-microglia marker that does not label PVM, while MRC1 is a PVM specific marker. Association with either Iba1 or MRC1 may shed light on whether the CD163+ microglia originated from the periphery, or are resident to the CNS. Fibrinogen was also used to test for damage to the integrity of the BBB.

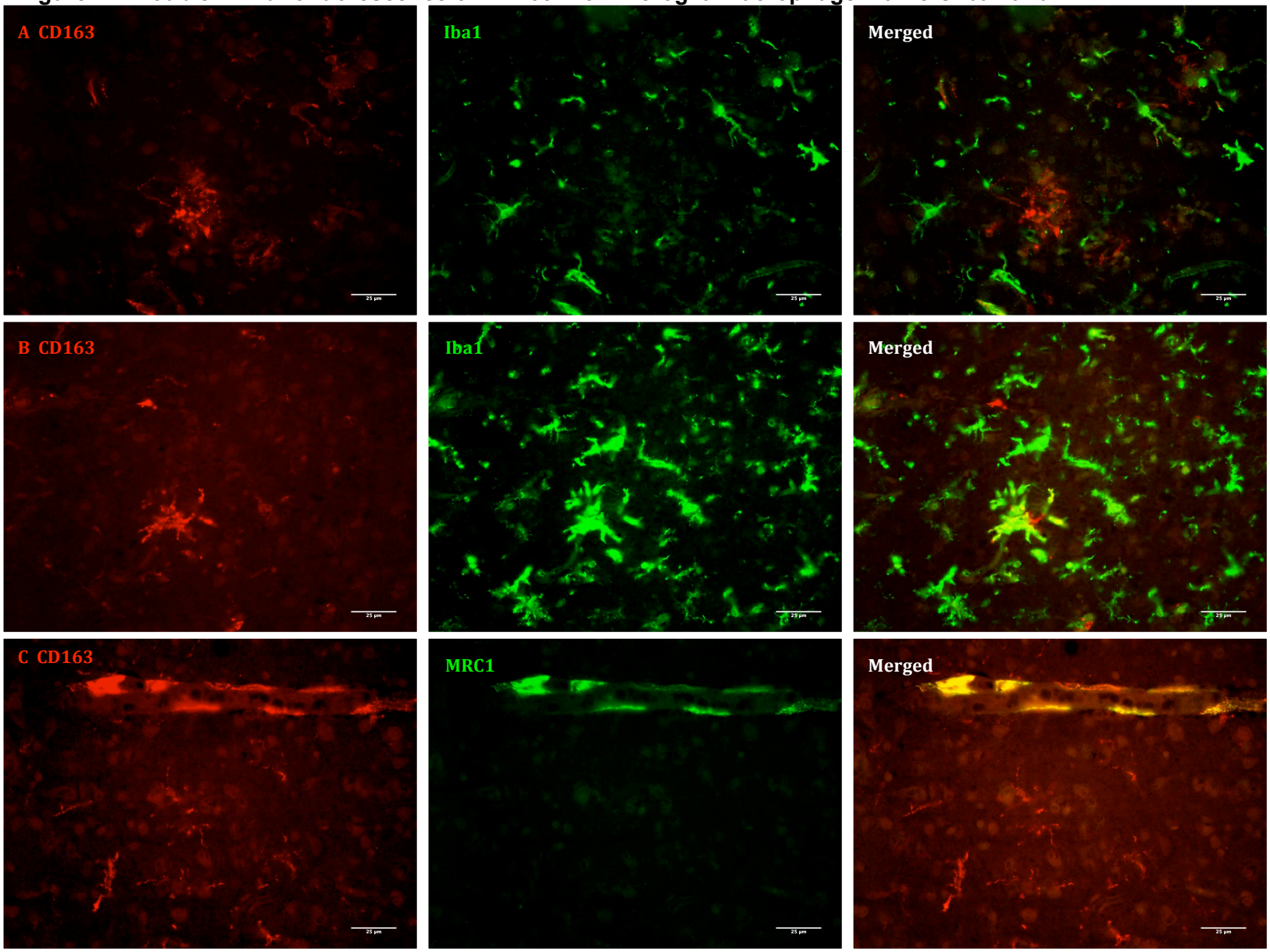
While it was expected that all CD163+ microglia would co-stain with Iba1, absence of Iba1 was observed in many of them. (Figure 27a, b) It was found that MRC1 positivity was strictly limited to PVM (Figure 27c). Staining for fibrinogen revealed perivascular leakage around small to medium sized vessels and around pial vessels in both AD and PD cases. (Figure 28) This was seen as a diffuse pattern that remained in a halo around the compromised vessels. Most vessels in both AD and PD were spared from BBB breakdown, as surmised from the presence of fibrinogen only within the blood vessel lumen. DIF showed CD163+ microglia had a tendency to be found in close proximity to sites of BBB damage. (Figure 29) These blood vessels with fibrinogen leakage were not associated with CAA, neither did CAA show any association with CD163+ microglia. (Figure 30)

5.4.5 Correlation between CD163 %area and clinical symptoms of LBD cases

Negative correlation was found between anxiety and CD163 %area in the cingulate cortex ($U= 27.0$, $p<0.05$), entorhinal cortex ($U= 36.0$, $p<0.05$), and LC ($U= 30.0$, $p<0.05$) in LBD cases. (Figure 31) These correlations remained statistically significant whether or not DLB cases were included in the statistical tests.

No correlation was found between CD163 %area and motor onset symptoms (tremor, rigidity, gait and balance impairment). There was also no correlation between Braak stages of PD and CD163 %area, and CD163 %area with age at death, disease onset age and duration of disease in both AD and LBD cases tested.

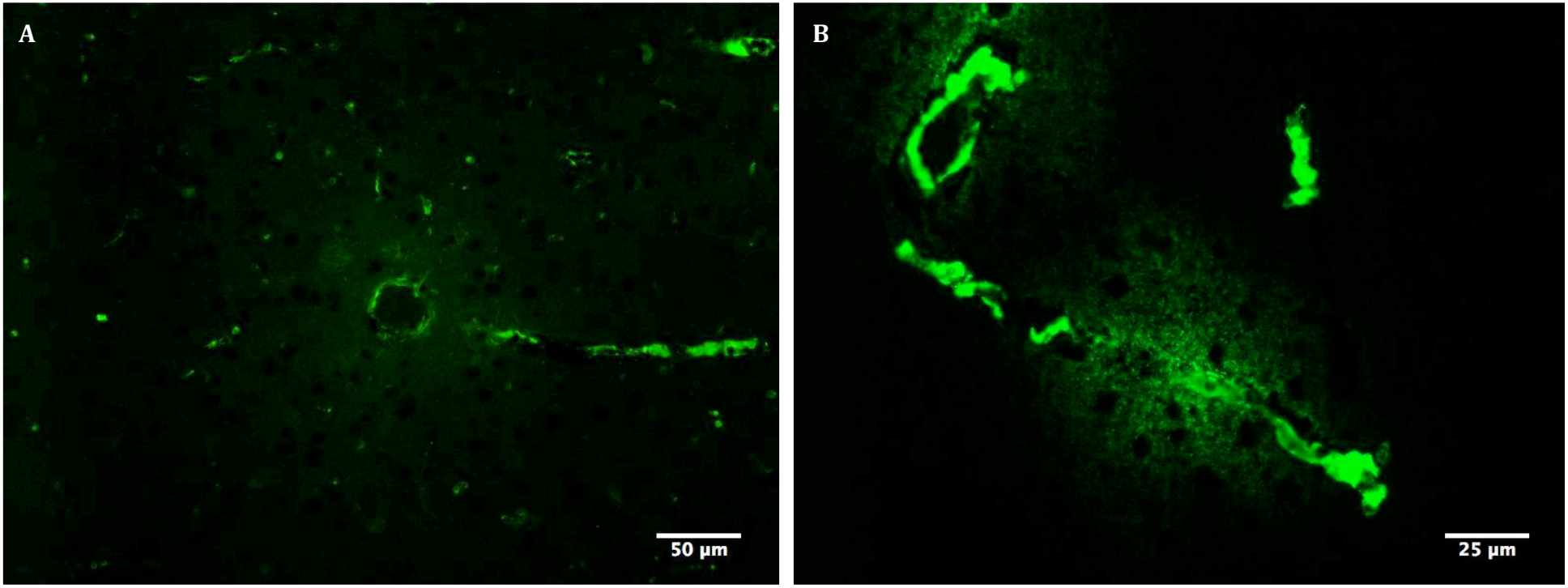
Figure 27: Double immunofluorescence of CD163 with microglia/macrophage markers Iba1 and MRC1



(A & B) DIF for CD163 (red) and Iba1 (green) in the occipital cortex of AD cases. Not all CD163+ microglia stained for Iba1 (centerfield), a marker highly specific for microglia. This suggests that CD163+ parenchyma microglia might originate from systemic cells that have yet to obtain an Iba1+ profile. The presence of Iba+ CD163+ microglia also indicates that resident microglia might be able to express CD163 with stimulation from the periphery. Magnification x40

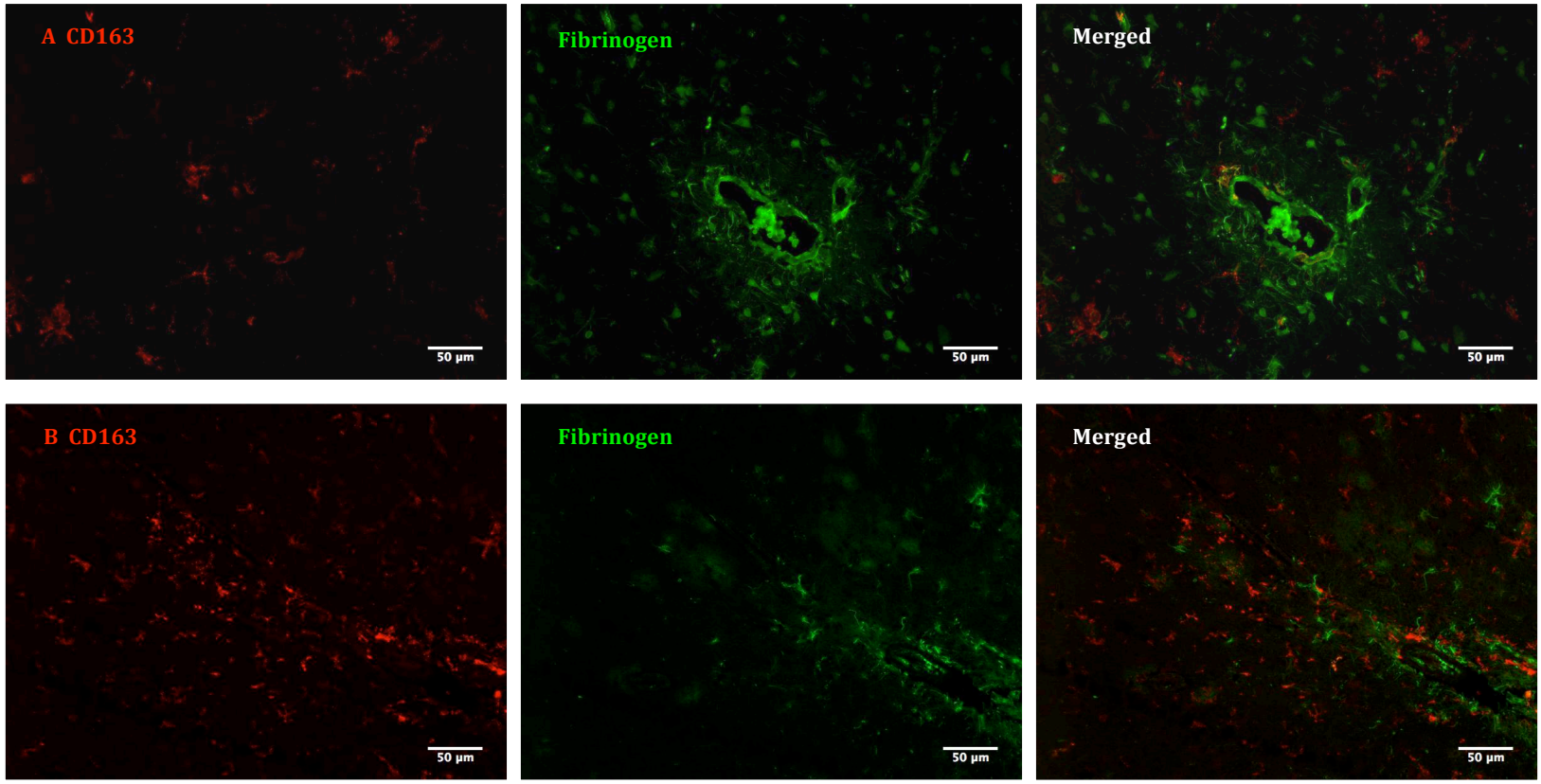
(C) DIF for CD163 (red) and MRC1 (green) in the occipital cortex of an AD case. CD163 co-stains PVM with MRC1. MRC1 expression is limited to PVM. This is in concordance with findings that MRC1 is restricted to PVM despite a clear BBB breakdown. Magnification x40

Figure 28: Immunofluorescence staining against fibrinogen



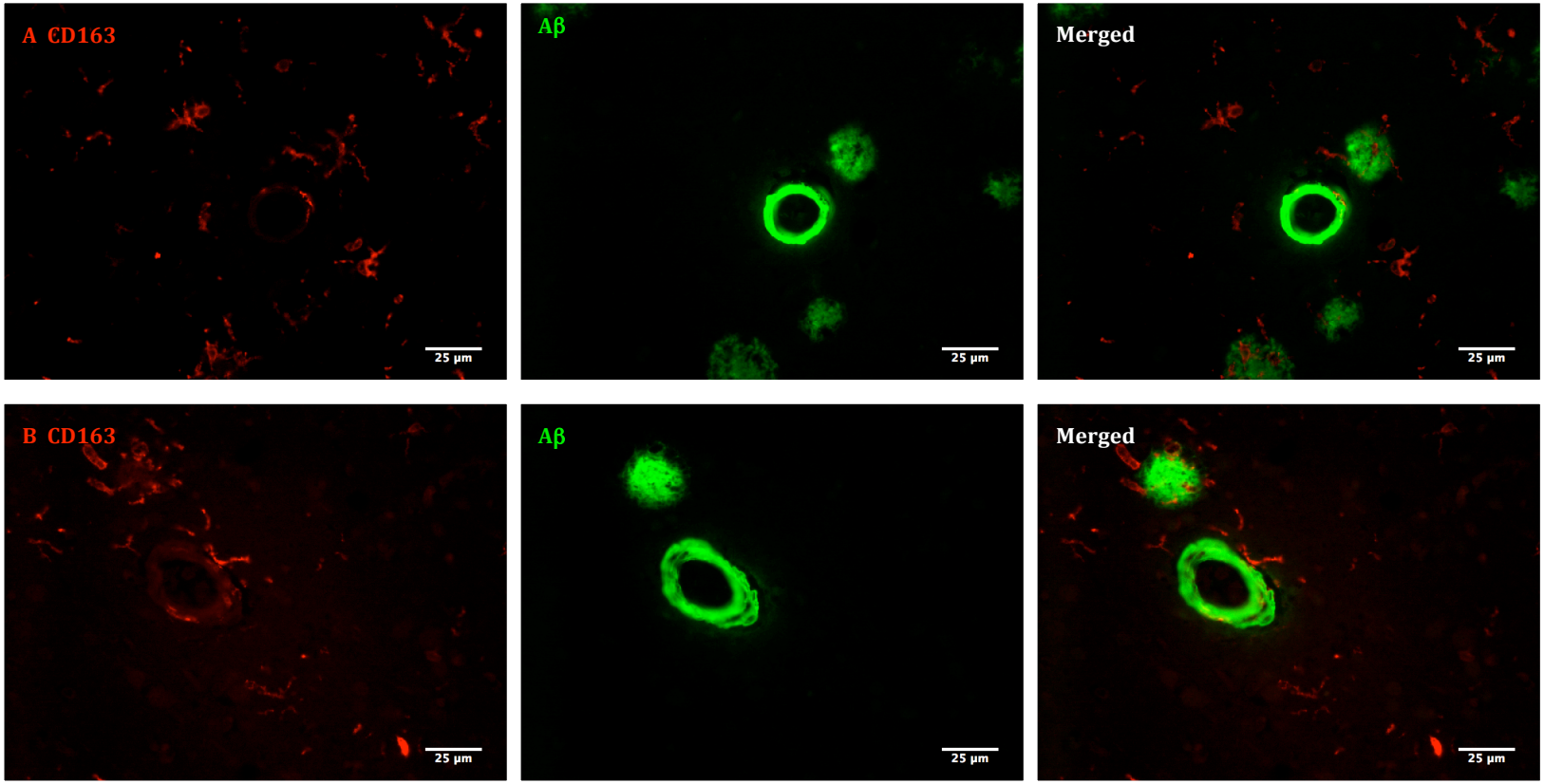
Fibrinogen was found to exude from compromised blood vessels in both AD and PD cases, but to a lesser extent in the PD cases. **(A)** occipital cortex of an AD case. Magnification x20. **(B)** cingulate cortex of a PD case. Magnification x40

Figure 29: Double immunofluorescence of CD163 with fibrinogen



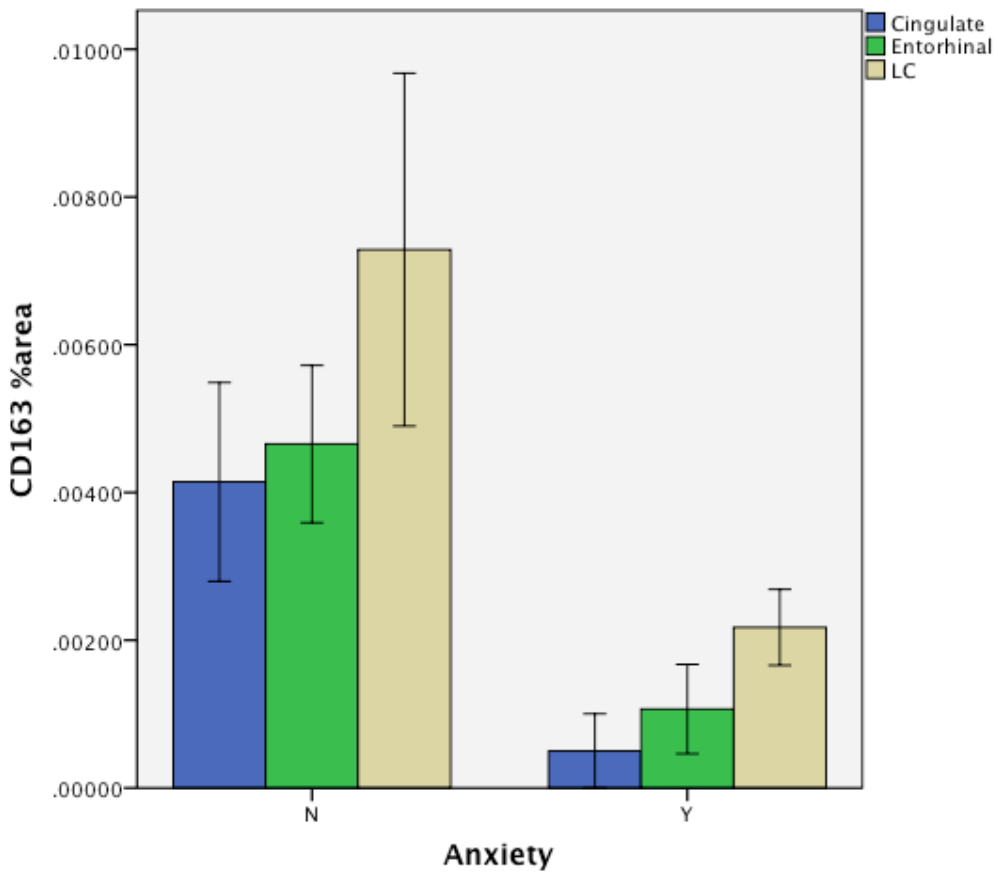
DIF for CD163 (red) and fibrinogen (green) in the **(A)** frontal and **(B)** occipital cortex of AD cases. CD163+ microglia were seen at close proximity to areas of fibrinogen leakage in the parenchyma. This raises the possibility that CD163+ microglia are a result of migration from the periphery. Astrocytes were also found to have fibrinogen immunoreactivity. Magnification x20

Figure 30: Double immunofluorescence detection of CD163 with CAA



Neither CD163+ PVM nor microglia appear to be overtly reacting to CAA in the occipital lobes of an AD case. Aβ plaques are observed in the vicinity of these vessels and clearly associated with CD163+ microglia. Magnification x40

Figure 31: Level of CD163 immunoreactivity in LBD patients with anxiety symptoms



Columns represent the mean %area of CD163+ microglia in the cingulate cortex (blue), entorhinal cortex (green), and locus coerulus (beige) of LBD cases (n=27). Out of all brain regions tested for LBD, associations between CD163 %area and presence of anxiety were found to be statistically significant in these three regions.

5.4.6 CD163+ microglia are associated with Aβ plaques, but not NFT or α-syn pathology

Using DIF, CD163+ microglia were found to co-localise with Aβ plaques. Microglia clusters were seen within plaques with most of them found in the core of neuritic plaques (Figure 32), and fewer of them within diffuse plaques. While many of these microglia retained their ramifications, some of them seemed to have taken on a more amoeboid shape, indicating phagocytosis. This was observed in AD, LBD and control cases. DIF was also performed using antibodies against CD68 and CD163, to confirm the phagocytic nature of CD163+ microglia. Many of the CD163+ microglia were positive for CD68, particularly those with a more amoeboid shape. (Figure 33a)

DIF was performed using antibodies to CD163 and NFT or α-syn, there was no visible signs of interaction between CD163+ microglia and intracellular NFT (Figure 33b) or α-syn. However, in rare cases, microglia were found to associate with extracellular α-syn. (Figure 33c)

5.4.7 Cytokines and neurotrophin levels do not correlate with CD163+ microglia load

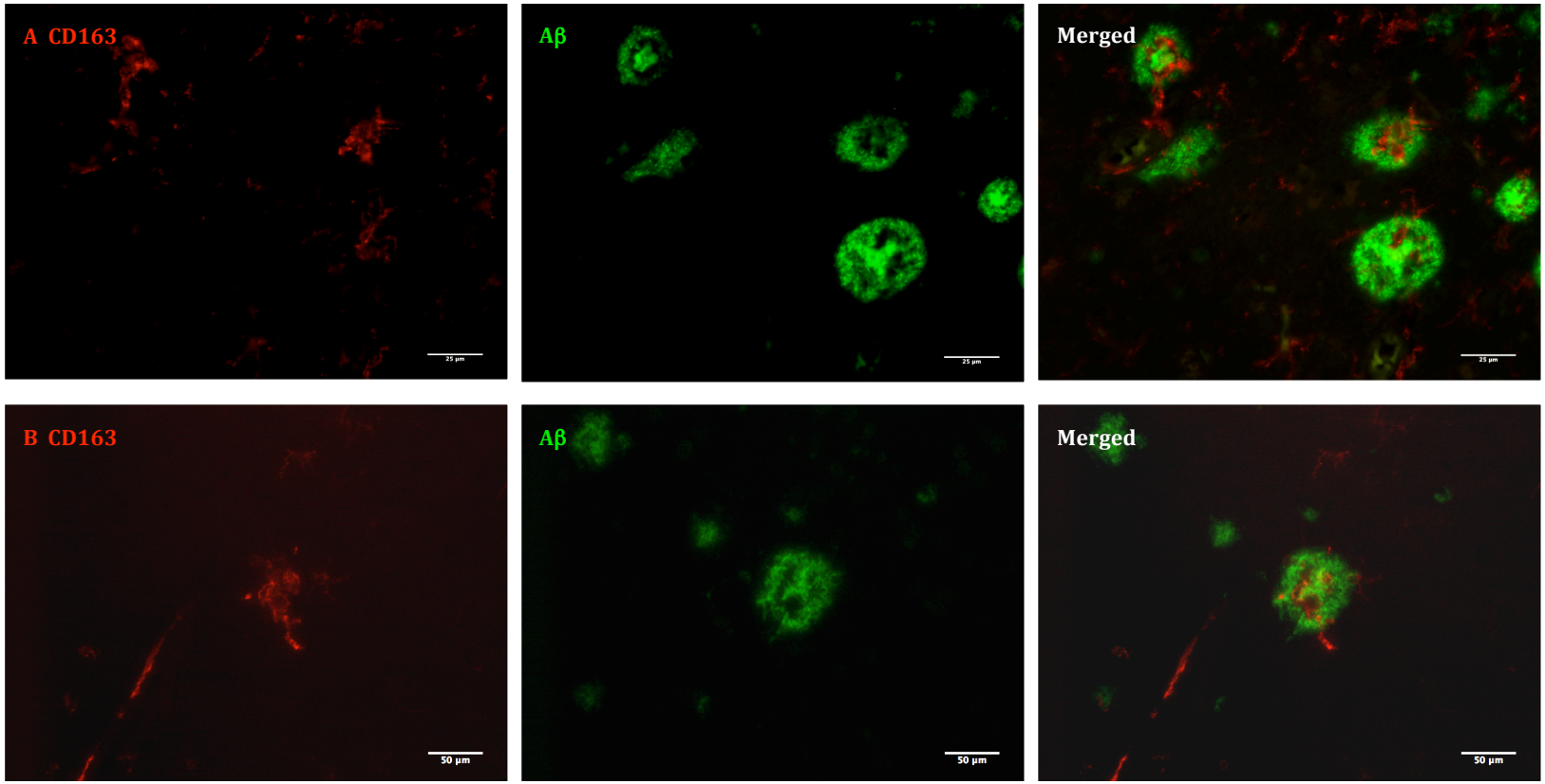
Expression levels of TNFα, IL-10 and BDNF detected using ELISA did not demonstrate statistically significant results with both the expression levels of CD163 as measured by Western blotting, and CD163 %area assessment on DAB-stained sections. However, a correlation between TNFα and IL-10 expression levels was observed ($\rho = 0.676$, $p<0.005$). (Figure 34)

5.4.8 Comparison between different methodologies of assessing CD163 immunoreactivity

Assessments of microglia immunoreactivity were carried out in the cingulate and entorhinal cortex of LBD cases. Using Pearson correlation, measurement of CD163 microglial immunoreactivity using microglia load (using scale 0-3) and %area yielded significant positive correlation in both the cingulate cortex ($r=0.779$, $p<0.001$) and the entorhinal cortex ($r=0.831$, $p<0.001$).

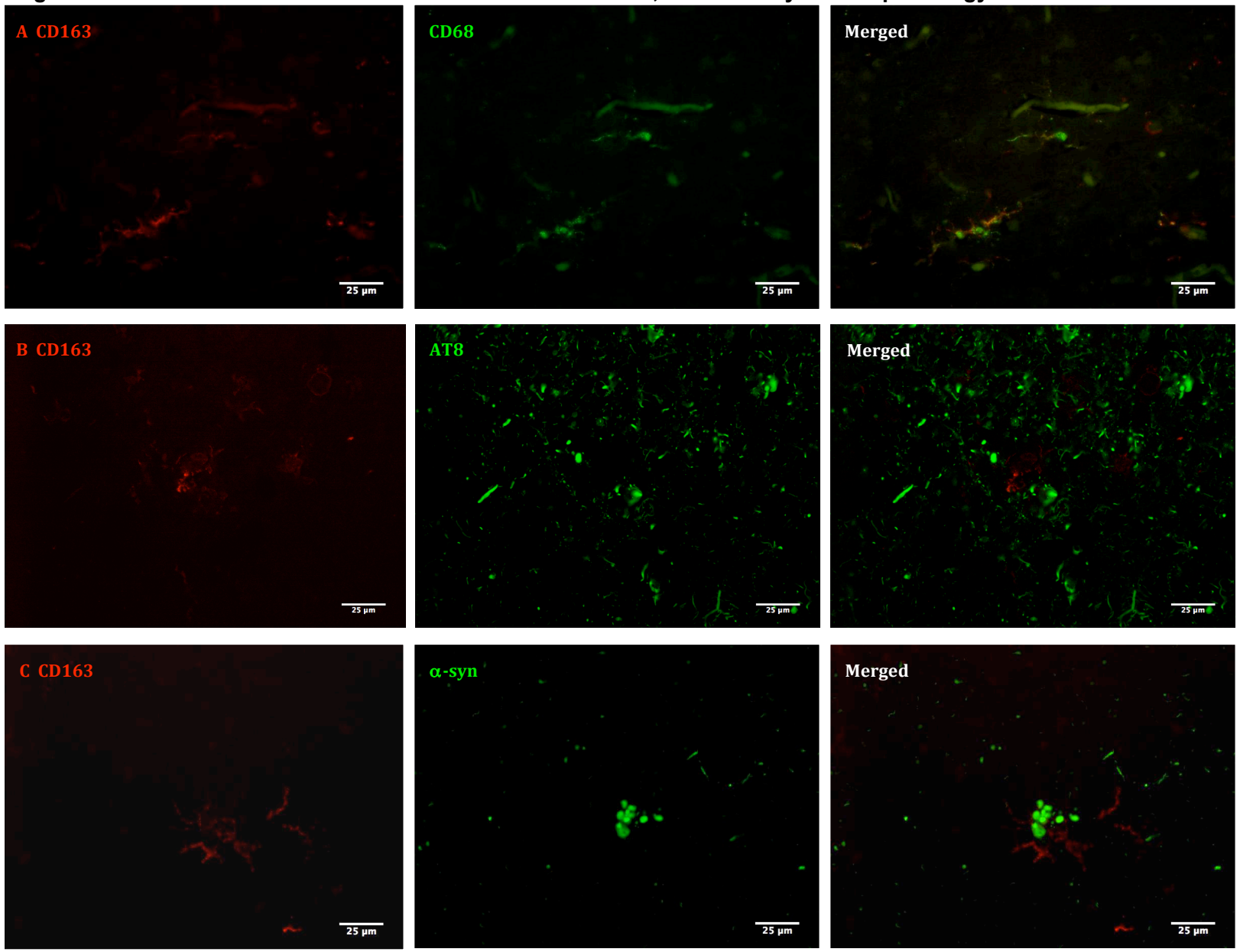
No statistically significant relationship was found between expression of CD163 (Western blot, refer to figure 35) and CD163 %area using Pearson correlation. Instead using Spearman correlation produced a statistically significant positive relationship ($\rho = 0.471$, $p<0.05$).

Figure 32: Double immunofluorescence of CD163 with Aβ pathology



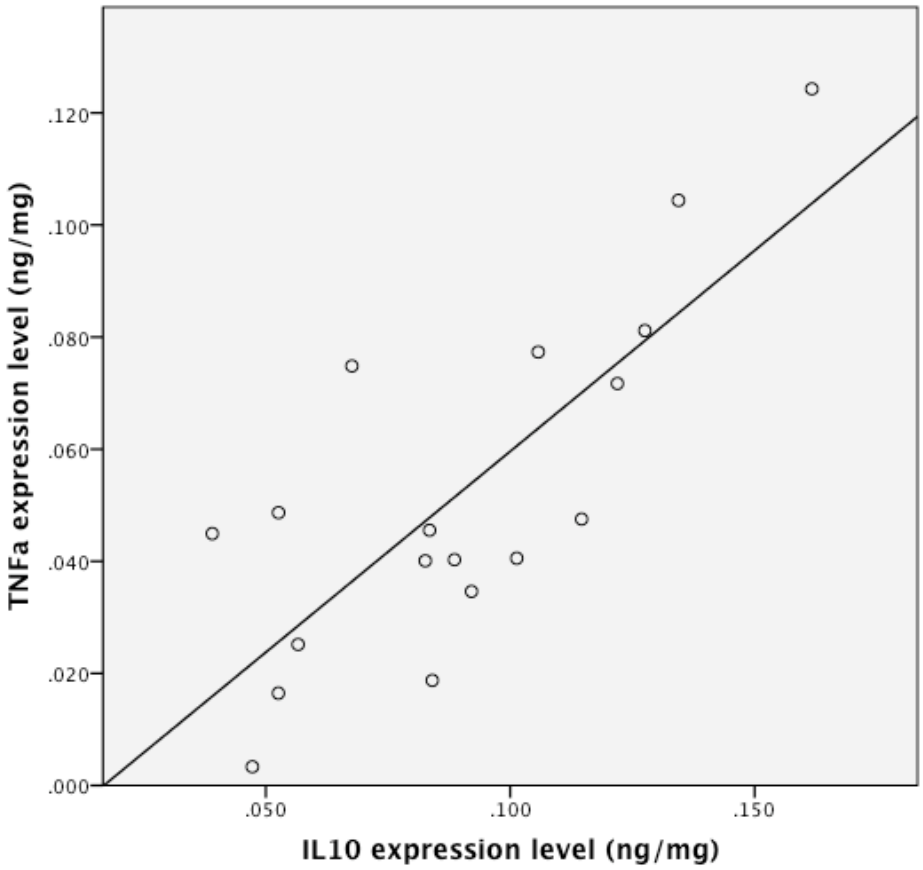
DIF for CD163 (red) and Aβ (green) in the **(A)** occipital cortex of an AD case, and the **(B)** frontal cortex of a PD case. CD163+ microglia were observed to cluster around neuritic plaques. Magnification at x40 and x20 respectively

Figure 33: Double immunofluorescence of CD163 with CD68, tau and α-synuclein pathology



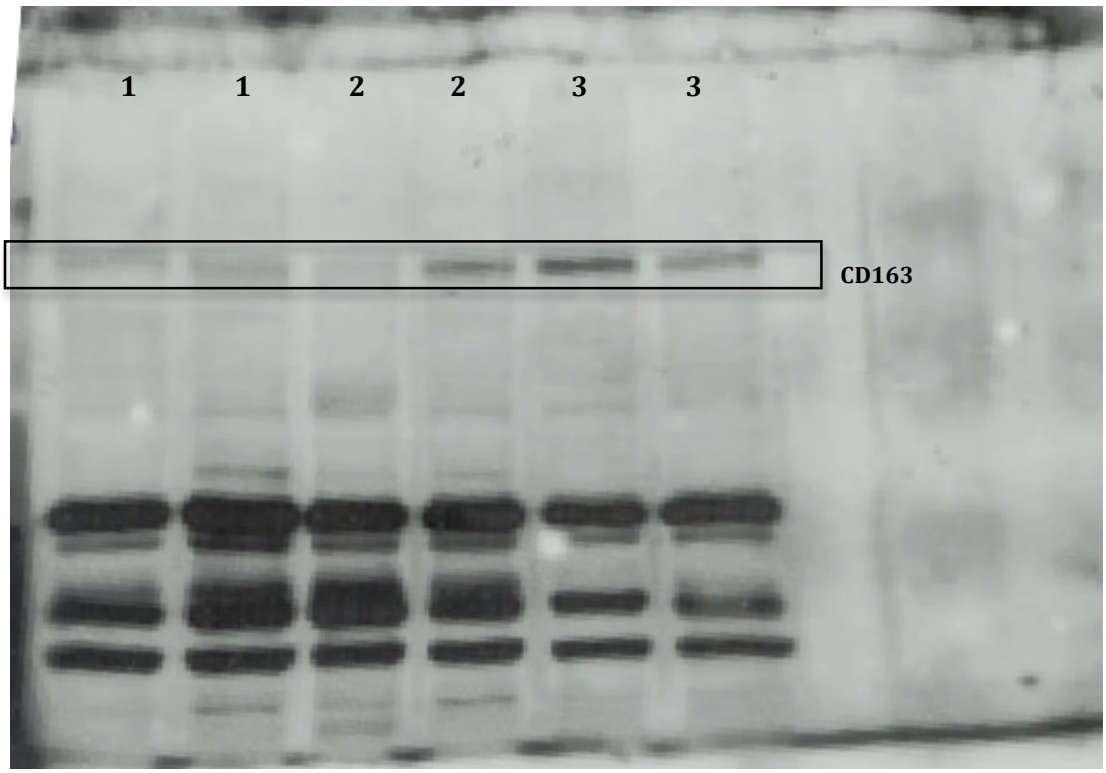
(A) DIF for CD163 (red) and CD68 (green) in the frontal cortex of an AD case. CD68 is a marker for lysosomes; co-localisation indicates phagocytic properties of CD163+ microglia, confirming its role as a scavenger receptor.
(B) DIF for CD163 (red) and AT8 (green) in the hippocampus of an AD case. CD163+ microglia are in close proximity with neuropil thread but with no specific associations.
(C) DIF for CD163 (red) and α-syn (green) in the frontal cortex of a PD case. CD163+ microglia cluster around extracellular LBs. Most of the LB were intracellular and did not coincide with CD163+ microglia. It is predictable that these microglia would react with abnormal aggregates of protein found extracellularly, not intracellularly. Magnification x40

Figure 34: Scatter plot of expression levels of IL-10 against TNFα in LBD cases



Expression levels of TNFα and IL-10 were measured in the frontal and cingulate cortices of LBD patients (n=10) using ELISA. Significant positive correlation between their expression levels was observed.

Figure 35: CD163 quantification using Western blot



A representative western blot of 6 different PD cases chosen based on their CD163 microglial load (1, 2, 3). Two samples were used for each microglial load, totaling 6 samples per blot.

5.4.9 Co-localisation of CD163 and CD14 immunoreactivities within the same microglial cells

CD163 and CD14 both represent phagocytic functions of macrophages/microglia, and DIF with both markers found that they co-localised within the same microglial cells. (Figure 36a,b) However, microglia immunoreactive for only one marker are also seen. CD14+ only microglia tend to take on a more ramified morphology, while CD163+ only microglia tend to be more amoeboid. PVM were immunopositive for both CD163 and CD14. (Figure 36c)

5.4.10 CD163 immunoreactivity in the parenchyma of a haemorrhage

Staining against CD163 labeled both microglia and macrophages in TBI. At the sites of lesion, intense amounts of peripheral macrophages immunoreactive for CD163 were observed. (Figure 37a, b) A gradient of morphological change was observed away from the lesion. Macrophages were usually found in close proximity to lesions, representing an influx from the periphery. At peri-contusional sites, ramified microglia increased in numbers while amoeboid macrophages/microglia decreased. (Figure 37c, d)

5.4.11 CD163+ microglia in non-lesional sites of TBI

Away from lesions and haemorrhages in the parenchyma, CD163+ microglia and macrophages were also observed in the parenchyma. (Figure 38a, b) The morphology of these microglia ranged from ramified to amoeboid, and they were upregulated in varying degrees ranging from mild to intense immunoreactivity.

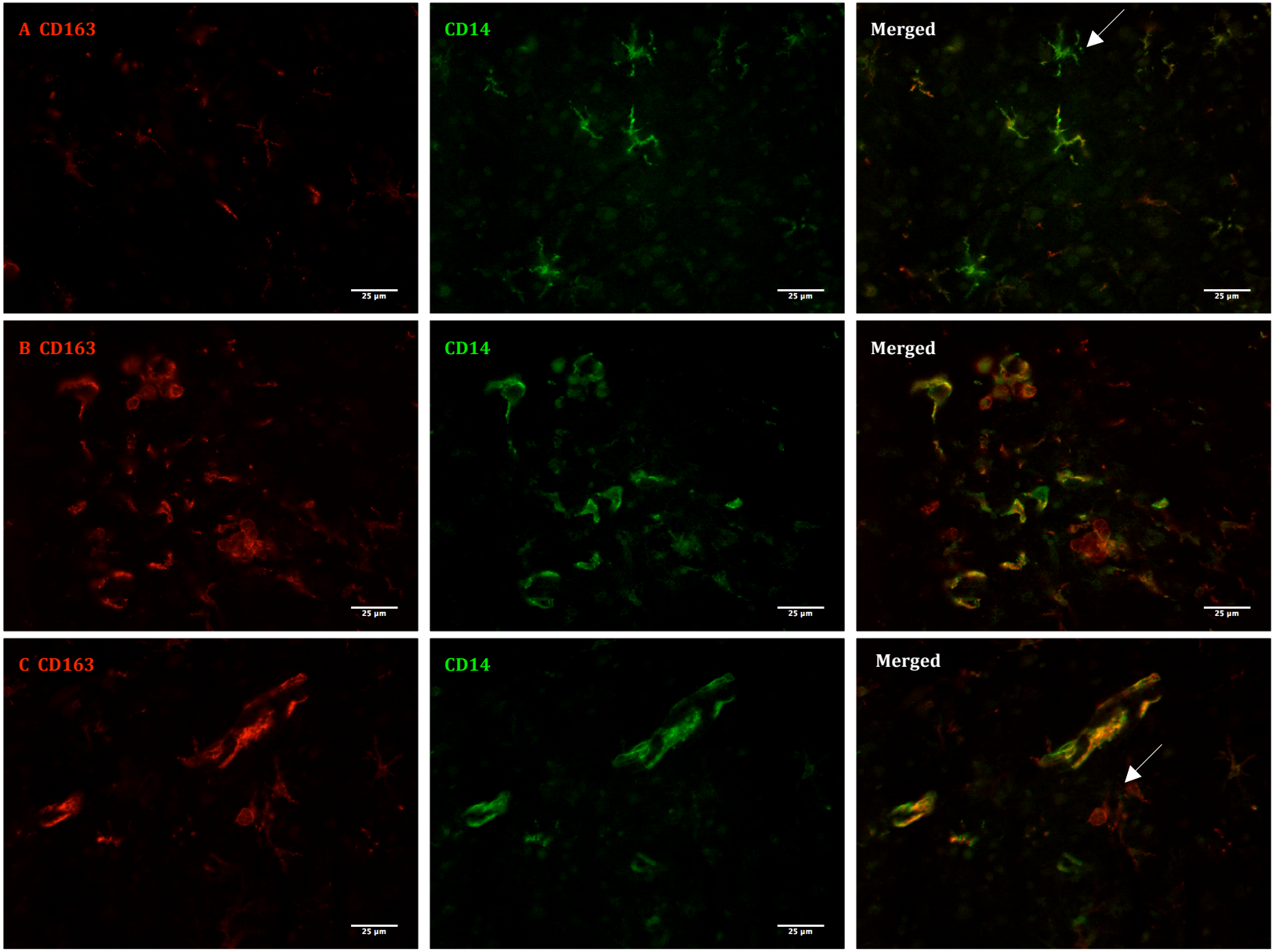
PVM strongly immunoreactive for CD163 were observed in vessels of all sizes. (Figure 38c) CD163+ microglia were also found close to meninges, where subpial macrophages exhibited intense upregulation of CD163. (Figure 38d)

5.4.12 Presence of β-amyloid protein in TBI cases relates to increasing age at death

There was a statistically significant positive correlation between the increasing age at death and the presence of Aβ plaques. ($U= 204.0$, $p< 0.05$) (Figure 39) This finding is not unexpected as Aβ plaques are observed in subjects of advanced age (Arsene and Ardeleanu 2010), however this was also observed in young TBI patients in our cohort (Gentleman et al. 1997), with the youngest one at age 16.

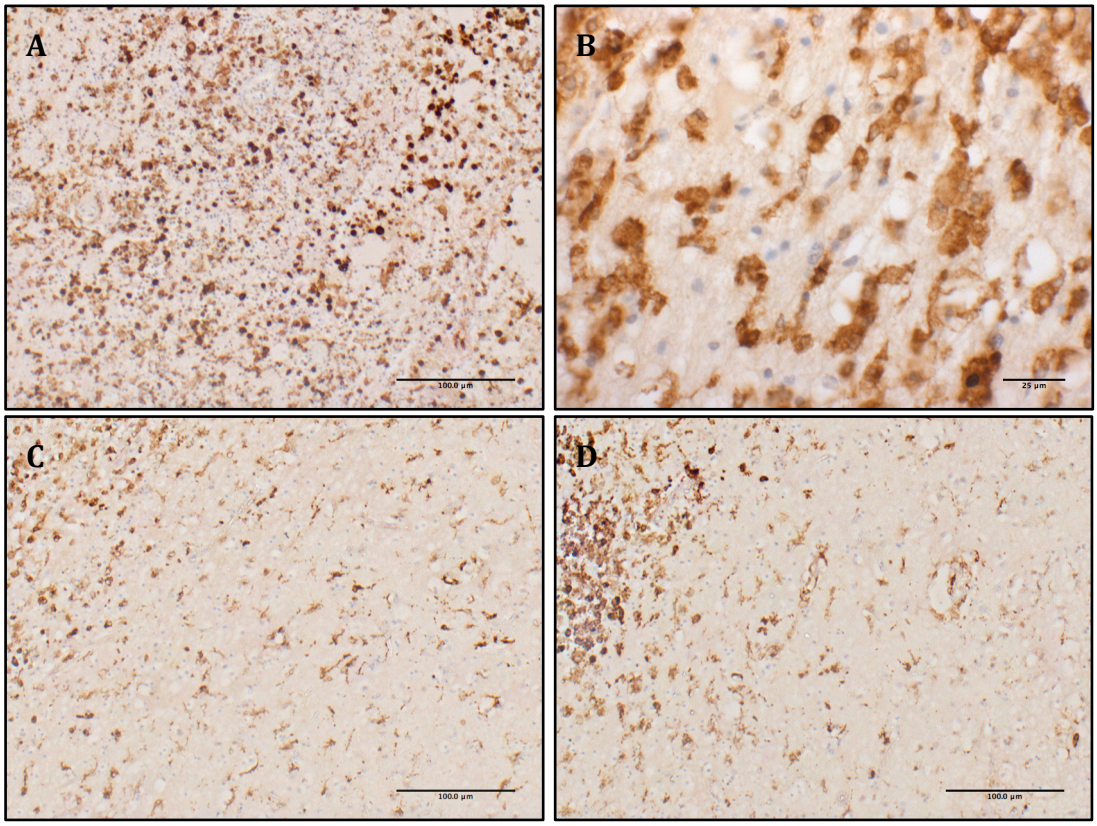
No correlation was found between CD163+ microglia load and presence of Aβ plaques.

Figure 36: Double immunofluorescence of CD163 and CD14



DIF against CD163 (red) and CD14 (green) show co-localisation in the same cells. Ramified and amoeboid microglia were immunopositive for CD163 and CD14. Occasional microglia were seen immunopositive for only one marker (arrows). In **(C)**, PVM were immunoreactive for both markers. All images were taken in the occipital cortex of an AD case. Magnification x40

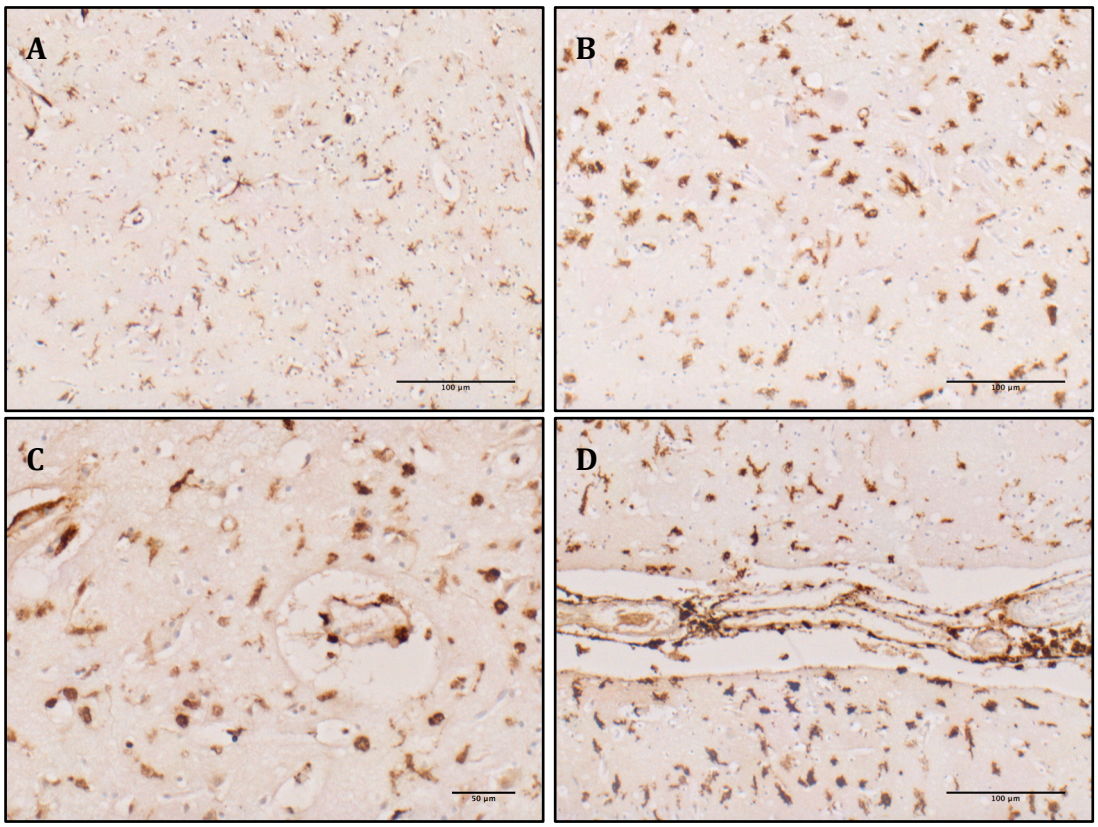
Figure 37: CD163 immunoreactivity in a haemorrhage



(A & B) Amoeboid macrophages immunoreactive for CD163 in a haemorrhagic lesion in the frontal cortex of a TBI case. Magnification x10 and x40 respectively

(C & D) Peri-contusional areas displaying the perimeter of the macrophage influx into the brain parenchyma in the frontal cortex of a TBI case. As distance increases from the lesion, a change in morphology (from amoeboid to ramified) and/or cell type (macrophage to microglia) is evident. Magnification x40

Figure 38: CD163 immunoreactivity in non-lesional areas of TBI

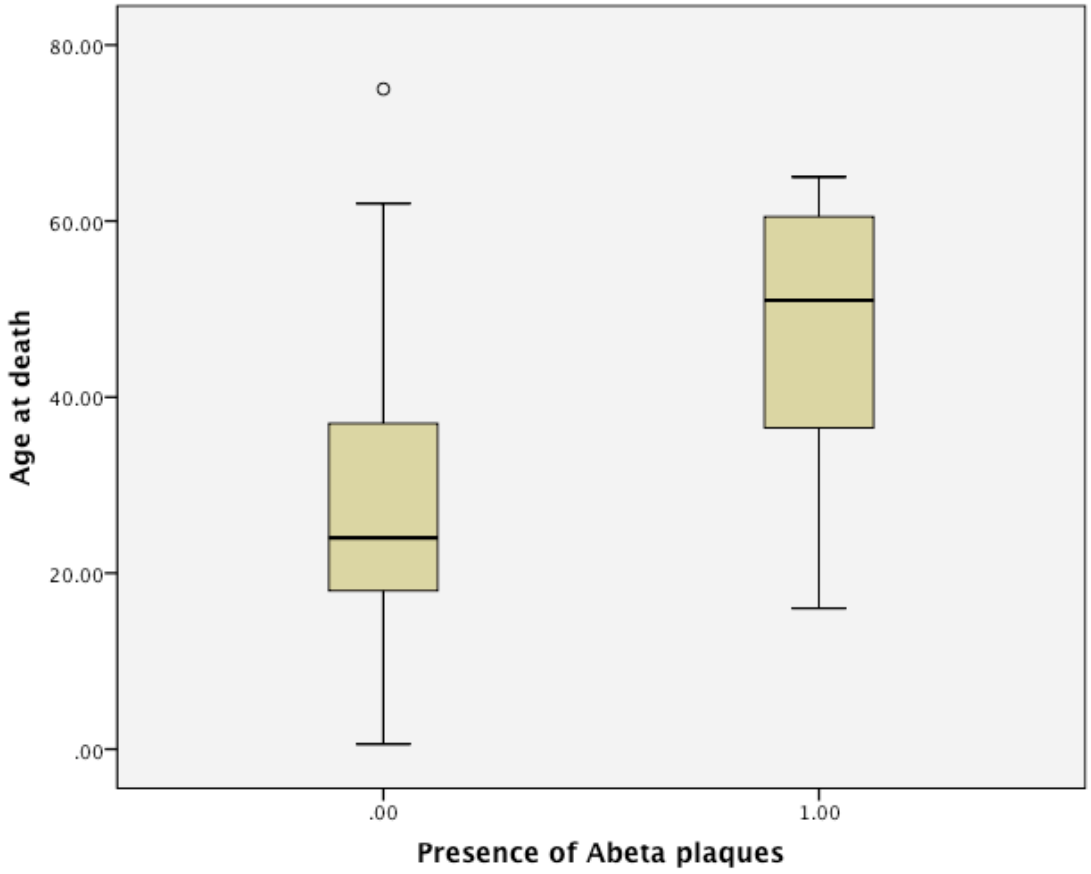


(A & B) CD163+ microglia were observed even in regions of the brain cortex spared from lesions and haemorrhages. These microglia appeared in a range of morphologies, from semi-ramified **(A)** to amoeboid **(B)**. Magnification x10

(C) PVM in the vessels of non-lesional areas were rounded and stained strongly for CD163. This is usually accompanied by CD163+ microglia and macrophages in the vicinity of the vessels, as seen in the cingulate cortex of a TBI case. Magnification x20

(D) Sub-pial and sub-arachnoid macrophages displayed strong immunostaining for CD163. Microglia/ macrophages were also observed close to the meninges, seen in the cortex of a TBI case. Magnification x10

Figure 39: Boxplot showing the association between the presence of Aβ plaques and age at death of TBI patients



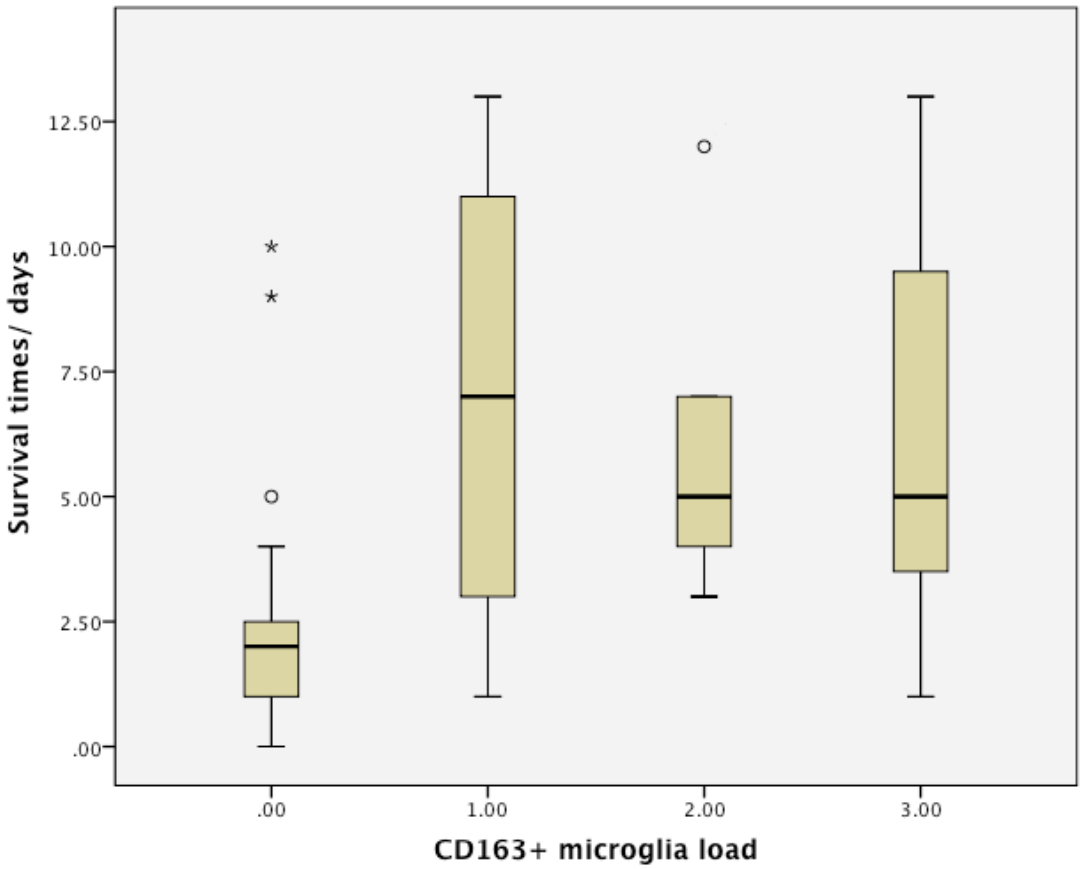
Presence of Aβ plaques was compared against the age at death of TBI cases (n=41), using a single brain region from each case. The cases with Aβ plaques had a later age at death compared to those without Aβ plaques.

5.4.13 Survival times and age at death correlate with CD163+ microglia load in TBI cases

CD163+ microglia load correlated positively with age at death (H= 8.218, p<0.05) and survival times (H= 13.817, p<0.005) of TBI patients. When controlled for survival time, the relationship between age at death and CD163+ microglia load lost its significance. However, when controlled for age at death, survival time continue to show significant correlation with CD163+ microglia load (p<0.007).

Jonckheere's test revealed a significant trend (J= 424.00, p<0.005) with regards to survival time only: CD163+ microglia load increases along with survival times. (Figure 40)

Figure 40: Boxplot showing the association between survival time and CD163+ microglia load in the cortices of TBI patients



The survival times was compared against CD163+ microglia load in TBI cases (n=41). CD163+ microglia load was found to increase with survival times.

5.5 Discussion

CD163 is generally considered to be a specific PVM marker (Fabriek et al. 2005, Borda et al. 2008) and indeed in this study its expression was restricted to PVM in the majority of control cases. However we found that in AD and PD, in addition to a more intense staining of PVM, it was also expressed by microglia in the parenchyma. Foamy macrophages and microglia in the brain parenchyma have previously been found to express CD163 in HIVE (Roberts et al. 2004), MS, and head injury tissue (Gentleman et al. 2004). In concordance with general consensus, these are inflammatory disorders, and the observation can be attributed to breakdown of the BBB and infiltration of peripheral monocytes (Fabriek et al. 2005, Kim et al. 2006, Borda et al. 2008). Therefore the reason for significant increases of CD163+ parenchymal microglia in AD could be an immune response to the pathology specific to AD (D'Andrea, Cole and Ard 2004, El Khoury and Luster 2008). We also observed CD163+ microglia in PD, although the number of microglia stained and intensity of staining did not reach the levels seen in AD. CD163+ microglia were most prominent in the brainstem of PD cases, coinciding with the regions affected in the earlier stages of the disease. While some of the CD163+ microglia coincided with plaques both in AD and PD, focal aggregations were also seen in the absence of plaques. Such an observation might suggest that CD163 can react to both neuronal debris and extracellular abnormal aggregates of protein, including Aβ plaques and occasional Lewy bodies.

Much interest has been shown in Aβ and its removal from the brain via natural methods or vaccination (Holmes et al. 2008). Microglia are capable of phagocytosis through immunization with $A\beta_{42}$ peptides in the Elan clinical trials. In these patients, microglia were found to increase their expression of CD68 and Aβ was found within lysosomes (Zotova et al. 2011). In this study, the observation that CD163+ microglia cluster around Aβ plaques supports the idea that they might serve a similar purpose. Co-localisation with CD68 indicates the possibility for a phagocytic function, that phagocytosis of Aβ takes place naturally in AD, and Aβ is a stimulant for microglia. As seen in our study, CD163+ microglia did not react with NFT or intracellular LB, only extracellular LB- further supporting a role in phagocytosis. In spite of this, most of them remain in a ramified state, albeit with shortened and thickened processes, which raises doubt with regards to their phagocytic function. One possibility might be that they are influenced by the chronic state of the disease and the persistent generation of Aβ plaques (in AD) (Hickman, Allison and El Khoury 2008).

Staining with MRC1 remains restricted to the perivascular spaces in AD and PD. MRC1 is a mannose receptor involved in adhesion, pathogen recognition and clearance, and a marker for PVM (Galea et al. 2005). This is similar to other findings that no other

MRC1 expression was found in the parenchyma, despite acknowledged BBB damage e.g. in MS; and also in excitotoxic damage, acute inflammation and other chronic neurodegeneration models (Fabriek et al. 2005, Galea et al. 2005). It demonstrates that although they might serve similar phagocytic functions, CD163 and MRC1 expression and consequently their targets might be different e.g. MRC1 is involved in the recognition and endocytosis of foreign pathogens (Musiani and Battelli 2003, Galea et al. 2005).

There has been doubt as to whether the BBB is intact in AD (Eikelenboom et al. 2006, El Khoury and Luster 2008). In this study most of the microglia were found near the meninges, which might indicate infiltration of PVM from the periphery. Furthermore, some of these microglia were found to coincide with fibrinogen leakage around vessel walls, an indication that there is a breakdown in BBB. Migration of CD163+ macrophages from the periphery into the parenchyma might stem from vasculature compromise. This theory corresponds with the observation that most of the CD163 microglia were not positive for Iba1. Iba1 is a calcium binding protein expressed in macrophages/microglia (Ito et al. 2001, Kanazawa et al. 2002), which labels all microglia and only certain types of PVM (Ahmed et al. 2007, Xu et al. 2007, Hawkes and McLaurin 2009, Mendes-Jorge et al. 2009). Signaling or infiltration through the BBB could strengthen the idea that systemic inflammation plays a part in the pathology of AD and PD (Perry 2004, Whitton 2007, Tansey and Goldberg 2010), and might explain the variations in CD163+ microglia load within each disease. Presence of microglia double positive for Iba1 and CD163 can be explained by the capability of resident parenchymal microglia to upregulate CD163, induced by signaling from the periphery. Vascular risk factors that are associated with an increased chance of developing AD might result in either an infiltration of peripheral macrophages into the brain, or increased signaling through the BBB to cause an abnormality in protein expression and upregulation.

With regards to histological techniques, there has been no marker singularly capable of distinguishing between blood derived macrophages and resident brain microglia. If these CD163+ microglia are indeed derived from the blood, it would be interesting to further confirm their origin using markers specific for systemic macrophages. Animal models can also be used to track CD163+ peripheral macrophages and their probable entry into the brain parenchyma with or without a breach of the BBB. More experiments can be done to delve deeper into the presence and function of CD163 in AD and PD, and the subset of microglia/macrophages that express CD163 in these diseases.

In TBI cases, macrophages that constitutively express CD163 were observed in parenchymal hemorrhagic lesions. This is especially relevant to the function of CD163,

which serves as a Hb scavenger. During hemolysis, Hb is freed, which can cause toxicity via oxidative damage through the production of ROS and oxidization of low-density lipoproteins, as well as inducing pro-inflammatory reactions in endothelial cells (Schaer et al. 2007, Weaver et al. 2007). The expression level of CD163 on human macrophages is directly proportionate to their uptake of Hp-Hb complexes (Schaer et al. 2002). On microglia, CD163 expression in microglia is very low under normal culture conditions, and only detectable by immunoprecipitation/ Western blotting. However treatment with Hp-Hb complexes causes upregulation of CD163 to levels detectable by IHC (Borda et al. 2008).

At peri-contusional sites, both amoeboid macrophages and activated microglia (albeit with ramifications) were observed. As distance increased away from lesions, the number of macrophages decreased and activated microglia increased. These findings present a possibility that peripheral macrophages are able to change their phenotype and structure to resemble microglia. Similar to AD, brain-resident microglia in TBI might also have upregulated CD163. The ability to upregulate a marker originally exclusive to PVM via signaling from peripheral macrophages might explain the spread of CD163 microglia immunoreactivity to brain regions remote from lesions. CD163 expressed by microglia/macrophages remote from haemorrhages most likely serve purposes other than the scavenging of hemoglobin. A confirmation of CD163+ microglia's phagocytic function can be carried out via double immunofluorescence with CD68.

A positive correlation was found between CD163+ microglia load and survival timing of TBI patients. These microglia were not associated with lesions and were found throughout the uninjured parenchyma of the neocortex. CD163 could potentially be used as a marker specific for labeling activated microglia in TBI cases.

Chapter 6: General discussion

The hypothesis of this project states that microglial phenotype differs in chronic neurodegenerative diseases AD and PD, as well as in acute brain disorder TBI. To carry out this investigation, a range of microglial markers was utilised. Many of these markers were characteristic of a particular microglial activation state i.e. classical or alternative activation, while some were of a more ambiguous nature. They were initially tested in AD cases in order to assess their usability in formalin-fixed, paraffin-embedded sections. Many of these markers did not work in brain tissue despite testing at a range of antibody concentrations and using a variety of pretreatments; either a lack of immunopositivity, or a very high background was observed.

Immunopositivity of CD14 and CD163 in parenchymal microglia proved to be intriguing as both markers were thought to be exclusive to PVM; this project sought to characterize them extensively in AD, PD and TBI. While these two markers are not as reliable at revealing the type of microglial activation state, they give an indication of phagocytic function. Their spatial and temporal distribution, and associations with pathological hallmarks were clearly varied in the disorders, which affirms the hypothesis that microglial phenotype differs between these three disorders.

There was consistently higher CD14 and CD163 immunoreactivity in AD than in PD, which might be attributed to the presence of CAA in AD that cause weakened vessel walls, a subsequent disruption of BBB and influx of peripheral macrophages into the brain parenchyma. Higher amount of extracellular protein aggregates i.e. Aβ plaques, might also serve as a stimulant for upregulation of CD14 and CD163 in AD, as compared to intracellular Lewy Bodies in PD. Similar to AD cases, TBI cases also exhibited increased CD163 immunoreactivity in parenchymal microglia even in brain regions remote from lesions.

Correlation between CD14 and CD163 microglia immunoreactivity and clinical symptoms in LBD cases, co-localisation with extracellular pathology, and upregulation in non-lesional areas of TBI cases indicate that the functions of these microglia are varied and diverse, and extend beyond phagocytosis.

6.1 Evaluation of microglia quantification methods

In chapter 4 and 5, two different methodologies were used to quantify CD14 and CD163 immunoreactivity in parenchymal microglia. CD14+ microglia were rated semi-quantitatively on the entire tissue section based on an impression scale of 0-3, while CD163+ microglia were rated using %area.

Both quantification methods produced consistently reliable results- significant correlation (Pearson r=0.779 and 0.831) was found. This was further confirmed when

the data on CD163 immunoreactivity obtained from both methods were correlated with clinical symptoms. CD163 immunoreactivity in the cingulate and entorhinal cortices was positively associated with anxiety symptoms in LBD patients using both methods.

The reliability of the semi-quantification methods was further ascertained with the usage of western blot. Using Spearman correlation, a statistically significant positive monotonic relationship was found between CD163 %area and protein expression. However, Pearson correlation did not find a significant linear relationship between the quantification methods. This might be due to the presence of PVM and microglia in the white matter, both of which are omitted during assessment on stained sections.

Quantifying microglia has always been a challenge especially when they are distributed in a non-homogenous fashion. Various methods exist to quantify microglia, including assessing %area of staining, manual counting, and measurement of intersections using a grid (Blackbeard et al. 2007). Counting the number of cells per field does not give information about the morphology or level of activation of these microglial cells, while grid method allows for horizontal or vertical intersections with cell processes that indicate morphological changes. Most studies have utilized %area as a quantification method for microglia protein load (Reinacher et al. 1999, Ryu and McLarnon 2009, Simpson et al. 2007, Zotova et al. 2011). Semi-quantitative assessment of microglia load based on a scale of mild, moderate, and high frequency (microglia load) correlates well with %area measurements and can therefore be used in its place. This finding is in agreement with that of Blackbeard et al 2007. Microglia load assessment provides a less time-consuming method for rating microglial immunoreactivity, and is more practical when a large number of cases have to be analysed.

6.2 Functions and inflammatory states of CD163 and CD14 immunoreactive microglia

In relation to aims 1 and 2, microglial markers including MHCII, Iba1, MRC1, IL-1α, CD68, Arg1 and 2, CD36, CD14, CD163, TNFα, BDNF and IL-10 were investigated in human brain tissue. Some markers did not work in paraffin-embedded tissue. Among the markers that worked successfully, immunostaining against CD14 and CD163 revealed interesting and novel upregulation in microglia of AD and LBD. Detailed analysis of the immunoreactivity of CD14 and CD163 in parenchymal microglia and their distribution was carried out to shed light on the alternative activation profile of microglia in two of the most common neurodegenerative diseases- AD and LBD, as well as in an acute disorder- TBI, which could possibly shed light on microglial phenotype during the early stages of disease development (AD or CTE).

Microglia are thought to be classically activated in both AD and LBD. Many studies utilising human postmortem tissue, transgenic animals and *in vitro* cell cultures point to Aβ plaques as the main culprit in inciting pro-inflammatory reactions (Lue et al. 2001, Lau et al. 2004, Fan et al. 2007, El Khoury and Luster 2008, Fabriek et al. 2009). Pro-inflammatory conditions are prolific in LBD too, with the spotlight on destruction of DA neurons in the SN and striatum, although widespread microglia activation is observed throughout the brain (Croisier et al. 2005, Ouchi et al. 2009, Qian and Flood 2008). Recently, the alternative activation state of microglia in AD has garnered attention. Colton et al. demonstrated the presence of mRNAs of alternative activation genes in postmortem specimens and cell cultures and transgenic mice models, although Walker et al. 2009 did not detect the mRNA of IL-4 and IL-13 in most of the brain samples examined. Phagocytosis is a function most fundamental to microglial alternative activation and/or acquired deactivation states.

CD14 and CD163 function as receptors that mediate phagocytosis- CD14 recognises microbial pathogens (Ulevitch and Tobias 1999), apoptotic cells (Schlegel et al. 1999), and fibrillar Aβ (Fassbender et al. 2004, Liu et al. 2005), while CD163 functions as a scavenger receptor for hemoglobin (Kristiansen et al. 2001), and also recognises and binds bacteria (Fabriek et al. 2009). CD14 is essential for the binding and clearance of Aβ plaques (Liu et al. 2005), and although phagocytosis is generally associated with an alternative activation state, the uptake of fibrillar Aβ via CD14 in culture conditions leads to the release of free radicals and ROS (Reed-Geaghan et al. 2009). Unexpectedly, CD14 knockout animal models show a reduced plaque burden (Reed-Geaghan et al. 2010). Inactivating mutations in TLR4, which forms a complex with CD14, also results in a decreased risk for AD (Minoretti et al. 2006). As CD14 mediates a pro-inflammatory reaction towards bacterial pathogens (Kielian 2006), sustained inflammation throughout life e.g. repeated infections resulting in peripheral or systemic inflammatory responses, predisposes both humans and animals towards neuronal dysfunction and the development of AD or AD-like symptoms (Sheng et al. 2003). CD14 knockout animals are spared from repeated bouts of inflammation, resulting in a neuroprotective outcome.

The specific molecular interactions between CD163 and Aβ or α-syn, and subsequent downstream signaling pathways have yet to be investigated. Whether the stimulation of CD163 results in a pro-inflammatory or anti-inflammatory reaction is also a matter for debate. CD163 levels are increased in wound healing and in late phases of acute and chronic inflammation e.g. experimental gingivitis (Topoll et al. 1989), indicating its role in the resolution of inflammation (Zwadlo et al. 1987, Zwadlo-Klarwasser et al. 1990, Sulahian et al. 2000, Roberts et al. 2004). It may also exert protective functions through

the removal of toxic Hb, and the subsequent stimulation of IL-10 release (Schaer et al. 2002, Zhang et al. 2012). CD163 has also been shown to bind and scavenge TWEAK (TNF-like weak inducer of apoptosis), which has been found to induce apoptosis and compromise the neurovascular unit (Bover et al. 2007, Zhang et al. 2007b). However when CD163 is activated/ cross-linked by Hp-Hb complexes or antibodies, it can also stimulate the production of inflammatory cytokines e.g. IL-1*β*, IL-6, GM-CSF and IL-10 (Van den Heuvel et al. 1999, Kristiansen et al. 2001, Ritter et al. 2001).

With respect to the 3rd aim, double immunofluorescence was carried out with to detect associations between CD14+ and CD163+ microglia with pathologies of AD and PD i.e. Aβ plaques, LB, NFT and NT pathology. Co-localisation of Aβ plaques and α-syn/ extracellular Lewy bodies with CD14 and CD163+ microglia might be due to the phagocytic nature of these microglia. Many of these microglia had a semi-ramified morphology- less ramified than a resting microglia with hypertrophied soma. The morphology of microglia provides less than adequate information about its activation state and function (Boche, Perry and Nicoll 2013). If these microglia were indeed phagocytosing plaques/ α-syn, why have they not taken on a more amoeboid morphology?

One possible reason for these microglia to appear semi-ramified is due to the resistance of fibrillar Aβ towards degradation. It is known that microglia are extremely inefficient at phagocytosing fibrillar Aβ and breaking them down (Koenigsknecht-Talboo and Landreth 2005, Kawahara et al. 2012). Diffuse Aβ plaques are more susceptible to degradation (Wilcock et al. 2003), which could justify the observation that a greater number of CD14+ microglia were co-localised with diffuse plaques instead of neuritic plaques.

As AD progresses through its stages, it is likely that microglia which have been chronically activated gradually lose their phagocytic ability. This is observed in APP transgenic mice models where microglia continue to produce pro-inflammatory cytokines but lose their Aβ-clearing ability. An overall downregulation in the expression of scavenger receptors e.g. scavenger receptor A and CD36 are observed in these mice (Hickman et al. 2008). Blood-derived monocytes and macrophages isolated from AD patients are unable to degrade Aβ and are susceptible to apoptosis (Fiala et al. 2005). Following the hypothesis that microglia in diseased patients are defective in their phagocytic capabilities, a change in morphology may follow suit.

Although CD163 is upregulated by IL-10 and downregulated by TNFα, no correlation was found between CD163 expression and cytokine expression. It is hypothesized that the aged brain has an impaired response towards IL-10; microglia from aged mice do not express high levels of IL-10 receptor (Fenn et al. 2012, Norden and Godbout 2013).

Failure of microglia to respond properly to IL-10 might explain the patchy distribution and weak upregulation of CD163 instead of a strong immunoreactivity and homogenous distribution as seen with other microglial activation markers e.g. MHCII.

6.3 Heterogeneity of microglial phenotype

The diversity of microglial phenotype is demonstrated with double staining against CD163 and CD14. Although both markers represent a phagocytic function, some microglia were immunopositive for both, while others were singly immunopositive for CD163 or CD14. The variation in their marker profile is probably not due to different stimuli as these microglia were observed in close proximity, but due to their innate differences e.g. the existence of subsets of microglia (Colton et al. 2006, Colton and Wilcock 2010), pre-conditioning or priming of microglia (Norden and Godbout 2013), or their sources- peripheral derived vs brain resident (Simard et al. 2006, Soulet and Rivest 2008, Gate et al. 2010). The functions and inflammatory cytokine profiles of these microglia are not known.

6.4 Origin of CD14 and CD163 immunoreactive microglia

CD14+ and CD163+ microglia, but especially CD163+ microglia, were found in higher densities around the meninges, and occasionally, compromised blood vessels. As discussed in the previous result chapters, the population of CD163+ microglia in AD and LBD are probably made up of resident microglia and peripheral macrophages. It is most likely the same for CD14+ microglia.

Work done on TBI cases gives additional insight into the origins of CD163+ microglia. The gradation of microglia morphology from lesions to peri-lesional areas, and the presence of CD163+ microglia in areas remote from the lesions, both point to the ability of brain-resident microglia to upregulate CD163. This upregulation might be stimulated via signaling from the periphery aggravated by a compromise in the BBB. Activated microglia are found in long-term survivors of TBI, and are thought to contribute to chronic neurodegeneration and development of AD (Fleminger et al. 2003) and CTE (McKee et al. 2009). Phagocytic microglia/macrophages have been found in the corpus callosum of TBI cases up to 18 years after injury (Johnson et al. 2013). The association between survival times and CD163+ microglia load provides evidence for the chronic inflammatory response that arises after the initial injury, which is thought to predispose long-term TBI survivors to neurodegeneration and subsequent neurological diseases (Smith 2013). While the longest survival period after injury used in this study is 13 days, CD163 upregulation by microglia throughout various brain regions is most likely to persist beyond this period.

6.5 Distribution of CD14 and CD163 immunoreactive microglia in AD, LBD and TBI

Aside from the obvious breakdown of the BBB in TBI cases, compromise of the BBB has also been shown in AD and a rat model of LBD, which was correlated with neuronal death (Rite et al. 2007, Popescu et al. 2009). This coincides with our findings that the highest amount of CD14+ and CD163+ microglia were found in brain regions most affected in AD and LBD, including the frontal and occipital lobes in AD, and SN, DMV, and striatum in LBD. The breakdown of BBB in these brain regions might explain the upregulation of CD14 and CD163 that are usually restricted to perivascular macrophages. Our observation of CD14 and CD163 positivity in the parenchyma gives an example of how systemic inflammation could affect the brain and aggravate disease progression (Holmes et al. 2008, Perry 2004).

6.6 CD163 immunoreactive microglia associated with anxiety symptoms in LBD patients

With regards to the 4th aim, upregulation of CD163+ microglia in the cingulate cortex, entorhinal cortex and LC of LBD cases were found to associate with an absence of anxiety symptoms. DA receptor polymorphisms have been linked to social anxiety disorder and obsessive-compulsive personality disorder, both of which are anxiety disorders (Light et al. 2006, Sareen et al. 2007). High frequency of anxiety symptoms in the premotor phase of LBD can be explained by the loss of noradrenergic neurons in the LC that precedes degeneration of DA neurons in the SN (Braak et al. 2006). This coincides with the significant association we found between anxiety symptoms and the presence of CD163+ microglia in the LC. In patients who are clinically anxious, or those diagnosed with social anxiety disorder, both the anterior cingulate cortex and the entorhinal cortex are affected (Hattingh et al. 2012, Klumpp et al. 2013), confirming the involvement of neurotransmitter systems in these brain regions with fear and anxiety regulation.

A study into the cytokine profile of CD163+ microglia and their interactions with common pathological protein aggregates e.g. tau, Aβ or α-syn in these brain regions might help to shed light on the cause of neuronal dysfunction resulting in anxiety. This finding is significant as it directly links inflammation to the clinical aspects of neurological diseases. Upregulation of microglia markers can also be useful in the mapping of clinical symptoms to specific brain regions.

6.7 Future studies

To bring the investigation forward on characterization of microglial alternative activation state in patients afflicted with neurological diseases, it is essential to continue to test for new markers that can help characterize the diverse microglia phenotypes in the brain. As many of these markers target antigens that are too labile or in low quantities, experiments can be carried out with frozen, unfixed tissue using IHC, ELISA or capture protein microarray.

Double staining experiments have been carried out with CD163 antibody were not done with CD14 antibody. A confirmation of the phagocytic capacity of CD14+ microglia in AD, PD and TBI cases can be tested using a double stain against CD14 and CD68. Antibodies highlighting BBB damage e.g. fibrinogen, occludin or von Willebrand factor can also be used in double staining experiments to look into the origins of these microglia.

While double immunofluorescence detects association between microglial cells and pathology, it does not provide information regarding the type of interaction. This problem can be solved with confocal microscopy, which can help in the visualization of internalized pathological protein within microglial cells. To confirm interaction between microglial markers and protein pathology, immunoprecipitation followed by Western blotting can be done to assess binding affinities.

No experiments have been done in AD, LBD, or TBI patients investigating the release of pro or anti-inflammatory cytokines via interactions between CD163 and CD14 and pathological protein aggregates (Gentleman 2013). Studying the cytokine expression in paraffin-embedded sections using IHC, or in frozen fresh tissue using Western blot have always proven difficult due to the cytokines' small quantity, low molecular weight and labile nature (Boche et al. 2013). A new technique using immunolaser capture microdissection in conjunction with microarray can be used to target a specific microglia subset from a heterogenous population, thus providing a way for transcriptome profiling (Waller et al. 2012) and a thorough characterization of microglia's specific responses to different types of pathology.

With regards to TBI cases, cases with survival period up to several years should be included in the testing as the cohort used in this study represents only the acute injury phase. A decrease in CD68 and CR3/43 immunoreactivity was found in those who survived more than a year after injury (Smith et al. 2012). Exploring a variety of microglial activation markers can help to define the evolution of microglial phenotype prior to the onset of AD or CTE.

Current PET assessment of microglial activation using [^{11}C](R)-PK11195 lacks accuracy as it measures the activation of both microglia and astrocytes. Our finding

that CD163 is upregulated by microglia in AD, PD and TBI cases could provide an alternative to the usage of mitochondrial translocator protein as a ligand for PET imaging.

References

Aarsland, D., K. Andersen, J. P. Larsen, A. Lolk & P. Kragh-Sorensen (2003) Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch Neurol,* 60**,** 387-92.

Abou-Sleiman, P. M., M. M. Muqit & N. W. Wood (2006) Expanding insights of mitochondrial dysfunction in Parkinson's disease. *Nat Rev Neurosci,* 7**,** 207-19.

Aboud, O., R. E. Mrak, F. Boop & S. T. Griffin (2012) Apolipoprotein epsilon 3 alleles are associated with indicators of neuronal resilience. *BMC Med,* 10**,** 35.

Ahmed, Z., G. Shaw, V. P. Sharma, C. Yang, E. McGowan & D. W. Dickson (2007) Actin-binding proteins coronin-1a and IBA-1 are effective microglial markers for immunohistochemistry. *J Histochem Cytochem,* 55**,** 687-700.

Aizenstein, H. J., R. D. Nebes, J. A. Saxton, J. C. Price, C. A. Mathis, N. D. Tsopelas, S. K. Ziolko, J. A. James, B. E. Snitz, P. R. Houck, W. Bi, A. D. Cohen, B. J. Lopresti, S. T. DeKosky, E. M. Halligan & W. E. Klunk (2008) Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol,* 65**,** 1509-17.

Ajami, B., J. L. Bennett, C. Krieger, W. Tetzlaff & F. M. Rossi (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci,* 10**,** 1538-43.

Ajmone-Cat, M. A., A. Nicolini & L. Minghetti (2003) Prolonged exposure of microglia to lipopolysaccharide modifies the intracellular signaling pathways and selectively promotes prostaglandin E2 synthesis. *J Neurochem,* 87**,** 1193-203.

Akila, P., V. Prashant, M. N. Suma, S. N. Prashant & T. R. Chaitra (2012) CD163 and its expanding functional repertoire. *Clin Chim Acta,* 413**,** 669-74.

Akiyama, H., T. Arai, H. Kondo, E. Tanno, C. Haga & K. Ikeda (2000) Cell mediators of inflammation in the Alzheimer disease brain. *Alzheimer Dis Assoc Disord,* 14 Suppl 1**,** S47-53.

Alafuzoff, I., T. Arzberger, S. Al-Sarraj, I. Bodi, N. Bogdanovic, H. Braak, O. Bugiani, K. Del-Tredici, I. Ferrer, E. Gelpi, G. Giaccone, M. B. Graeber, P. Ince, W. Kamphorst, A. King, P. Korkolopoulou, G. G. Kovacs, S. Larionov, D. Meyronet, C. Monoranu, P. Parchi, E. Patsouris, W. Roggendorf, D. Seilhean, F. Tagliavini, C. Stadelmann, N. Streichenberger, D. R. Thal, S. B. Wharton & H. Kretzschmar (2008a) Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. *Brain Pathol,* 18**,** 484-96.

Alafuzoff, I., P. G. Ince, T. Arzberger, S. Al-Sarraj, J. Bell, I. Bodi, N. Bogdanovic, O. Bugiani, I. Ferrer, E. Gelpi, S. Gentleman, G. Giaccone, J. W. Ironside, N.

Kavantzas, A. King, P. Korkolopoulou, G. G. Kovacs, D. Meyronet, C. Monoranu, P. Parchi, L. Parkkinen, E. Patsouris, W. Roggendorf, A. Rozemuller, C. Stadelmann-Nessler, N. Streichenberger, D. R. Thal & H. Kretzschmar (2009) Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. *Acta Neuropathol,* 117**,** 635-52.

Alafuzoff, I., M. Pikkarainen, S. Al-Sarraj, T. Arzberger, J. Bell, I. Bodi, N. Bogdanovic, H. Budka, O. Bugiani, I. Ferrer, E. Gelpi, G. Giaccone, M. B. Graeber, J. J. Hauw, W. Kamphorst, A. King, N. Kopp, P. Korkolopoulou, G. G. Kovacs, D. Meyronet, P. Parchi, E. Patsouris, M. Preusser, R. Ravid, W. Roggendorf, D. Seilhean, N. Streichenberger, D. R. Thal & H. Kretzschmar (2006) Interlaboratory comparison of assessments of Alzheimer disease-related lesions: a study of the BrainNet Europe Consortium. *J Neuropathol Exp Neurol,* 65**,** 740-57.

Alafuzoff, I., M. Pikkarainen, T. Arzberger, D. R. Thal, S. Al-Sarraj, J. Bell, I. Bodi, H. Budka, E. Capetillo-Zarate, I. Ferrer, E. Gelpi, S. Gentleman, G. Giaccone, N. Kavantzas, A. King, P. Korkolopoulou, G. G. Kovacs, D. Meyronet, C. Monoranu, P. Parchi, E. Patsouris, W. Roggendorf, C. Stadelmann, N. Streichenberger, F. Tagliavini & H. Kretzschmar (2008b) Inter-laboratory comparison of neuropathological assessments of beta-amyloid protein: a study of the BrainNet Europe consortium. *Acta Neuropathol,* 115**,** 533-46.

Allavena, P., M. Chieppa, P. Monti & L. Piemonti (2004) From pattern recognition receptor to regulator of homeostasis: the double-faced macrophage mannose receptor. *Crit Rev Immunol,* 24**,** 179-92.

Allinson, T. M., E. T. Parkin, A. J. Turner & N. M. Hooper (2003) ADAMs family members as amyloid precursor protein alpha-secretases. *J Neurosci Res,* 74**,** 342-52.

Alonso, A. D. C., Li, B., Grundke-Iqbal, I., Iqbal, K. (2006) Polymerization of hyperphosphorylated tau into filaments eliminates its inhibitory activity. *PNAS,* 103**,** 8864-8869.

Alves, G., J. P. Larsen, M. Emre, T. Wentzel-Larsen & D. Aarsland (2006) Changes in motor subtype and risk for incident dementia in Parkinson's disease. *Mov Disord,* 21**,** 1123-30.

Andorfer, C., C. M. Acker, Y. Kress, P. R. Hof, K. Duff & P. Davies (2005) Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J Neurosci,* 25**,** 5446-54.

Arimon, M., I. Diez-Perez, M. J. Kogan, N. Durany, E. Giralt, F. Sanz & X. Fernandez-Busquets (2005) Fine structure study of Abeta1-42 fibrillogenesis with atomic force microscopy. *FASEB J,* 19**,** 1344-6.

Armstrong, R. A. (2003) Quantifying the pathology of neurodegenerative disorders: quantitative measurements, sampling strategies and data analysis. *Histopathology,* 42**,** 521-9.

Arriagada, P. V., J. H. Growdon, E. T. Hedley-Whyte & B. T. Hyman (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology,* 42**,** 631-9.

Arsene, D. & C. Ardeleanu (2010) Neurodegenerative changes in human aging brain. An autopsy study. *Rom J Morphol Embryol,* 51**,** 55-60.

Asanuma, M., S. Nishibayashi-Asanuma, I. Miyazaki, M. Kohno & N. Ogawa (2001) Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. *J Neurochem,* 76**,** 1895-904.

Attems, J., K. Jellinger, D. R. Thal & W. Van Nostrand (2011) Review: sporadic cerebral amyloid angiopathy. *Neuropathol Appl Neurobiol,* 37**,** 75-93.

Attems, J. & K. A. Jellinger (2004) Only cerebral capillary amyloid angiopathy correlates with Alzheimer pathology--a pilot study. *Acta Neuropathol,* 107**,** 83-90.

Attems, J., M. Quass, K. A. Jellinger & F. Lintner (2007) Topographical distribution of cerebral amyloid angiopathy and its effect on cognitive decline are influenced by Alzheimer disease pathology. *J Neurol Sci,* 257**,** 49-55.

Auluck, P. K., H. Y. Chan, J. Q. Trojanowski, V. M. Lee & N. M. Bonini (2002) Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science,* 295**,** 865-8.

Bachis, A., A. M. Colangelo, S. Vicini, P. P. Doe, M. A. De Bernardi, G. Brooker & I. Mocchetti (2001) Interleukin-10 prevents glutamate-mediated cerebellar granule cell death by blocking caspase-3-like activity. *J Neurosci,* 21**,** 3104-12.

Bamberger, M. E., M. E. Harris, D. R. McDonald, J. Husemann & G. E. Landreth (2003) A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. *J Neurosci,* 23**,** 2665-74.

Barkhoudarian, G., D. A. Hovda & C. C. Giza (2011) The molecular pathophysiology of concussive brain injury. *Clin Sports Med,* 30**,** 33-48, vii-iii.

Barnes, D. E., G. S. Alexopoulos, O. L. Lopez, J. D. Williamson & K. Yaffe (2006) Depressive symptoms, vascular disease, and mild cognitive impairment: findings from the Cardiovascular Health Study. *Arch Gen Psychiatry,* 63**,** 273-9.

Batchelor, P. E., G. T. Liberatore, J. Y. Wong, M. J. Porritt, F. Frerichs, G. A. Donnan & D. W. Howells (1999) Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor. *J Neurosci,* 19**,** 1708-16.

Beach, T. G., C. H. Adler, L. Lue, L. I. Sue, J. Bachalakuri, J. Henry-Watson, J. Sasse, S. Boyer, S. Shirohi, R. Brooks, J. Eschbacher, C. L. White, 3rd, H. Akiyama, J. Caviness, H. A. Shill, D. J. Connor, M. N. Sabbagh, D. G. Walker & C. Arizona Parkinson's Disease (2009) Unified staging system for Lewy body disorders: correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathol,* 117**,** 613-34.

Becher, B., V. Fedorowicz & J. P. Antel (1996) Regulation of CD14 expression on human adult central nervous system-derived microglia. *J Neurosci Res,* 45**,** 375-81.

Berardelli, A., J. C. Rothwell, P. D. Thompson & M. Hallett (2001) Pathophysiology of bradykinesia in Parkinson's disease. *Brain,* 124**,** 2131-46.

Beyer, K. & A. Ariza (2013) Alpha-synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. *Mol Neurobiol,* 47**,** 509-24.

Bianchi, M. E. (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol,* 81**,** 1-5.

Bierer, L. M., P. R. Hof, D. P. Purohit, L. Carlin, J. Schmeidler, K. L. Davis & D. P. Perl (1995) Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease. *Arch Neurol,* 52**,** 81-8.

Biswas, A., A. Bhattacharya, S. Kar & P. K. Das (2011) Expression of IL-10-triggered STAT3-dependent IL-4Ralpha is required for induction of arginase 1 in visceral leishmaniasis. *Eur J Immunol,* 41**,** 992-1003.

Bitan, G., M. D. Kirkitadze, A. Lomakin, S. S. Vollers, G. B. Benedek & D. B. Teplow (2003) Amyloid beta -protein (Abeta) assembly: Abeta 40 and Abeta 42 oligomerize through distinct pathways. *Proc Natl Acad Sci U S A,* 100**,** 330-5.

Blackbeard, J., K. P. O'Dea, V. C. Wallace, A. Segerdahl, T. Pheby, M. Takata, M. J. Field & A. S. Rice (2007) Quantification of the rat spinal microglial response to peripheral nerve injury as revealed by immunohistochemical image analysis and flow cytometry. *J Neurosci Methods,* 164**,** 207-17.

Blennow, K., J. Hardy & H. Zetterberg (2012) The neuropathology and neurobiology of traumatic brain injury. *Neuron,* 76**,** 886-99.

Blochl, A. & C. Sirrenberg (1996) Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and p75Lntr receptors. *J Biol Chem,* 271**,** 21100-7.

Boche, D., V. H. Perry & J. A. Nicoll (2013) Review: activation patterns of microglia and their identification in the human brain. *Neuropathol Appl Neurobiol,* 39**,** 3-18.

Bogen, S. A., K. Vani & S. R. Sompuram (2009) Molecular mechanisms of antigen retrieval: antigen retrieval reverses steric interference caused by formalin-induced cross-links. *Biotech Histochem,* 84**,** 207-15.

Bonnet, A. M., M. F. Jutras, V. Czernecki, J. C. Corvol & M. Vidailhet (2012) Nonmotor symptoms in Parkinson's disease in 2012: relevant clinical aspects. *Parkinsons Dis,* 2012**,** 198316.

Borda, J. T., X. Alvarez, M. Mohan, A. Hasegawa, A. Bernardino, S. Jean, P. Aye & A. A. Lackner (2008) CD163, a marker of perivascular macrophages, is up-regulated by microglia in simian immunodeficiency virus encephalitis after haptoglobin-hemoglobin complex stimulation and is suggestive of breakdown of the blood-brain barrier. *Am J Pathol,* 172**,** 725-37.

Bover, L. C., M. Cardo-Vila, A. Kuniyasu, J. Sun, R. Rangel, M. Takeya, B. B. Aggarwal, W. Arap & R. Pasqualini (2007) A previously unrecognized protein-protein interaction between TWEAK and CD163: potential biological implications. *J Immunol,* 178**,** 8183-94.

Braak, H., I. Alafuzoff, T. Arzberger, H. Kretzschmar & K. Del Tredici (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol,* 112**,** 389-404.

Braak, H., K. Del Tredici, U. Rub, R. A. de Vos, E. N. Jansen Steur & E. Braak (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging,* 24**,** 197-211.

Brecht, W. J., F. M. Harris, S. Chang, I. Tesseur, G. Q. Yu, Q. Xu, J. Dee Fish, T. Wyss-Coray, M. Buttini, L. Mucke, R. W. Mahley & Y. Huang (2004) Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. *J Neurosci,* 24**,** 2527-34.

Britschgi, M., C. E. Olin, H. T. Johns, Y. Takeda-Uchimura, M. C. LeMieux, K. Rufibach, J. Rajadas, H. Zhang, B. Tomooka, W. H. Robinson, C. M. Clark, A. M. Fagan, D. R. Galasko, D. M. Holtzman, M. Jutel, J. A. Kaye, C. A. Lemere, J. Leszek, G. Li, E. R. Peskind, J. F. Quinn, J. A. Yesavage, J. A. Ghiso & T. Wyss-Coray (2009) Neuroprotective natural antibodies to assemblies of amyloidogenic peptides decrease with normal aging and advancing Alzheimer's disease. *Proc Natl Acad Sci U S A,* 106**,** 12145-50.

Brodie, C., N. Goldreich, T. Haiman & G. Kazimirsky (1998) Functional IL-4 receptors on mouse astrocytes: IL-4 inhibits astrocyte activation and induces NGF secretion. *J Neuroimmunol,* 81**,** 20-30.

Brown, T. P., P. C. Rumsby, A. C. Capleton, L. Rushton & L. S. Levy (2006) Pesticides and Parkinson's disease--is there a link? *Environ Health Perspect,* 114**,** 156-64.

Bu, G., J. Cam & C. Zerbinatti (2006) LRP in amyloid-beta production and metabolism. *Ann N Y Acad Sci,* 1086**,** 35-53.

Buchhave, P., L. Minthon, H. Zetterberg, A. K. Wallin, K. Blennow & O. Hansson (2012) Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry,* 69**,** 98-106.

Buechler, C., M. Ritter, E. Orso, T. Langmann, J. Klucken & G. Schmitz (2000) Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. *J Leukoc Biol,* 67**,** 97-103.

Burke, R. E., M. Antonelli & D. Sulzer (1998) Glial cell line-derived neurotrophic growth factor inhibits apoptotic death of postnatal substantia nigra dopamine neurons in primary culture. *J Neurochem,* 71**,** 517-25.

Cadavid, D., H. Mena, K. Koeller & R. A. Frommelt (2000) Cerebral beta amyloid angiopathy is a risk factor for cerebral ischemic infarction. A case control study in human brain biopsies. *J Neuropathol Exp Neurol,* 59**,** 768-73.

Caille, I., B. Allinquant, E. Dupont, C. Bouillot, A. Langer, U. Muller & A. Prochiantz (2004) Soluble form of amyloid precursor protein regulates proliferation of progenitors in the adult subventricular zone. *Development,* 131**,** 2173-81.

Cannon, J. R., V. Tapias, H. M. Na, A. S. Honick, R. E. Drolet & J. T. Greenamyre (2009) A highly reproducible rotenone model of Parkinson's disease. *Neurobiol Dis,* 34**,** 279-90.

Cantu, R. C. (1996) Head injuries in sport. *Br J Sports Med,* 30**,** 289-96.

Carare, R. O., M. Bernardes-Silva, T. A. Newman, A. M. Page, J. A. Nicoll, V. H. Perry & R. O. Weller (2008) Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. *Neuropathol Appl Neurobiol,* 34**,** 131-44.

Caselli, R. J., T. G. Beach, R. Yaari & E. M. Reiman (2006) Alzheimer's disease a century later. *J Clin Psychiatry,* 67**,** 1784-800.

Cataldo, J. K. & S. A. Glantz (2010) Smoking cessation and Alzheimer's disease: facts, fallacies and promise. *Expert Rev Neurother,* 10**,** 629-31.

Chabot, S., G. Williams, M. Hamilton, G. Sutherland & V. W. Yong (1999) Mechanisms of IL-10 production in human microglia-T cell interaction. *J Immunol,* 162**,** 6819-28.

Chakrabarty, P., K. Jansen-West, A. Beccard, C. Ceballos-Diaz, Y. Levites, C. Verbeeck, A. C. Zubair, D. Dickson, T. E. Golde & P. Das (2010) Massive gliosis induced by interleukin-6 suppresses Abeta deposition in vivo: evidence against inflammation as a driving force for amyloid deposition. *FASEB J,* 24**,** 548-59.

Charidimou, A., Q. Gang & D. J. Werring (2012) Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. *J Neurol Neurosurg Psychiatry,* 83**,** 124-37.

Chaudhuri, K. R., D. G. Healy, A. H. Schapira & E. National Institute for Clinical (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurol,* 5**,** 235-45.

Chen, C., Z. Zhou, M. Li, M. Qu, Q. Ma, M. Zhong, Y. Zhang & Z. Yu (2012) Presenilin-2 polymorphisms and risk of sporadic AD: evidence from a meta-analysis. *Gene,* 503**,** 194-9.

Chen, H., E. O'Reilly, M. L. McCullough, C. Rodriguez, M. A. Schwarzschild, E. E. Calle, M. J. Thun & A. Ascherio (2007) Consumption of dairy products and risk of Parkinson's disease. *Am J Epidemiol,* 165**,** 998-1006.

Chen, K. H., E. A. Reese, H. W. Kim, S. I. Rapoport & J. S. Rao (2011) Disturbed neurotransmitter transporter expression in Alzheimer's disease brain. *J Alzheimers Dis,* 26**,** 755-66.

Chen, X. H., V. E. Johnson, K. Uryu, J. Q. Trojanowski & D. H. Smith (2009) A lack of amyloid beta plaques despite persistent accumulation of amyloid beta in axons of long-term survivors of traumatic brain injury. *Brain Pathol,* 19**,** 214-23.

Chen, X. H., R. Siman, A. Iwata, D. F. Meaney, J. Q. Trojanowski & D. H. Smith (2004) Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. *Am J Pathol,* 165**,** 357-71.

Cheng, B. & M. P. Mattson (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. *Brain Res,* 640**,** 56-67.

Chieppa, M., G. Bianchi, A. Doni, A. Del Prete, M. Sironi, G. Laskarin, P. Monti, L. Piemonti, A. Biondi, A. Mantovani, M. Introna & P. Allavena (2003) Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. *J Immunol,* 171**,** 4552-60.

Choi, S. H., B. H. Lee, S. Kim, D. S. Hahm, J. H. Jeong, S. J. Yoon, Y. Jeong, C. K. Ha & D. L. Nab (2003) Interchanging scores between clinical dementia rating scale and global deterioration scale. *Alzheimer Dis Assoc Disord,* 17**,** 98-105.

Churchyard, A. & A. J. Lees (1997) The relationship between dementia and direct involvement of the hippocampus and amygdala in Parkinson's disease. *Neurology,* 49**,** 1570-6.

Clare, R., V. G. King, M. Wirenfeldt & H. V. Vinters (2010) Synapse loss in dementias. *J Neurosci Res,* 88**,** 2083-90.

Cleary, J. P., D. M. Walsh, J. J. Hofmeister, G. M. Shankar, M. A. Kuskowski, D. J. Selkoe & K. H. Ashe (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci,* 8**,** 79-84.

Coles, J. P., T. D. Fryer, P. Smielewski, K. Rice, J. C. Clark, J. D. Pickard & D. K. Menon (2004) Defining ischemic burden after traumatic brain injury using 15O PET imaging of cerebral physiology. *J Cereb Blood Flow Metab,* 24**,** 191-201.

Colton, C. & D. M. Wilcock (2010) Assessing activation states in microglia. *CNS Neurol Disord Drug Targets,* 9**,** 174-91.

Colton, C. A. (2009) Heterogeneity of microglial activation in the innate immune response in the brain. *J Neuroimmune Pharmacol,* 4**,** 399-418.

Colton, C. A., R. T. Mott, H. Sharpe, Q. Xu, W. E. Van Nostrand & M. P. Vitek (2006) Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. *J Neuroinflammation,* 3**,** 27.

Conway, K. A., S. J. Lee, J. C. Rochet, T. T. Ding, R. E. Williamson & P. T. Lansbury, Jr. (2000) Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc Natl Acad Sci U S A,* 97**,** 571-6.

Coraci, I. S., J. Husemann, J. W. Berman, C. Hulette, J. H. Dufour, G. K. Campanella, A. D. Luster, S. C. Silverstein & J. B. El-Khoury (2002) CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. *Am J Pathol,* 160**,** 101-12.

Corbo, R. M. & R. Scacchi (1999) Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? *Ann Hum Genet,* 63**,** 301-10.

Corder, E. H., A. M. Saunders, N. J. Risch, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, Jr., J. B. Rimmler, P. A. Locke, P. M. Conneally, K. E. Schmader & et al. (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet,* 7**,** 180-4.

Correale, J. & A. Villa (2004) The neuroprotective role of inflammation in nervous system injuries. *J Neurol,* 251**,** 1304-16.

Corsellis, J. A., C. J. Bruton & D. Freeman-Browne (1973) The aftermath of boxing. *Psychol Med,* 3**,** 270-303.

Cortes-Canteli, M. & S. Strickland (2009) Fibrinogen, a possible key player in Alzheimer's disease. *J Thromb Haemost,* 7 Suppl 1**,** 146-50.

Cosenza, M. A., M. L. Zhao, Q. Si & S. C. Lee (2002) Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis. *Brain Pathol,* 12**,** 442-55.

Costa, F. H., A. L. Rosso, H. Maultasch, D. H. Nicaretta & M. B. Vincent (2012) Depression in Parkinson's disease: diagnosis and treatment. *Arq Neuropsiquiatr,* 70**,** 617-20.

Craft, S., L. Teri, S. D. Edland, W. A. Kukull, G. Schellenberg, W. C. McCormick, J. D. Bowen & E. B. Larson (1998) Accelerated decline in apolipoprotein E-epsilon4 homozygotes with Alzheimer's disease. *Neurology,* 51**,** 149-53.

Croisier, E., M. R. DE, M. Deprez, K. Goldring, D. T. Dexter, R. K. Pearce, M. B. Graeber & F. Roncaroli (2006) Comparative study of commercially available anti-alpha-synuclein antibodies. *Neuropathol Appl Neurobiol,* 32**,** 351-6.

Croisier, E., L. B. Moran, D. T. Dexter, R. K. Pearce & M. B. Graeber (2005) Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. *J Neuroinflammation,* 2**,** 14.

Cruchaga, C., G. Haller, S. Chakraverty, K. Mayo, F. L. Vallania, R. D. Mitra, K. Faber, J. Williamson, T. Bird, R. Diaz-Arrastia, T. M. Foroud, B. F. Boeve, N. R. Graff-Radford, P. St Jean, M. Lawson, M. G. Ehm, R. Mayeux, A. M. Goate & N.-L. N. F. S. Consortium (2012) Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. *PLoS One,* 7**,** e31039.

Cruts, M., C. M. van Duijn, H. Backhovens, M. Van den Broeck, A. Wehnert, S. Serneels, R. Sherrington, M. Hutton, J. Hardy, P. H. St George-Hyslop, A. Hofman & C. Van Broeckhoven (1998) Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. *Hum Mol Genet,* 7**,** 43-51.

Cunningham, C., D. C. Wilcockson, S. Campion, K. Lunnon & V. H. Perry (2005) Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci,* 25**,** 9275-84.

D'Andrea, M. R., G. M. Cole & M. D. Ard (2004) The microglial phagocytic role with specific plaque types in the Alzheimer disease brain. *Neurobiol Aging,* 25**,** 675-83.

da Silva, R. P. & S. Gordon (1999) Phagocytosis stimulates alternative glycosylation of macrosialin (mouse CD68), a macrophage-specific endosomal protein. *Biochem J,* 338 (Pt 3)**,** 687-94.

Dahlgren, K. N., A. M. Manelli, W. B. Stine, Jr., L. K. Baker, G. A. Krafft & M. J. LaDu (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem,* 277**,** 32046-53.

Dale, G. E., A. Probst, P. Luthert, J. Martin, B. H. Anderton & P. N. Leigh (1992) Relationships between Lewy bodies and pale bodies in Parkinson's disease. *Acta Neuropathol,* 83**,** 525-9.

Davalos, D., J. Grutzendler, G. Yang, J. V. Kim, Y. Zuo, S. Jung, D. R. Littman, M. L. Dustin & W. B. Gan (2005) ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci,* 8**,** 752-8.

Davidson, W. S., A. Jonas, D. F. Clayton & J. M. George (1998) Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J Biol Chem,* 273**,** 9443-9.

de Haas, A. H., H. W. Boddeke & K. Biber (2008) Region-specific expression of immunoregulatory proteins on microglia in the healthy CNS. *Glia,* 56**,** 888-94.

de la Monte, S. M. & J. R. Wands (2006) Molecular indices of oxidative stress and mitochondrial dysfunction occur early and often progress with severity of Alzheimer's disease. *J Alzheimers Dis,* 9**,** 167-81.

de Lau, L. M. & M. M. Breteler (2006) Epidemiology of Parkinson's disease. *Lancet Neurol,* 5**,** 525-35.

De Vera, M., M. M. Rahman, J. Rankin, J. Kopec, X. Gao & H. Choi (2008) Gout and the risk of Parkinson's disease: a cohort study. *Arthritis Rheum,* 59**,** 1549-54.

Deane, R., Z. Wu & B. V. Zlokovic (2004) RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. *Stroke,* 35**,** 2628-31.

Deleidi, M. & W. Maetzler (2012) Protein clearance mechanisms of alpha-synuclein and amyloid-Beta in lewy body disorders. *Int J Alzheimers Dis,* 2012**,** 391438.

Deng-Bryant, Y., I. N. Singh, K. M. Carrico & E. D. Hall (2008) Neuroprotective effects of tempol, a catalytic scavenger of peroxynitrite-derived free radicals, in a mouse traumatic brain injury model. *J Cereb Blood Flow Metab,* 28**,** 1114-26.

Desplats, P., H. J. Lee, E. J. Bae, C. Patrick, E. Rockenstein, L. Crews, B. Spencer, E. Masliah & S. J. Lee (2009) Inclusion formation and neuronal cell death through

neuron-to-neuron transmission of alpha-synuclein. *Proc Natl Acad Sci U S A,* 106**,** 13010-5.

Devitt, A., O. D. Moffatt, C. Raykundalia, J. D. Capra, D. L. Simmons & C. D. Gregory (1998) Human CD14 mediates recognition and phagocytosis of apoptotic cells. *Nature,* 392**,** 505-9.

Dick, F. D. (2006) Parkinson's disease and pesticide exposures. *Br Med Bull,* 79-80**,** 219-31.

Dickson, D. W., H. Braak, J. E. Duda, C. Duyckaerts, T. Gasser, G. M. Halliday, J. Hardy, J. B. Leverenz, K. Del Tredici, Z. K. Wszolek & I. Litvan (2009) Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol,* 8**,** 1150-7.

Dickson, D. W., H. Fujishiro, A. DelleDonne, J. Menke, Z. Ahmed, K. J. Klos, K. A. Josephs, R. Frigerio, M. Burnett, J. E. Parisi & J. E. Ahlskog (2008) Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. *Acta Neuropathol,* 115**,** 437-44.

Dickson, T. C. & J. C. Vickers (2001) The morphological phenotype of beta-amyloid plaques and associated neuritic changes in Alzheimer's disease. *Neuroscience,* 105**,** 99-107.

Dilger, R. N. & R. W. Johnson (2008) Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. *J Leukoc Biol,* 84**,** 932-9.

Dixit, R., J. L. Ross, Y. E. Goldman & E. L. Holzbaur (2008) Differential regulation of dynein and kinesin motor proteins by tau. *Science,* 319**,** 1086-9.

Driver, J. A., G. Logroscino, J. M. Gaziano & T. Kurth (2009) Incidence and remaining lifetime risk of Parkinson disease in advanced age. *Neurology,* 72**,** 432-8.

Dubois, B., H. H. Feldman, C. Jacova, S. T. Dekosky, P. Barberger-Gateau, J. Cummings, A. Delacourte, D. Galasko, S. Gauthier, G. Jicha, K. Meguro, J. O'Brien, F. Pasquier, P. Robert, M. Rossor, S. Salloway, Y. Stern, P. J. Visser & P. Scheltens (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol,* 6**,** 734-46.

Dunker, N., N. Schuster & K. Krieglstein (2001) TGF-beta modulates programmed cell death in the retina of the developing chick embryo. *Development,* 128**,** 1933-42.

Dunn, N., M. Mullee, V. H. Perry & C. Holmes (2005) Association between dementia and infectious disease: evidence from a case-control study. *Alzheimer Dis Assoc Disord,* 19**,** 91-4.

Edbauer, D., E. Winkler, J. T. Regula, B. Pesold, H. Steiner & C. Haass (2003) Reconstitution of gamma-secretase activity. *Nat Cell Biol,* 5**,** 486-8.

Eikelenboom, P., R. Veerhuis, W. Scheper, A. J. Rozemuller, W. A. van Gool & J. J. Hoozemans (2006) The significance of neuroinflammation in understanding Alzheimer's disease. *J Neural Transm,* 113**,** 1685-95.

Ekdahl, C. T., Z. Kokaia & O. Lindvall (2009) Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience,* 158**,** 1021-9.

El Khoury, J. & A. D. Luster (2008) Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications. *Trends Pharmacol Sci,* 29**,** 626-32.

El Khoury, J. B., K. J. Moore, T. K. Means, J. Leung, K. Terada, M. Toft, M. W. Freeman & A. D. Luster (2003) CD36 mediates the innate host response to beta-amyloid. *J Exp Med,* 197**,** 1657-66.

Emmerson, E., L. Campbell, G. S. Ashcroft & M. J. Hardman (2009) Unique and synergistic roles for 17beta-estradiol and macrophage migration inhibitory factor during cutaneous wound closure are cell type specific. *Endocrinology,* 150**,** 2749-57.

Emre, M., D. Aarsland, R. Brown, D. J. Burn, C. Duyckaerts, Y. Mizuno, G. A. Broe, J. Cummings, D. W. Dickson, S. Gauthier, J. Goldman, C. Goetz, A. Korczyn, A. Lees, R. Levy, I. Litvan, I. McKeith, W. Olanow, W. Poewe, N. Quinn, C. Sampaio, E. Tolosa & B. Dubois (2007) Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord,* 22**,** 1689-707; quiz 1837.

Eskow Jaunarajs, K. L., M. Angoa-Perez, D. M. Kuhn & C. Bishop (2011) Potential mechanisms underlying anxiety and depression in Parkinson's disease: consequences of l-DOPA treatment. *Neurosci Biobehav Rev,* 35**,** 556-64.

Etminan, M., Gill, S., Samil, A. (2003) Effect of non-steroidal anti-inflammatory drugs on risk of Alzheimer's disease: systematic review and meta-analysis of observational studies. *BMJ,* 327**,** 128-131.

Eyal, A., R. Szargel, E. Avraham, E. Liani, J. Haskin, R. Rott & S. Engelender (2006) Synphilin-1A: an aggregation-prone isoform of synphilin-1 that causes neuronal death and is present in aggregates from alpha-synucleinopathy patients. *Proc Natl Acad Sci U S A,* 103**,** 5917-22.

Fabriek, B. O., R. van Bruggen, D. M. Deng, A. J. Ligtenberg, K. Nazmi, K. Schornagel, R. P. Vloet, C. D. Dijkstra & T. K. van den Berg (2009) The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. *Blood,* 113**,** 887-92.

Fabriek, B. O., E. S. Van Haastert, I. Galea, M. M. Polfliet, E. D. Dopp, M. M. Van Den Heuvel, T. K. Van Den Berg, C. J. De Groot, P. Van Der Valk & C. D. Dijkstra

(2005) CD163-positive perivascular macrophages in the human CNS express molecules for antigen recognition and presentation. *Glia,* 51**,** 297-305.

Factor, S. S., Weiner, W.J. 2002. *Parkinson's Disease Diagnosis and Clinical Management*.

Fagan, A. M., C. M. Roe, C. Xiong, M. A. Mintun, J. C. Morris & D. M. Holtzman (2007) Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol,* 64**,** 343-9.

Fan, R., F. Xu, M. L. Previti, J. Davis, A. M. Grande, J. K. Robinson & W. E. Van Nostrand (2007) Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid. *J Neurosci,* 27**,** 3057-63.

Farooqui, A. A., L. A. Horrocks & T. Farooqui (2007) Modulation of inflammation in brain: a matter of fat. *J Neurochem,* 101**,** 577-99.

Farooqui, T. & A. A. Farooqui (2011) Lipid-mediated oxidative stress and inflammation in the pathogenesis of Parkinson's disease. *Parkinsons Dis,* 2011**,** 247467.

Fassbender, K., S. Walter, S. Kuhl, R. Landmann, K. Ishii, T. Bertsch, A. K. Stalder, F. Muehlhauser, Y. Liu, A. J. Ulmer, S. Rivest, A. Lentschat, E. Gulbins, M. Jucker, M. Staufenbiel, K. Brechtel, J. Walter, G. Multhaup, B. Penke, Y. Adachi, T. Hartmann & K. Beyreuther (2004) The LPS receptor (CD14) links innate immunity with Alzheimer's disease. *FASEB J,* 18**,** 203-5.

Febbraio, M., D. P. Hajjar & R. L. Silverstein (2001) CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J Clin Invest,* 108**,** 785-91.

Fenelon, G. & G. Alves (2010) Epidemiology of psychosis in Parkinson's disease. *J Neurol Sci,* 289**,** 12-7.

Fenelon, G., F. Mahieux, R. Huon & M. Ziegler (2000) Hallucinations in Parkinson's disease: prevalence, phenomenology and risk factors. *Brain,* 123 (Pt 4)**,** 733-45.

Fenn, A. M., C. J. Henry, Y. Huang, A. Dugan & J. P. Godbout (2012) Lipopolysaccharide-induced interleukin (IL)-4 receptor-alpha expression and corresponding sensitivity to the M2 promoting effects of IL-4 are impaired in microglia of aged mice. *Brain Behav Immun,* 26**,** 766-77.

Fernandez, J. A., L. Rojo, R. O. Kuljis & R. B. Maccioni (2008) The damage signals hypothesis of Alzheimer's disease pathogenesis. *J Alzheimers Dis,* 14**,** 329-33.

Ferreiro, E., I. Baldeiras, I. L. Ferreira, R. O. Costa, A. C. Rego, C. F. Pereira & C. R. Oliveira (2012) Mitochondrial- and endoplasmic reticulum-associated oxidative

stress in Alzheimer's disease: from pathogenesis to biomarkers. *Int J Cell Biol,* 2012**,** 735206.

Fiala, M., J. Lin, J. Ringman, V. Kermani-Arab, G. Tsao, A. Patel, A. S. Lossinsky, M. C. Graves, A. Gustavson, J. Sayre, E. Sofroni, T. Suarez, F. Chiappelli & G. Bernard (2005) Ineffective phagocytosis of amyloid-beta by macrophages of Alzheimer's disease patients. *J Alzheimers Dis,* 7**,** 221-32; discussion 255-62.

Fiala, M., P. T. Liu, A. Espinosa-Jeffrey, M. J. Rosenthal, G. Bernard, J. M. Ringman, J. Sayre, L. Zhang, J. Zaghi, S. Dejbakhsh, B. Chiang, J. Hui, M. Mahanian, A. Baghaee, P. Hong & J. Cashman (2007) Innate immunity and transcription of MGAT-III and Toll-like receptors in Alzheimer's disease patients are improved by bisdemethoxycurcumin. *Proc Natl Acad Sci U S A,* 104**,** 12849-54.

Fillenbaum, G. G., G. van Belle, J. C. Morris, R. C. Mohs, S. S. Mirra, P. C. Davis, P. N. Tariot, J. M. Silverman, C. M. Clark, K. A. Welsh-Bohmer & A. Heyman (2008) Consortium to Establish a Registry for Alzheimer's Disease (CERAD): the first twenty years. *Alzheimers Dement,* 4**,** 96-109.

Finch, C. E., N. J. Laping, T. E. Morgan, N. R. Nichols & G. M. Pasinetti (1993) TGF-beta 1 is an organizer of responses to neurodegeneration. *J Cell Biochem,* 53**,** 314-22.

Firestone, J. A., J. I. Lundin, K. M. Powers, T. Smith-Weller, G. M. Franklin, P. D. Swanson, W. T. Longstreth, Jr. & H. Checkoway (2010) Occupational factors and risk of Parkinson's disease: A population-based case-control study. *Am J Ind Med,* 53**,** 217-23.

Fleminger, S., D. L. Oliver, S. Lovestone, S. Rabe-Hesketh & A. Giora (2003) Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *J Neurol Neurosurg Psychiatry,* 74**,** 857-62.

Floden, A. M., S. Li & C. K. Combs (2005) Beta-amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor alpha and NMDA receptors. *J Neurosci,* 25**,** 2566-75.

Fong, T. G., S. R. Tulebaev & S. K. Inouye (2009) Delirium in elderly adults: diagnosis, prevention and treatment. *Nat Rev Neurol,* 5**,** 210-20.

Forno, L. S., J. W. Langston, L. E. DeLanney, I. Irwin & G. A. Ricaurte (1986) Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. *Ann Neurol,* 20**,** 449-55.

Forsaa, E. B., J. P. Larsen, T. Wentzel-Larsen, C. G. Goetz, G. T. Stebbins, D. Aarsland & G. Alves (2010) A 12-year population-based study of psychosis in Parkinson disease. *Arch Neurol,* 67**,** 996-1001.

Frisina, P. G., V. Haroutunian & L. S. Libow (2009) The neuropathological basis for depression in Parkinson's disease. *Parkinsonism Relat Disord,* 15**,** 144-8.

Galea, I., K. Palin, T. A. Newman, N. Van Rooijen, V. H. Perry & D. Boche (2005) Mannose receptor expression specifically reveals perivascular macrophages in normal, injured, and diseased mouse brain. *Glia,* 49**,** 375-84.

Gao, H. M., J. S. Hong, W. Zhang & B. Liu (2003) Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. *J Neurosci,* 23**,** 1228-36.

Gao, H. M., P. T. Kotzbauer, K. Uryu, S. Leight, J. Q. Trojanowski & V. M. Lee (2008) Neuroinflammation and oxidation/nitration of alpha-synuclein linked to dopaminergic neurodegeneration. *J Neurosci,* 28**,** 7687-98.

Garantziotis, S., D. M. Brass, J. Savov, J. W. Hollingsworth, E. McElvania-TeKippe, K. Berman, J. K. Walker & D. A. Schwartz (2006) Leukocyte-derived IL-10 reduces subepithelial fibrosis associated with chronically inhaled endotoxin. *Am J Respir Cell Mol Biol,* 35**,** 662-7.

Gate, D., K. Rezai-Zadeh, D. Jodry, A. Rentsendorj & T. Town (2010) Macrophages in Alzheimer's disease: the blood-borne identity. *J Neural Transm,* 117**,** 961-70.

Gentleman, S. M. (2013) Review: microglia in protein aggregation disorders: friend or foe? *Neuropathol Appl Neurobiol,* 39**,** 45-50.

Gentleman, S. M., B. D. Greenberg, M. J. Savage, M. Noori, S. J. Newman, G. W. Roberts, W. S. Griffin & D. I. Graham (1997) A beta 42 is the predominant form of amyloid beta-protein in the brains of short-term survivors of head injury. *Neuroreport,* 8**,** 1519-22.

Gentleman, S. M., P. D. Leclercq, L. Moyes, D. I. Graham, C. Smith, W. S. Griffin & J. A. Nicoll (2004) Long-term intracerebral inflammatory response after traumatic brain injury. *Forensic Sci Int,* 146**,** 97-104.

Gentleman, S. M., M. J. Nash, C. J. Sweeting, D. I. Graham & G. W. Roberts (1993) Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. *Neurosci Lett,* 160**,** 139-44.

Gerhard, A., N. Pavese, G. Hotton, F. Turkheimer, M. Es, A. Hammers, K. Eggert, W. Oertel, R. B. Banati & D. J. Brooks (2006) In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis,* 21**,** 404-12.

Gerlach, M., P. Riederer & K. L. Double (2008) Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease. *Parkinsonism Relat Disord,* 14 Suppl 2**,** S185-8.

Gilman, S., M. Koller, R. S. Black, L. Jenkins, S. G. Griffith, N. C. Fox, L. Eisner, L. Kirby, M. B. Rovira, F. Forette, J. M. Orgogozo & A. N. S. Team (2005) Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology,* 64**,** 1553-62.

Goate, A., M. C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James & et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature,* 349**,** 704-6.

Goedert, M., R. Jakes & E. Vanmechelen (1995) Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205. *Neurosci Lett,* 189**,** 167-9.

Gordon, S. (2003) Alternative activation of macrophages. *Nat Rev Immunol,* 3**,** 23-35.

Gordon, S. & P. R. Taylor (2005) Monocyte and macrophage heterogeneity. *Nat Rev Immunol,* 5**,** 953-64.

Gosselin, D. & S. Rivest (2007) Role of IL-1 and TNF in the brain: twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system. *Brain Behav Immun,* 21**,** 281-9.

Gotz, J. & L. M. Ittner (2008) Animal models of Alzheimer's disease and frontotemporal dementia. *Nat Rev Neurosci,* 9**,** 532-44.

Gotz, J., L. M. Ittner, N. Schonrock & R. Cappai (2008) An update on the toxicity of Abeta in Alzheimer's disease. *Neuropsychiatr Dis Treat,* 4**,** 1033-42.

Gouras, G. K., C. G. Almeida & R. H. Takahashi (2005) Intraneuronal Abeta accumulation and origin of plaques in Alzheimer's disease. *Neurobiol Aging,* 26**,** 1235-44.

Graeber, M. B., K. Bise & P. Mehraein (1994) CR3/43, a marker for activated human microglia: application to diagnostic neuropathology. *Neuropathol Appl Neurobiol,* 20**,** 406-8.

Gralle, M. & S. T. Ferreira (2007) Structure and functions of the human amyloid precursor protein: the whole is more than the sum of its parts. *Prog Neurobiol,* 82**,** 11-32.

Graversen, J. H., M. Madsen & S. K. Moestrup (2002) CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. *Int J Biochem Cell Biol,* 34**,** 309-14.

Gregory, C. D. (2000) CD14-dependent clearance of apoptotic cells: relevance to the immune system. *Curr Opin Immunol,* 12**,** 27-34.

Gregory, C. D. & A. Devitt (2004) The macrophage and the apoptotic cell: an innate immune interaction viewed simplistically? *Immunology,* 113**,** 1-14.

Griffin, W. S. & R. E. Mrak (2002) Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. *J Leukoc Biol,* 72**,** 233-8.

Griffin, W. S., J. G. Sheng, M. C. Royston, S. M. Gentleman, J. E. McKenzie, D. I. Graham, G. W. Roberts & R. E. Mrak (1998) Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol,* 8**,** 65-72.

Grilli, M., I. Barbieri, H. Basudev, R. Brusa, C. Casati, G. Lozza & E. Ongini (2000) Interleukin-10 modulates neuronal threshold of vulnerability to ischaemic damage. *Eur J Neurosci,* 12**,** 2265-72.

Guillemin, G. J. & B. J. Brew (2004) Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. *J Leukoc Biol,* 75**,** 388-97.

Guo, J. P., T. Arai, J. Miklossy & P. L. McGeer (2006) Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease. *Proc Natl Acad Sci U S A,* 103**,** 1953-8.

Guo, Z., L. A. Cupples, A. Kurz, S. H. Auerbach, L. Volicer, H. Chui, R. C. Green, A. D. Sadovnick, R. Duara, C. DeCarli, K. Johnson, R. C. Go, J. H. Growdon, J. L. Haines, W. A. Kukull & L. A. Farrer (2000) Head injury and the risk of AD in the MIRAGE study. *Neurology,* 54**,** 1316-23.

Gustafson, D., E. Rothenberg, K. Blennow, B. Steen & I. Skoog (2003) An 18-year follow-up of overweight and risk of Alzheimer disease. *Arch Intern Med,* 163**,** 1524-8.

Haass, C., A. Y. Hung, M. G. Schlossmacher, D. B. Teplow & D. J. Selkoe (1993) beta-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. *J Biol Chem,* 268**,** 3021-4.

Haass, C., A. Y. Hung, D. J. Selkoe & D. B. Teplow (1994) Mutations associated with a locus for familial Alzheimer's disease result in alternative processing of amyloid beta-protein precursor. *J Biol Chem,* 269**,** 17741-8.

Haass, C. & D. J. Selkoe (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol,* 8**,** 101-12.

Hall, R. C. & M. J. Chapman (2005) Definition, diagnosis, and forensic implications of postconcussional syndrome. *Psychosomatics,* 46**,** 195-202.

Halliday, G. M., J. L. Holton, T. Revesz & D. W. Dickson (2011) Neuropathology underlying clinical variability in patients with synucleinopathies. *Acta Neuropathol,* 122**,** 187-204.

Halliday, G. M. & H. McCann (2008) Human-based studies on alpha-synuclein deposition and relationship to Parkinson's disease symptoms. *Exp Neurol,* 209**,** 12-21.

Halliday, G. M. & C. H. Stevens (2011) Glia: initiators and progressors of pathology in Parkinson's disease. *Mov Disord,* 26**,** 6-17.

Hamos, J. E., L. J. DeGennaro & D. A. Drachman (1989) Synaptic loss in Alzheimer's disease and other dementias. *Neurology,* 39**,** 355-61.

Hanger, D. P., B. H. Anderton & W. Noble (2009) Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med,* 15**,** 112-9.

Hansen, C., E. Angot, A. L. Bergstrom, J. A. Steiner, L. Pieri, G. Paul, T. F. Outeiro, R. Melki, P. Kallunki, K. Fog, J. Y. Li & P. Brundin (2011) alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J Clin Invest,* 121**,** 715-25.

Hardy, J. & D. J. Selkoe (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science,* 297**,** 353-6.

Hartings, J. A., T. Watanabe, M. R. Bullock, D. O. Okonkwo, M. Fabricius, J. Woitzik, J. P. Dreier, A. Puccio, L. A. Shutter, C. Pahl, A. J. Strong & D. Co-Operative Study on Brain Injury (2011) Spreading depolarizations have prolonged direct current shifts and are associated with poor outcome in brain trauma. *Brain,* 134**,** 1529-40.

Hattingh, C. J., J. Ipser, S. A. Tromp, S. Syal, C. Lochner, S. J. Brooks & D. J. Stein (2012) Functional magnetic resonance imaging during emotion recognition in social anxiety disorder: an activation likelihood meta-analysis. *Front Hum Neurosci,* 6**,** 347.

Hawkes, C. A. & J. McLaurin (2009) Selective targeting of perivascular macrophages for clearance of beta-amyloid in cerebral amyloid angiopathy. *Proc Natl Acad Sci U S A,* 106**,** 1261-6.

Hely, M. A., J. G. Morris, W. G. Reid & R. Trafficante (2005) Sydney Multicenter Study of Parkinson's disease: non-L-dopa-responsive problems dominate at 15 years. *Mov Disord,* 20**,** 190-9.

Henrich-Noack, P., J. H. Prehn & J. Krieglstein (1996) TGF-beta 1 protects hippocampal neurons against degeneration caused by transient global ischemia. Dose-response relationship and potential neuroprotective mechanisms. *Stroke,* 27**,** 1609-14; discussion 1615.

Herrera-Molina, R. & R. von Bernhardi (2005) Transforming growth factor-beta 1 produced by hippocampal cells modulates microglial reactivity in culture. *Neurobiol Dis,* 19**,** 229-36.

Hickman, S. E., E. K. Allison & J. El Khoury (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J Neurosci,* 28**,** 8354-60.

Hiler, M., M. Czosnyka, P. Hutchinson, M. Balestreri, P. Smielewski, B. Matta & J. D. Pickard (2006) Predictive value of initial computerized tomography scan, intracranial pressure, and state of autoregulation in patients with traumatic brain injury. *J Neurosurg,* 104**,** 731-7.

Hippius, H. & G. Neundorfer (2003) The discovery of Alzheimer's disease. *Dialogues Clin Neurosci,* 5**,** 101-8.

Hirsch, E. C. (2009) Iron transport in Parkinson's disease. *Parkinsonism Relat Disord,* 15 Suppl 3**,** S209-11.

Hirsch, E. C., T. Breidert, E. Rousselet, S. Hunot, A. Hartmann & P. P. Michel (2003) The role of glial reaction and inflammation in Parkinson's disease. *Ann N Y Acad Sci,* 991**,** 214-28.

Hirsch, E. C. & S. Hunot (2009) Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol,* 8**,** 382-97.

Hoerndli, F. J., S. Pelech, A. Papassotiropoulos & J. Gotz (2007) Abeta treatment and P301L tau expression in an Alzheimer's disease tissue culture model act synergistically to promote aberrant cell cycle re-entry. *Eur J Neurosci,* 26**,** 60-72.

Hogger, P., J. Dreier, A. Droste, F. Buck & C. Sorg (1998) Identification of the integral membrane protein RM3/1 on human monocytes as a glucocorticoid-inducible member of the scavenger receptor cysteine-rich family (CD163). *J Immunol,* 161**,** 1883-90.

Holmes, C., D. Boche, D. Wilkinson, G. Yadegarfar, V. Hopkins, A. Bayer, R. W. Jones, R. Bullock, S. Love, J. W. Neal, E. Zotova & J. A. Nicoll (2008) Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet,* 372**,** 216-23.

Holtzman, D. M., K. R. Bales, T. Tenkova, A. M. Fagan, M. Parsadanian, L. J. Sartorius, B. Mackey, J. Olney, D. McKeel, D. Wozniak & S. M. Paul (2000) Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A,* 97**,** 2892-7.

Holtzman, D. M., J. C. Morris & A. M. Goate (2011) Alzheimer's disease: the challenge of the second century. *Sci Transl Med,* 3**,** 77sr1.

Hong, D. P., A. L. Fink & V. N. Uversky (2009) Smoking and Parkinson's disease: does nicotine affect alpha-synuclein fibrillation? *Biochim Biophys Acta,* 1794**,** 282-90.

Hornykiewicz, O. (2006) The discovery of dopamine deficiency in the parkinsonian brain. *J Neural Transm Suppl*, 9-15.

Houlden, H., M. Baker, E. McGowan, P. Lewis, M. Hutton, R. Crook, N. W. Wood, S. Kumar-Singh, J. Geddes, M. Swash, F. Scaravilli, J. L. Holton, T. Lashley, T. Tomita, T. Hashimoto, A. Verkkoniemi, H. Kalimo, M. Somer, A. Paetau, J. J. Martin, C. Van Broeckhoven, T. Golde, J. Hardy, M. Haltia & T. Revesz (2000) Variant Alzheimer's disease with spastic paraparesis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-beta concentrations. *Ann Neurol,* 48, 806-8.

Hu, G., S. Bidel, P. Jousilahti, R. Antikainen & J. Tuomilehto (2007a) Coffee and tea consumption and the risk of Parkinson's disease. *Mov Disord,* 22, 2242-8.

Hu, G., P. Jousilahti, S. Bidel, R. Antikainen & J. Tuomilehto (2007b) Type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care,* 30, 842-7.

Hu, G., P. Jousilahti, A. Nissinen, R. Antikainen, M. Kivipelto & J. Tuomilehto (2006) Body mass index and the risk of Parkinson disease. *Neurology,* 67, 1955-9.

Hunot, S., F. Boissiere, B. Faucheux, B. Brugg, A. Mouatt-Prigent, Y. Agid & E. C. Hirsch (1996) Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience,* 72, 355-63.

Hurtig, H. I., J. Q. Trojanowski, J. Galvin, D. Ewbank, M. L. Schmidt, V. M. Lee, C. M. Clark, G. Glosser, M. B. Stern, S. M. Gollomp & S. E. Arnold (2000) Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. *Neurology,* 54, 1916-21.

Husemann, J., J. D. Loike, R. Anankov, M. Febbraio & S. C. Silverstein (2002) Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia,* 40, 195-205.

Hutton, M. (2000) Molecular genetics of chromosome 17 tauopathies. *Ann N Y Acad Sci,* 920, 63-73.

Hyder, A. A., C. A. Wunderlich, P. Puvanachandra, G. Gururaj & O. C. Kobusingye (2007) The impact of traumatic brain injuries: a global perspective. *NeuroRehabilitation,* 22, 341-53.

Hyman, C., M. Juhasz, C. Jackson, P. Wright, N. Y. Ip & R. M. Lindsay (1994) Overlapping and distinct actions of the neurotrophins BDNF, NT-3, and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. *J Neurosci,* 14, 335-47.

Iadecola, C. (2003) Cerebrovascular effects of amyloid-beta peptides: mechanisms and implications for Alzheimer's dementia. *Cell Mol Neurobiol,* 23, 681-9.

Imai, Y., I. Ibata, D. Ito, K. Ohsawa & S. Kohsaka (1996) A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. *Biochem Biophys Res Commun,* 224**,** 855-62.

Imamura, K., N. Hishikawa, M. Sawada, T. Nagatsu, M. Yoshida & Y. Hashizume (2003) Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol,* 106**,** 518-26.

Iosif, R. E., H. Ahlenius, C. T. Ekdahl, V. Darsalia, P. Thored, S. Jovinge, Z. Kokaia & O. Lindvall (2008) Suppression of stroke-induced progenitor proliferation in adult subventricular zone by tumor necrosis factor receptor 1. *J Cereb Blood Flow Metab,* 28**,** 1574-87.

Ishihara, L. & C. Brayne (2006) A systematic review of depression and mental illness preceding Parkinson's disease. *Acta Neurol Scand,* 113**,** 211-20.

Ishihara, L. S., A. Cheesbrough, C. Brayne & A. Schrag (2007) Estimated life expectancy of Parkinson's patients compared with the UK population. *J Neurol Neurosurg Psychiatry,* 78**,** 1304-9.

Itakura, E., R. R. Huang, D. R. Wen, E. Paul, P. H. Wunsch & A. J. Cochran (2011) IL-10 expression by primary tumor cells correlates with melanoma progression from radial to vertical growth phase and development of metastatic competence. *Mod Pathol,* 24**,** 801-9.

Ito, D., Y. Imai, K. Ohsawa, K. Nakajima, Y. Fukuuchi & S. Kohsaka (1998) Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res,* 57**,** 1-9.

Ito, D., K. Tanaka, S. Suzuki, T. Dembo & Y. Fukuuchi (2001) Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain. *Stroke,* 32**,** 1208-15.

Jack, C. R., Jr., D. S. Knopman, W. J. Jagust, L. M. Shaw, P. S. Aisen, M. W. Weiner, R. C. Petersen & J. Q. Trojanowski (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol,* 9**,** 119-28.

Jacque, C. M., C. Vinner, M. Kujas, M. Raoul, J. Racadot & N. A. Baumann (1978) Determination of glial fibrillary acidic protein (GFAP) in human brain tumors. *J Neurol Sci,* 35**,** 147-55.

Janabi, M., S. Yamashita, K. Hirano, N. Sakai, H. Hiraoka, K. Matsumoto, Z. Zhang, S. Nozaki & Y. Matsuzawa (2000) Oxidized LDL-induced NF-kappa B activation and subsequent expression of proinflammatory genes are defective in

monocyte-derived macrophages from CD36-deficient patients. *Arterioscler Thromb Vasc Biol,* 20**,** 1953-60.

Jankovic, J. (2008) Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry,* 79**,** 368-76.

Jellinger, K. A. (2002) Alzheimer disease and cerebrovascular pathology: an update. *J Neural Transm,* 109**,** 813-36.

Jimenez, S., D. Baglietto-Vargas, C. Caballero, I. Moreno-Gonzalez, M. Torres, R. Sanchez-Varo, D. Ruano, M. Vizuete, A. Gutierrez & J. Vitorica (2008) Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: age-dependent switch in the microglial phenotype from alternative to classic. *J Neurosci,* 28**,** 11650-61.

Johnson, V. E., J. E. Stewart, F. D. Begbie, J. Q. Trojanowski, D. H. Smith & W. Stewart (2013) Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain,* 136**,** 28-42.

Johnson, V. E., W. Stewart & D. H. Smith (2010) Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? *Nat Rev Neurosci,* 11**,** 361-70.

--- (2012) Axonal pathology in traumatic brain injury. *Exp Neurol*.

Johnston, J. A., R. F. Cowburn, S. Norgren, B. Wiehager, N. Venizelos, B. Winblad, C. Vigo-Pelfrey, D. Schenk, L. Lannfelt & C. O'Neill (1994) Increased beta-amyloid release and levels of amyloid precursor protein (APP) in fibroblast cell lines from family members with the Swedish Alzheimer's disease APP670/671 mutation. *FEBS Lett,* 354**,** 274-8.

Joshi, A., J. M. Ringman, A. S. Lee, K. O. Juarez & M. F. Mendez (2012) Comparison of clinical characteristics between familial and non-familial early onset Alzheimer's disease. *J Neurol,* 259**,** 2182-8.

Kalaria, R. N. (2010) Vascular basis for brain degeneration: faltering controls and risk factors for dementia. *Nutr Rev,* 68 Suppl 2**,** S74-87.

Kanazawa, H., K. Ohsawa, Y. Sasaki, S. Kohsaka & Y. Imai (2002) Macrophage/microglia-specific protein Iba1 enhances membrane ruffling and Rac activation via phospholipase C-gamma -dependent pathway. *J Biol Chem,* 277**,** 20026-32.

Kauwe, J. S., S. Jacquart, S. Chakraverty, J. Wang, K. Mayo, A. M. Fagan, D. M. Holtzman, J. C. Morris & A. M. Goate (2007) Extreme cerebrospinal fluid amyloid beta levels identify family with late-onset Alzheimer's disease presenilin 1 mutation. *Ann Neurol,* 61**,** 446-53.

Kawahara, K., M. Suenobu, A. Yoshida, K. Koga, A. Hyodo, H. Ohtsuka, A. Kuniyasu, N. Tamamaki, Y. Sugimoto & H. Nakayama (2012) Intracerebral microinjection

of interleukin-4/interleukin-13 reduces beta-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. *Neuroscience,* 207**,** 243-60.

Keeney, P. M., J. Xie, R. A. Capaldi & J. P. Bennett, Jr. (2006) Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. *J Neurosci,* 26**,** 5256-64.

Kelley, S. L., T. Lukk, S. K. Nair & R. I. Tapping (2013) The crystal structure of human soluble CD14 reveals a bent solenoid with a hydrophobic amino-terminal pocket. *J Immunol,* 190**,** 1304-11.

Kempster, P. A., B. Hurwitz & A. J. Lees (2007) A new look at James Parkinson's Essay on the Shaking Palsy. *Neurology,* 69**,** 482-5.

Kettenmann, H., U. K. Hanisch, M. Noda & A. Verkhratsky (2011) Physiology of microglia. *Physiol Rev,* 91**,** 461-553.

Kielian, T. (2006) Toll-like receptors in central nervous system glial inflammation and homeostasis. *J Neurosci Res,* 83**,** 711-30.

Kim, W. G., R. P. Mohney, B. Wilson, G. H. Jeohn, B. Liu & J. S. Hong (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J Neurosci,* 20**,** 6309-16.

Kim, W. K., X. Alvarez, J. Fisher, B. Bronfin, S. Westmoreland, J. McLaurin & K. Williams (2006) CD163 identifies perivascular macrophages in normal and viral encephalitic brains and potential precursors to perivascular macrophages in blood. *Am J Pathol,* 168**,** 822-34.

Kim, Y. S., S. S. Kim, J. J. Cho, D. H. Choi, O. Hwang, D. H. Shin, H. S. Chun, M. F. Beal & T. H. Joh (2005) Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. *J Neurosci,* 25**,** 3701-11.

Kirschner, P. B., B. G. Jenkins, J. B. Schulz, S. P. Finkelstein, R. T. Matthews, B. R. Rosen & M. F. Beal (1996) NGF, BDNF and NT-5, but not NT-3 protect against MPP+ toxicity and oxidative stress in neonatal animals. *Brain Res,* 713**,** 178-85.

Kitamura, Y., T. Taniguchi, H. Kimura, Y. Nomura & P. J. Gebicke-Haerter (2000) Interleukin-4-inhibited mRNA expression in mixed rat glial and in isolated microglial cultures. *J Neuroimmunol,* 106**,** 95-104.

Kitazawa, M., S. Oddo, T. R. Yamasaki, K. N. Green & F. M. LaFerla (2005) Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. *J Neurosci,* 25**,** 8843-53.

Kivipelto, M., E. L. Helkala, T. Hanninen, M. P. Laakso, M. Hallikainen, K. Alhainen, H. Soininen, J. Tuomilehto & A. Nissinen (2001a) Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. *Neurology,* 56**,** 1683-9.

Kivipelto, M., E. L. Helkala, M. P. Laakso, T. Hanninen, M. Hallikainen, K. Alhainen, H. Soininen, J. Tuomilehto & A. Nissinen (2001b) Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. *BMJ,* 322**,** 1447-51.

Klein, C. & A. Westenberger (2012) Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med,* 2**,** a008888.

Klumpp, H., D. Post, M. Angstadt, D. A. Fitzgerald & K. L. Phan (2013) Anterior cingulate cortex and insula response during indirect and direct processing of emotional faces in generalized social anxiety disorder. *Biol Mood Anxiety Disord,* 3**,** 7.

Knopman, D. S., J. E. Parisi, A. Salviati, M. Floriach-Robert, B. F. Boeve, R. J. Ivnik, G. E. Smith, D. W. Dickson, K. A. Johnson, L. E. Petersen, W. C. McDonald, H. Braak & R. C. Petersen (2003) Neuropathology of cognitively normal elderly. *J Neuropathol Exp Neurol,* 62**,** 1087-95.

Knott, C., G. Stern, A. Kingsbury, A. A. Welcher & G. P. Wilkin (2002) Elevated glial brain-derived neurotrophic factor in Parkinson's diseased nigra. *Parkinsonism Relat Disord,* 8**,** 329-41.

Knott, C., G. Stern & G. P. Wilkin (2000) Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. *Mol Cell Neurosci,* 16**,** 724-39.

Koenigsknecht, J. & G. Landreth (2004) Microglial phagocytosis of fibrillar beta-amyloid through a beta1 integrin-dependent mechanism. *J Neurosci,* 24**,** 9838-46.

Koenigsknecht-Talboo, J. & G. E. Landreth (2005) Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. *J Neurosci,* 25**,** 8240-9.

Kordower, J. H., Y. Chu, R. A. Hauser, T. B. Freeman & C. W. Olanow (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med,* 14**,** 504-6.

Kordower, J. H., S. Palfi, E. Y. Chen, S. Y. Ma, T. Sendera, E. J. Cochran, E. J. Mufson, R. Penn, C. G. Goetz & C. D. Comella (1999) Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with Parkinson's disease. *Ann Neurol,* 46**,** 419-24.

Koronyo-Hamaoui, M., M. K. Ko, Y. Koronyo, D. Azoulay, A. Seksenyan, G. Kunis, M. Pham, J. Bakhsheshian, P. Rogeri, K. L. Black, D. L. Farkas & M. Schwartz (2009) Attenuation of AD-like neuropathology by harnessing peripheral immune cells: local elevation of IL-10 and MMP-9. *J Neurochem,* 111**,** 1409-24.

Kostic, V. S., F. Agosta, I. Petrovic, S. Galantucci, V. Spica, M. Jecmenica-Lukic & M. Filippi (2010) Regional patterns of brain tissue loss associated with depression in Parkinson disease. *Neurology,* 75**,** 857-63.

Kounnas, M. Z., A. M. Danks, S. Cheng, C. Tyree, E. Ackerman, X. Zhang, K. Ahn, P. Nguyen, D. Comer, L. Mao, C. Yu, D. Pleynet, P. J. Digregorio, G. Velicelebi, K. A. Stauderman, W. T. Comer, W. C. Mobley, Y. M. Li, S. S. Sisodia, R. E. Tanzi & S. L. Wagner (2010) Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *Neuron,* 67**,** 769-80.

Kreutzberg, G. W. (1995) Microglia, the first line of defence in brain pathologies. *Arzneimittelforschung,* 45**,** 357-60.

Krieglstein, K., S. Richter, L. Farkas, N. Schuster, N. Dunker, R. W. Oppenheim & K. Unsicker (2000) Reduction of endogenous transforming growth factors beta prevents ontogenetic neuron death. *Nat Neurosci,* 3**,** 1085-90.

Kril, J. J. (2009) Alzheimer disease: Alzheimer disease neuropathology in the oldest old. *Nat Rev Neurol,* 5**,** 411-2.

Kristiansen, M., J. H. Graversen, C. Jacobsen, O. Sonne, H. J. Hoffman, S. K. Law & S. K. Moestrup (2001) Identification of the haemoglobin scavenger receptor. *Nature,* 409**,** 198-201.

Kuo, S. H., P. Y. Yeh, L. T. Chen, M. S. Wu, C. W. Lin, K. H. Yeh, Y. S. Tzeng, J. Y. Chen, P. N. Hsu, J. T. Lin & A. L. Cheng (2008) Overexpression of B cell-activating factor of TNF family (BAFF) is associated with Helicobacter pylori-independent growth of gastric diffuse large B-cell lymphoma with histologic evidence of MALT lymphoma. *Blood,* 112**,** 2927-34.

Kuzuhara, S., H. Mori, N. Izumiyama, M. Yoshimura & Y. Ihara (1988) Lewy bodies are ubiquitinated. A light and electron microscopic immunocytochemical study. *Acta Neuropathol,* 75**,** 345-53.

Laboratories, C. S. H. DSM-IV criteria for Alzheimer's disease.

LaFerla, F. M., K. N. Green & S. Oddo (2007) Intracellular amyloid-beta in Alzheimer's disease. *Nat Rev Neurosci,* 8**,** 499-509.

Lambert, C., J. Desbarats, N. Arbour, J. A. Hall, A. Olivier, A. Bar-Or & J. P. Antel (2008) Dendritic cell differentiation signals induce anti-inflammatory properties in human adult microglia. *J Immunol,* 181**,** 8288-97.

Lambert, J. C. & P. Amouyel (2007) Genetic heterogeneity of Alzheimer's disease: complexity and advances. *Psychoneuroendocrinology,* 32 Suppl 1**,** S62-70.

Langston, J. W. (2006) The Parkinson's complex: parkinsonism is just the tip of the iceberg. *Ann Neurol,* 59**,** 591-6.

Langston, J. W., P. Ballard, J. W. Tetrud & I. Irwin (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science,* 219**,** 979-80.

Lassmann, H., M. Schmied, K. Vass & W. F. Hickey (1993) Bone marrow derived elements and resident microglia in brain inflammation. *Glia,* 7**,** 19-24.

Lau, S. K., P. G. Chu & L. M. Weiss (2004) CD163: a specific marker of macrophages in paraffin-embedded tissue samples. *Am J Clin Pathol,* 122**,** 794-801.

Ledeboer, A., J. J. Breve, S. Poole, F. J. Tilders & A. M. Van Dam (2000) Interleukin-10, interleukin-4, and transforming growth factor-beta differentially regulate lipopolysaccharide-induced production of pro-inflammatory cytokines and nitric oxide in co-cultures of rat astroglial and microglial cells. *Glia,* 30**,** 134-42.

Lee, C. Y. & G. E. Landreth (2010) The role of microglia in amyloid clearance from the AD brain. *J Neural Transm,* 117**,** 949-60.

Lee, H. G., G. Casadesus, X. Zhu, A. Takeda, G. Perry & M. A. Smith (2004) Challenging the amyloid cascade hypothesis: senile plaques and amyloid-beta as protective adaptations to Alzheimer disease. *Ann N Y Acad Sci,* 1019**,** 1-4.

Lee, H. J., J. E. Suk, E. J. Bae & S. J. Lee (2008) Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. *Biochem Biophys Res Commun,* 372**,** 423-8.

Lee, T. S. & L. Y. Chau (2002) Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat Med,* 8**,** 240-6.

Lei, P., S. Ayton, D. I. Finkelstein, P. A. Adlard, C. L. Masters & A. I. Bush (2010) Tau protein: relevance to Parkinson's disease. *Int J Biochem Cell Biol,* 42**,** 1775-8.

Lemke, M. R. (2008) Depressive symptoms in Parkinson's disease. *Eur J Neurol,* 15 Suppl 1**,** 21-5.

Letiembre, M., Y. Liu, S. Walter, W. Hao, T. Pfander, A. Wrede, W. Schulz-Schaeffer & K. Fassbender (2009) Screening of innate immune receptors in neurodegenerative diseases: a similar pattern. *Neurobiol Aging,* 30**,** 759-68.

Leverenz, J. B., R. Hamilton, D. W. Tsuang, A. Schantz, D. Vavrek, E. B. Larson, W. A. Kukull, O. Lopez, D. Galasko, E. Masliah, J. Kaye, R. Woltjer, C. Clark, J. Q. Trojanowski & T. J. Montine (2008) Empiric refinement of the pathologic assessment of Lewy-related pathology in the dementia patient. *Brain Pathol,* 18**,** 220-4.

Levi, G., L. Minghetti & F. Aloisi (1998) Regulation of prostanoid synthesis in microglial cells and effects of prostaglandin E2 on microglial functions. *Biochimie,* 80**,** 899-904.

Levin, H. & D. Smith (2013) Traumatic brain injury: networks and neuropathology. *Lancet Neurol,* 12**,** 15-6.

Levy, G., M. X. Tang, E. D. Louis, L. J. Cote, B. Alfaro, H. Mejia, Y. Stern & K. Marder (2002) The association of incident dementia with mortality in PD. *Neurology,* 59**,** 1708-13.

Levy-Lahad, E., W. Wasco, P. Poorkaj, D. M. Romano, J. Oshima, W. H. Pettingell, C. E. Yu, P. D. Jondro, S. D. Schmidt, K. Wang & et al. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science,* 269**,** 973-7.

Li, A. A., P. J. Mink, L. J. McIntosh, M. J. Teta & B. Finley (2005) Evaluation of epidemiologic and animal data associating pesticides with Parkinson's disease. *J Occup Environ Med,* 47**,** 1059-87.

Li, J., J. Y. Yang, X. C. Yao, X. Xue, Q. C. Zhang, X. X. Wang, L. L. Ding & C. F. Wu (2013) Oligomeric Abeta-induced microglial activation is possibly mediated by NADPH oxidase. *Neurochem Res,* 38**,** 443-52.

Li, M. O., Y. Y. Wan, S. Sanjabi, A. K. Robertson & R. A. Flavell (2006) Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol,* 24**,** 99-146.

Light, K. J., P. R. Joyce, S. E. Luty, R. T. Mulder, C. M. Frampton, L. R. Joyce, A. L. Miller & M. A. Kennedy (2006) Preliminary evidence for an association between a dopamine D3 receptor gene variant and obsessive-compulsive personality disorder in patients with major depression. *Am J Med Genet B Neuropsychiatr Genet,* 141B**,** 409-13.

Lindersson, E., R. Beedholm, P. Hojrup, T. Moos, W. Gai, K. B. Hendil & P. H. Jensen (2004) Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J Biol Chem,* 279**,** 12924-34.

Ling, G., F. Bandak, R. Armonda, G. Grant & J. Ecklund (2009) Explosive blast neurotrauma. *J Neurotrauma,* 26**,** 815-25.

Litteljohn, D., E. Mangano, M. Clarke, J. Bobyn, K. Moloney & S. Hayley (2010) Inflammatory mechanisms of neurodegeneration in toxin-based models of Parkinson's disease. *Parkinsons Dis,* 2011**,** 713517.

Liu, B. (2006) Modulation of microglial pro-inflammatory and neurotoxic activity for the treatment of Parkinson's disease. *AAPS J,* 8**,** E606-21.

Liu, L., O. Aboud, R. A. Jones, R. E. Mrak, W. S. Griffin & S. W. Barger (2011a) Apolipoprotein E expression is elevated by interleukin 1 and other interleukin 1-induced factors. *J Neuroinflammation,* 8**,** 175.

Liu, Y., S. Walter, M. Stagi, D. Cherny, M. Letiembre, W. Schulz-Schaeffer, H. Heine, B. Penke, H. Neumann & K. Fassbender (2005) LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. *Brain,* 128**,** 1778-89.

Liu, Z., A. Turkoz, E. N. Jackson, J. C. Corbo, J. A. Engelbach, J. R. Garbow, D. R. Piwnica-Worms & R. Kopan (2011b) Notch1 loss of heterozygosity causes vascular tumors and lethal hemorrhage in mice. *J Clin Invest,* 121**,** 800-8.

Lodge, P. A. & S. Sriram (1996) Regulation of microglial activation by TGF-beta, IL-10, and CSF-1. *J Leukoc Biol,* 60**,** 502-8.

Loffler, J. & G. Huber (1992) Beta-amyloid precursor protein isoforms in various rat brain regions and during brain development. *J Neurochem,* 59**,** 1316-24.

Lomakin, A., D. B. Teplow, D. A. Kirschner & G. B. Benedek (1997) Kinetic theory of fibrillogenesis of amyloid beta-protein. *Proc Natl Acad Sci U S A,* 94**,** 7942-7.

Lotharius, J. & P. Brundin (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. *Nat Rev Neurosci,* 3**,** 932-42.

Louis, E. D., K. Marder, L. Cote, M. Tang & R. Mayeux (1997) Mortality from Parkinson disease. *Arch Neurol,* 54**,** 260-4.

Lozza, C., R. M. Marie & J. C. Baron (2002) The metabolic substrates of bradykinesia and tremor in uncomplicated Parkinson's disease. *Neuroimage,* 17**,** 688-99.

Luchsinger, J. A., M. X. Tang, Y. Stern, S. Shea & R. Mayeux (2001) Diabetes mellitus and risk of Alzheimer's disease and dementia with stroke in a multiethnic cohort. *Am J Epidemiol,* 154**,** 635-41.

Lue, L. F., L. Brachova, W. H. Civin & J. Rogers (1996) Inflammation, A beta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. *J Neuropathol Exp Neurol,* 55**,** 1083-8.

Lue, L. F., D. G. Walker & J. Rogers (2001) Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. *Neurobiol Aging,* 22**,** 945-56.

Maccioni, R. B., G. Farias, I. Morales & L. Navarrete (2010) The revitalized tau hypothesis on Alzheimer's disease. *Arch Med Res,* 41**,** 226-31.

Maeda, S., N. Sahara, Y. Saito, M. Murayama, Y. Yoshiike, H. Kim, T. Miyasaka, S. Murayama, A. Ikai & A. Takashima (2007) Granular tau oligomers as intermediates of tau filaments. *Biochemistry,* 46**,** 3856-61.

Maezawa, I., P. I. Zimin, H. Wulff & L. W. Jin (2011) Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity. *J Biol Chem,* 286**,** 3693-706.

Mahley, R. W. (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science,* 240**,** 622-30.

Makwana, M., L. L. Jones, D. Cuthill, H. Heuer, M. Bohatschek, M. Hristova, S. Friedrichsen, I. Ormsby, D. Bueringer, A. Koppius, K. Bauer, T. Doetschman & G. Raivich (2007) Endogenous transforming growth factor beta 1 suppresses inflammation and promotes survival in adult CNS. *J Neurosci,* 27**,** 11201-13.

Malm, T. M., J. Magga, G. F. Kuh, T. Vatanen, M. Koistinaho & J. Koistinaho (2008) Minocycline reduces engraftment and activation of bone marrow-derived cells but sustains their phagocytic activity in a mouse model of Alzheimer's disease. *Glia,* 56**,** 1767-79.

Mangat, H. S. (2012) Severe traumatic brain injury. *Continuum (Minneap Minn),* 18**,** 532-46.

Mann, D. M., P. O. Yates & J. Hawkes (1982) The noradrenergic system in Alzheimer and multi-infarct dementias. *J Neurol Neurosurg Psychiatry,* 45**,** 113-9.

Manning-Bog, A. B., A. L. McCormack, J. Li, V. N. Uversky, A. L. Fink & D. A. Di Monte (2002) The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J Biol Chem,* 277**,** 1641-4.

Marsh, L., J. R. Williams, M. Rocco, S. Grill, C. Munro & T. M. Dawson (2004) Psychiatric comorbidities in patients with Parkinson disease and psychosis. *Neurology,* 63**,** 293-300.

Martinez, F. O., L. Helming & S. Gordon (2009) Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol,* 27**,** 451-83.

Mattson, M. P., B. Cheng, A. R. Culwell, F. S. Esch, I. Lieberburg & R. E. Rydel (1993) Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron,* 10**,** 243-54.

Mayer, A. R., J. Ling, M. V. Mannell, C. Gasparovic, J. P. Phillips, D. Doezema, R. Reichard & R. A. Yeo (2010) A prospective diffusion tensor imaging study in mild traumatic brain injury. *Neurology,* 74**,** 643-50.

McCrory, P., W. Meeuwisse, K. Johnston, J. Dvorak, M. Aubry, M. Molloy & R. Cantu (2009) Consensus statement on concussion in sport - the Third International Conference on Concussion in Sport held in Zurich, November 2008. *Phys Sportsmed,* 37**,** 141-59.

McGeer, P. L. & E. G. McGeer (2008) Glial reactions in Parkinson's disease. *Mov Disord,* 23**,** 474-83.

McGowan, E., F. Pickford, J. Kim, L. Onstead, J. Eriksen, C. Yu, L. Skipper, M. P. Murphy, J. Beard, P. Das, K. Jansen, M. Delucia, W. L. Lin, G. Dolios, R. Wang, C. B. Eckman, D. W. Dickson, M. Hutton, J. Hardy & T. Golde (2005) Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron,* 47**,** 191-9.

McGreal, E. P., J. L. Miller & S. Gordon (2005) Ligand recognition by antigen-presenting cell C-type lectin receptors. *Curr Opin Immunol,* 17**,** 18-24.

McKee, A. C., R. C. Cantu, C. J. Nowinski, E. T. Hedley-Whyte, B. E. Gavett, A. E. Budson, V. E. Santini, H. S. Lee, C. A. Kubilus & R. A. Stern (2009) Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol,* 68**,** 709-35.

McKeith, I. G., D. W. Dickson, J. Lowe, M. Emre, J. T. O'Brien, H. Feldman, J. Cummings, J. E. Duda, C. Lippa, E. K. Perry, D. Aarsland, H. Arai, C. G. Ballard, B. Boeve, D. J. Burn, D. Costa, T. Del Ser, B. Dubois, D. Galasko, S. Gauthier, C. G. Goetz, E. Gomez-Tortosa, G. Halliday, L. A. Hansen, J. Hardy, T. Iwatsubo, R. N. Kalaria, D. Kaufer, R. A. Kenny, A. Korczyn, K. Kosaka, V. M. Lee, A. Lees, I. Litvan, E. Londos, O. L. Lopez, S. Minoshima, Y. Mizuno, J. A. Molina, E. B. Mukaetova-Ladinska, F. Pasquier, R. H. Perry, J. B. Schulz, J. Q. Trojanowski & M. Yamada (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology,* 65**,** 1863-72.

McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Price & E. M. Stadlan (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology,* 34**,** 939-44.

McNaught, K. S. & P. Jenner (2001) Proteasomal function is impaired in substantia nigra in Parkinson's disease. *Neurosci Lett,* 297**,** 191-4.

Mendes-Jorge, L., D. Ramos, M. Luppo, C. Llombart, G. Alexandre-Pires, V. Nacher, V. Melgarejo, M. Correia, M. Navarro, A. Carretero, S. Tafuro, A. Rodriguez-Baeza, J. A. Esperanca-Pina, F. Bosch & J. Ruberte (2009) Scavenger function of resident autofluorescent perivascular macrophages and their contribution to the maintenance of the blood-retinal barrier. *Invest Ophthalmol Vis Sci,* 50**,** 5997-6005.

Menon, D. K., K. Schwab, D. W. Wright, A. I. Maas, Demographics, I. Clinical Assessment Working Group of the, I. Interagency Initiative toward Common Data Elements for Research on Traumatic Brain & H. Psychological (2010) Position statement: definition of traumatic brain injury. *Arch Phys Med Rehabil,* 91**,** 1637-40.

Milatovic, D., S. Zaja-Milatovic, K. S. Montine, F. S. Shie & T. J. Montine (2004) Neuronal oxidative damage and dendritic degeneration following activation of CD14-dependent innate immune response in vivo. *J Neuroinflammation,* 1**,** 20.

Mildner, A., H. Schmidt, M. Nitsche, D. Merkler, U. K. Hanisch, M. Mack, M. Heikenwalder, W. Bruck, J. Priller & M. Prinz (2007) Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci,* 10**,** 1544-53.

Miller, D. W., S. M. Hague, J. Clarimon, M. Baptista, K. Gwinn-Hardy, M. R. Cookson & A. B. Singleton (2004) Alpha-synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. *Neurology,* 62**,** 1835-8.

Milward, E. A., R. Papadopoulos, S. J. Fuller, R. D. Moir, D. Small, K. Beyreuther & C. L. Masters (1992) The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. *Neuron,* 9**,** 129-37.

Minghetti, L., M. A. Ajmone-Cat, M. A. De Berardinis & R. De Simone (2005) Microglial activation in chronic neurodegenerative diseases: roles of apoptotic neurons and chronic stimulation. *Brain Res Brain Res Rev,* 48**,** 251-6.

Minoretti, P., C. Gazzaruso, C. D. Vito, E. Emanuele, M. Bianchi, E. Coen, M. Reino & D. Geroldi (2006) Effect of the functional toll-like receptor 4 Asp299Gly polymorphism on susceptibility to late-onset Alzheimer's disease. *Neurosci Lett,* 391**,** 147-9.

Mintun, M. A., G. N. Larossa, Y. I. Sheline, C. S. Dence, S. Y. Lee, R. H. Mach, W. E. Klunk, C. A. Mathis, S. T. DeKosky & J. C. Morris (2006) [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. *Neurology,* 67**,** 446-52.

Mitsumoto, A. & Y. Nakagawa (2001) DJ-1 is an indicator for endogenous reactive oxygen species elicited by endotoxin. *Free Radic Res,* 35**,** 885-93.

Miyoshi, K. (2009) What is 'early onset dementia'? *Psychogeriatrics,* 9**,** 67-72.

Mogi, M., M. Harada, H. Narabayashi, H. Inagaki, M. Minami & T. Nagatsu (1996) Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett,* 211**,** 13-6.

Mogi, M., A. Togari, T. Kondo, Y. Mizuno, O. Komure, S. Kuno, H. Ichinose & T. Nagatsu (1999) Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. *Neurosci Lett,* 270**,** 45-8.

--- (2000) Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. *J Neural Transm,* 107**,** 335-41.

Molina-Holgado, F., R. Grencis & N. J. Rothwell (2001) Actions of exogenous and endogenous IL-10 on glial responses to bacterial LPS/cytokines. *Glia,* 33**,** 97-106.

Montaner, L. J., R. P. da Silva, J. Sun, S. Sutterwala, M. Hollinshead, D. Vaux & S. Gordon (1999) Type 1 and type 2 cytokine regulation of macrophage endocytosis: differential activation by IL-4/IL-13 as opposed to IFN-gamma or IL-10. *J Immunol,* 162**,** 4606-13.

Moore, K. W., R. de Waal Malefyt, R. L. Coffman & A. O'Garra (2001) Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol,* 19**,** 683-765.

Morales, I., G. Farias & R. B. Maccioni (2010) Neuroimmunomodulation in the pathogenesis of Alzheimer's disease. *Neuroimmunomodulation,* 17**,** 202-4.

Moretti, L., I. Cristofori, S. M. Weaver, A. Chau, J. N. Portelli & J. Grafman (2012) Cognitive decline in older adults with a history of traumatic brain injury. *Lancet Neurol,* 11**,** 1103-12.

Mori, F., K. Tanji, M. Yoshimoto, H. Takahashi & K. Wakabayashi (2002) Demonstration of alpha-synuclein immunoreactivity in neuronal and glial cytoplasm in normal human brain tissue using proteinase K and formic acid pretreatment. *Exp Neurol,* 176**,** 98-104.

Morris, J. C., C. M. Roe, C. Xiong, A. M. Fagan, A. M. Goate, D. M. Holtzman & M. A. Mintun (2010) APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol,* 67**,** 122-31.

Morris, J. C., M. Storandt, D. W. McKeel, Jr., E. H. Rubin, J. L. Price, E. A. Grant & L. Berg (1996) Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. *Neurology,* 46**,** 707-19.

Morris, S. M., Jr. (2007) Arginine metabolism: boundaries of our knowledge. *J Nutr,* 137**,** 1602S-1609S.

Mortimer, J. A., L. R. French, J. T. Hutton & L. M. Schuman (1985) Head injury as a risk factor for Alzheimer's disease. *Neurology,* 35**,** 264-7.

Mosser, D. M. & X. Zhang (2008) Interleukin-10: new perspectives on an old cytokine. *Immunol Rev,* 226**,** 205-18.

Mrak, R. E. (2012) Microglia in Alzheimer brain: a neuropathological perspective. *Int J Alzheimers Dis,* 2012**,** 165021.

Mrak, R. E. & W. S. Griffin (2001) Interleukin-1, neuroinflammation, and Alzheimer's disease. *Neurobiol Aging,* 22**,** 903-8.

Mueller, S. G., N. Schuff, K. Yaffe, C. Madison, B. Miller & M. W. Weiner (2010) Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. *Hum Brain Mapp,* 31**,** 1339-47.

Murer, M. G., Q. Yan & R. Raisman-Vozari (2001) Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog Neurobiol,* 63**,** 71-124.

Murphy, D. D., S. M. Rueter, J. Q. Trojanowski & V. M. Lee (2000) Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J Neurosci,* 20**,** 3214-20.

Musiani, S. & M. G. Battelli (2003) Mannose receptor determination by an ELISA-like method. *J Biochem Biophys Methods,* 55**,** 121-5.

Nagatsu, T., M. Mogi, H. Ichinose & A. Togari (2000) Changes in cytokines and neurotrophins in Parkinson's disease. *J Neural Transm Suppl***,** 277-90.

Neumann, H. (2003) Molecular mechanisms of axonal damage in inflammatory central nervous system diseases. *Curr Opin Neurol,* 16**,** 267-73.

Neumann, H., T. Misgeld, K. Matsumuro & H. Wekerle (1998) Neurotrophins inhibit major histocompatibility class II inducibility of microglia: involvement of the p75 neurotrophin receptor. *Proc Natl Acad Sci U S A,* 95**,** 5779-84.

Nguyen, T. T., E. J. Schwartz, R. B. West, R. A. Warnke, D. A. Arber & Y. Natkunam (2005) Expression of CD163 (hemoglobin scavenger receptor) in normal tissues, lymphomas, carcinomas, and sarcomas is largely restricted to the monocyte/macrophage lineage. *Am J Surg Pathol,* 29**,** 617-24.

Nicoll, J. A., D. Wilkinson, C. Holmes, P. Steart, H. Markham & R. O. Weller (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med,* 9**,** 448-52.

Nimmerjahn, F. & J. V. Ravetch (2006) Fcgamma receptors: old friends and new family members. *Immunity,* 24**,** 19-28.

Nolan, S. (2005) Traumatic brain injury: a review. *Crit Care Nurs Q,* 28**,** 188-94.

Norden, D. M. & J. P. Godbout (2013) Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol,* 39**,** 19-34.

Nuti, A., R. Ceravolo, A. Piccinni, G. Dell'Agnello, G. Bellini, G. Gambaccini, C. Rossi, C. Logi, L. Dell'Osso & U. Bonuccelli (2004) Psychiatric comorbidity in a population of Parkinson's disease patients. *Eur J Neurol,* 11**,** 315-20.

O'Callaghan, J. P., K. Sriram & D. B. Miller (2008) Defining "neuroinflammation". *Ann N Y Acad Sci,* 1139**,** 318-30.

O'Keefe, G. M., V. T. Nguyen & E. N. Benveniste (1999) Class II transactivator and class II MHC gene expression in microglia: modulation by the cytokines TGF-beta, IL-4, IL-13 and IL-10. *Eur J Immunol,* 29**,** 1275-85.

Oddo, S., A. Caccamo, M. Kitazawa, B. P. Tseng & F. M. LaFerla (2003a) Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging,* 24**,** 1063-70.

Oddo, S., A. Caccamo, J. D. Shepherd, M. P. Murphy, T. E. Golde, R. Kayed, R. Metherate, M. P. Mattson, Y. Akbari & F. M. LaFerla (2003b) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron,* 39**,** 409-21.

Oddo, S., A. Caccamo, L. Tran, M. P. Lambert, C. G. Glabe, W. L. Klein & F. M. LaFerla (2006) Temporal profile of amyloid-beta (Abeta) oligomerization in an in vivo model of Alzheimer disease. A link between Abeta and tau pathology. *J Biol Chem,* 281**,** 1599-604.

Odegaard, J. I., R. R. Ricardo-Gonzalez, M. H. Goforth, C. R. Morel, V. Subramanian, L. Mukundan, A. Red Eagle, D. Vats, F. Brombacher, A. W. Ferrante & A. Chawla (2007) Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature,* 447**,** 1116-20.

Ogden, C. A., J. D. Pound, B. K. Batth, S. Owens, I. Johannessen, K. Wood & C. D. Gregory (2005) Enhanced apoptotic cell clearance capacity and B cell survival factor production by IL-10-activated macrophages: implications for Burkitt's lymphoma. *J Immunol,* 174**,** 3015-23.

Ohsawa, K., Y. Imai, Y. Sasaki & S. Kohsaka (2004) Microglia/macrophage-specific protein Iba1 binds to fimbrin and enhances its actin-bundling activity. *J Neurochem,* 88**,** 844-56.

Olichney, J. M., L. A. Hansen, D. Galasko, T. Saitoh, C. R. Hofstetter, R. Katzman & L. J. Thal (1996) The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. *Neurology,* 47**,** 190-6.

Orrison, W. W., E. H. Hanson, T. Alamo, D. Watson, M. Sharma, T. G. Perkins & R. D. Tandy (2009) Traumatic brain injury: a review and high-field MRI findings in 100 unarmed combatants using a literature-based checklist approach. *J Neurotrauma,* 26**,** 689-701.

Ott, A., R. P. Stolk, F. van Harskamp, H. A. Pols, A. Hofman & M. M. Breteler (1999) Diabetes mellitus and the risk of dementia: The Rotterdam Study. *Neurology,* 53**,** 1937-42.

Ouchi, Y., S. Yagi, M. Yokokura & M. Sakamoto (2009) Neuroinflammation in the living brain of Parkinson's disease. *Parkinsonism Relat Disord,* 15 Suppl 3**,** S200-4.

Ouchi, Y., E. Yoshikawa, Y. Sekine, M. Futatsubashi, T. Kanno, T. Ogusu & T. Torizuka (2005) Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol,* 57**,** 168-75.

Pacher, P., J. S. Beckman & L. Liaudet (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol Rev,* 87**,** 315-424.

Padurariu, M., A. Ciobica, I. Mavroudis, D. Fotiou & S. Baloyannis (2012) Hippocampal neuronal loss in the CA1 and CA3 areas of Alzheimer's disease patients. *Psychiatr Danub,* 24**,** 152-8.

Parachikova, A., M. G. Agadjanyan, D. H. Cribbs, M. Blurton-Jones, V. Perreau, J. Rogers, T. G. Beach & C. W. Cotman (2007) Inflammatory changes parallel the early stages of Alzheimer disease. *Neurobiol Aging,* 28**,** 1821-33.

Parain, K., M. G. Murer, Q. Yan, B. Faucheux, Y. Agid, E. Hirsch & R. Raisman-Vozari (1999) Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport,* 10**,** 557-61.

Paris, D., K. Townsend, A. Quadros, J. Humphrey, J. Sun, S. Brem, M. Wotoczek-Obadia, A. DelleDonne, N. Patel, D. F. Obregon, R. Crescentini, L. Abdullah, D. Coppola, A. M. Rojiani, F. Crawford, S. M. Sebti & M. Mullan (2004) Inhibition of angiogenesis by Abeta peptides. *Angiogenesis,* 7**,** 75-85.

Parkkinen, L., T. Pirttila, M. Tervahauta & I. Alafuzoff (2005) Widespread and abundant alpha-synuclein pathology in a neurologically unimpaired subject. *Neuropathology,* 25**,** 304-14.

Parkkinen, L., H. Soininen, M. Laakso & I. Alafuzoff (2001) Alpha-synuclein pathology is highly dependent on the case selection. *Neuropathol Appl Neurobiol,* 27**,** 314-25.

Patel, N. K., M. Bunnage, P. Plaha, C. N. Svendsen, P. Heywood & S. S. Gill (2005) Intraputamenal infusion of glial cell line-derived neurotrophic factor in PD: a two-year outcome study. *Ann Neurol,* 57**,** 298-302.

Patton, R. L., W. M. Kalback, C. L. Esh, T. A. Kokjohn, G. D. Van Vickle, D. C. Luehrs, Y. M. Kuo, J. Lopez, D. Brune, I. Ferrer, E. Masliah, A. J. Newel, T. G. Beach, E. M. Castano & A. E. Roher (2006) Amyloid-beta peptide remnants in AN-1792-immunized Alzheimer's disease patients: a biochemical analysis. *Am J Pathol,* 169**,** 1048-63.

Pedersen, K. F., G. Alves, D. Aarsland & J. P. Larsen (2009) Occurrence and risk factors for apathy in Parkinson disease: a 4-year prospective longitudinal study. *J Neurol Neurosurg Psychiatry,* 80**,** 1279-82.

Pendlebury, S. T. & P. M. Rothwell (2009) Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. *Lancet Neurol,* 8**,** 1006-18.

Pereira, J. B., C. Junque, D. Bartres-Faz, B. Ramirez-Ruiz, M. J. Marti & E. Tolosa (2013) Regional vulnerability of hippocampal subfields and memory deficits in Parkinson's disease. *Hippocampus***,** 0.

Perier, C., K. Tieu, C. Guegan, C. Caspersen, V. Jackson-Lewis, V. Carelli, A. Martinuzzi, M. Hirano, S. Przedborski & M. Vila (2005) Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. *Proc Natl Acad Sci U S A,* 102**,** 19126-31.

Perl, D. P. (2010) Neuropathology of Alzheimer's disease. *Mt Sinai J Med,* 77**,** 32-42.

Perneczky, R., S. Wagenpfeil, K. Komossa, T. Grimmer, J. Diehl & A. Kurz (2006) Mapping scores onto stages: mini-mental state examination and clinical dementia rating. *Am J Geriatr Psychiatry,* 14**,** 139-44.

Perrin, R. J., A. M. Fagan & D. M. Holtzman (2009) Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature,* 461**,** 916-22.

Perry, V. H. (2004) The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease. *Brain Behav Immun,* 18**,** 407-13.

Perry, V. H., C. Cunningham & C. Holmes (2007) Systemic infections and inflammation affect chronic neurodegeneration. *Nat Rev Immunol,* 7**,** 161-7.

Perry, V. H., J. A. Nicoll & C. Holmes (2010) Microglia in neurodegenerative disease. *Nat Rev Neurol,* 6**,** 193-201.

Peterson, A. L. & J. G. Nutt (2008) Treatment of Parkinson's disease with trophic factors. *Neurotherapeutics,* 5**,** 270-80.

Pfeifer, L. A., L. R. White, G. W. Ross, H. Petrovitch & L. J. Launer (2002) Cerebral amyloid angiopathy and cognitive function: the HAAS autopsy study. *Neurology,* 58**,** 1629-34.

Pioli, P. A., K. E. Goonan, K. Wardwell & P. M. Guyre (2004) TGF-beta regulation of human macrophage scavenger receptor CD163 is Smad3-dependent. *J Leukoc Biol,* 76**,** 500-8.

Plassman, B. L., R. J. Havlik, D. C. Steffens, M. J. Helms, T. N. Newman, D. Drosdick, C. Phillips, B. A. Gau, K. A. Welsh-Bohmer, J. R. Burke, J. M. Guralnik & J. C.

Breitner (2000) Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology,* 55**,** 1158-66.

Polazzi, E., L. E. Altamira, S. Eleuteri, R. Barbaro, C. Casadio, A. Contestabile & B. Monti (2009) Neuroprotection of microglial conditioned medium on 6-hydroxydopamine-induced neuronal death: role of transforming growth factor beta-2. *J Neurochem,* 110**,** 545-56.

Politis, M., K. Wu, C. Loane, F. E. Turkheimer, S. Molloy, D. J. Brooks & P. Piccini (2010) Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. *Neurology,* 75**,** 1920-7.

Polymeropoulos, M. H., C. Lavedan, E. Leroy, S. E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, E. S. Stenroos, S. Chandrasekharappa, A. Athanassiadou, T. Papapetropoulos, W. G. Johnson, A. M. Lazzarini, R. C. Duvoisin, G. Di Iorio, L. I. Golbe & R. L. Nussbaum (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science,* 276**,** 2045-7.

Popescu, B. O., E. C. Toescu, L. M. Popescu, O. Bajenaru, D. F. Muresanu, M. Schultzberg & N. Bogdanovic (2009) Blood-brain barrier alterations in ageing and dementia. *J Neurol Sci,* 283**,** 99-106.

Porcheray, F., S. Viaud, A. C. Rimaniol, C. Leone, B. Samah, N. Dereuddre-Bosquet, D. Dormont & G. Gras (2005) Macrophage activation switching: an asset for the resolution of inflammation. *Clin Exp Immunol,* 142**,** 481-9.

Pott Godoy, M. C., R. Tarelli, C. C. Ferrari, M. I. Sarchi & F. J. Pitossi (2008) Central and systemic IL-1 exacerbates neurodegeneration and motor symptoms in a model of Parkinson's disease. *Brain,* 131**,** 1880-94.

Poussin, C., M. Foti, J. L. Carpentier & J. Pugin (1998) CD14-dependent endotoxin internalization via a macropinocytic pathway. *J Biol Chem,* 273**,** 20285-91.

Preston, S. D., P. V. Steart, A. Wilkinson, J. A. Nicoll & R. O. Weller (2003) Capillary and arterial cerebral amyloid angiopathy in Alzheimer's disease: defining the perivascular route for the elimination of amyloid beta from the human brain. *Neuropathol Appl Neurobiol,* 29**,** 106-17.

Price, J. L. & J. C. Morris (1999) Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol,* 45**,** 358-68.

Priller, C., T. Bauer, G. Mitteregger, B. Krebs, H. A. Kretzschmar & J. Herms (2006) Synapse formation and function is modulated by the amyloid precursor protein. *J Neurosci,* 26**,** 7212-21.

Pritchard, S. M., P. J. Dolan, A. Vitkus & G. V. Johnson (2011) The toxicity of tau in Alzheimer disease: turnover, targets and potential therapeutics. *J Cell Mol Med,* 15**,** 1621-35.

Probst, A., A. Bloch & M. Tolnay (2008) New insights into the pathology of Parkinson's disease: does the peripheral autonomic system become central? *Eur J Neurol,* 15 Suppl 1**,** 1-4.

Pulford, K., K. Micklem, S. McCarthy, J. Cordell, M. Jones & D. Y. Mason (1992) A monocyte/macrophage antigen recognized by the four antibodies GHI/61, Ber-MAC3, Ki-M8 and SM4. *Immunology,* 75**,** 588-95.

Qian, L., M. L. Block, S. J. Wei, C. F. Lin, J. Reece, H. Pang, B. Wilson, J. S. Hong & P. M. Flood (2006) Interleukin-10 protects lipopolysaccharide-induced neurotoxicity in primary midbrain cultures by inhibiting the function of NADPH oxidase. *J Pharmacol Exp Ther,* 319**,** 44-52.

Qian, L. & P. M. Flood (2008) Microglial cells and Parkinson's disease. *Immunol Res,* 41**,** 155-64.

Qin, L., X. Wu, M. L. Block, Y. Liu, G. R. Breese, J. S. Hong, D. J. Knapp & F. T. Crews (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia,* 55**,** 453-62.

Quik, M., X. A. Perez & T. Bordia (2012) Nicotine as a potential neuroprotective agent for Parkinson's disease. *Mov Disord,* 27**,** 947-57.

Rabinowitz, S. S. & S. Gordon (1991) Macrosialin, a macrophage-restricted membrane sialoprotein differentially glycosylated in response to inflammatory stimuli. *J Exp Med,* 174**,** 827-36.

Ramaker, C., J. Marinus, A. M. Stiggelbout & B. J. Van Hilten (2002) Systematic evaluation of rating scales for impairment and disability in Parkinson's disease. *Mov Disord,* 17**,** 867-76.

Ramirez, A., A. Heimbach, J. Grundemann, B. Stiller, D. Hampshire, L. P. Cid, I. Goebel, A. F. Mubaidin, A. L. Wriekat, J. Roeper, A. Al-Din, A. M. Hillmer, M. Karsak, B. Liss, C. G. Woods, M. I. Behrens & C. Kubisch (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet,* 38**,** 1184-91.

Ramlackhansingh, A. F., D. J. Brooks, R. J. Greenwood, S. K. Bose, F. E. Turkheimer, K. M. Kinnunen, S. Gentleman, R. A. Heckemann, K. Gunanayagam, G. Gelosa & D. J. Sharp (2011) Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol,* 70**,** 374-83.

Ransohoff, R. M., P. Kivisakk & G. Kidd (2003) Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol,* 3**,** 569-81.

Reale, M., C. Iarlori, A. Thomas, D. Gambi, B. Perfetti, M. Di Nicola & M. Onofrj (2009) Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun,* 23**,** 55-63.

Reddy, P. H., G. Mani, B. S. Park, J. Jacques, G. Murdoch, W. Whetsell, Jr., J. Kaye & M. Manczak (2005) Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J Alzheimers Dis,* 7**,** 103-17; discussion 173-80.

Reed-Geaghan, E. G., Q. W. Reed, P. E. Cramer & G. E. Landreth (2010) Deletion of CD14 attenuates Alzheimer's disease pathology by influencing the brain's inflammatory milieu. *J Neurosci,* 30**,** 15369-73.

Reed-Geaghan, E. G., J. C. Savage, A. G. Hise & G. E. Landreth (2009) CD14 and toll-like receptors 2 and 4 are required for fibrillar A{beta}-stimulated microglial activation. *J Neurosci,* 29**,** 11982-92.

Reinacher, P. C., C. Blum, P. Gass, C. P. Karger & J. Debus (1999) Quantification of microglial late reaction to stereotactic irradiation of the rat brain using computer-aided image analysis. *Exp Neurol,* 160**,** 117-23.

Reitz, C., C. Brayne & R. Mayeux (2011) Epidemiology of Alzheimer disease. *Nat Rev Neurol,* 7**,** 137-52.

Remy, P., M. Doder, A. Lees, N. Turjanski & D. Brooks (2005) Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system. *Brain,* 128**,** 1314-22.

Revesz, T., J. Ghiso, T. Lashley, G. Plant, A. Rostagno, B. Frangione & J. L. Holton (2003) Cerebral amyloid angiopathies: a pathologic, biochemical, and genetic view. *J Neuropathol Exp Neurol,* 62**,** 885-98.

Rey Nores, J. E., A. Bensussan, N. Vita, F. Stelter, M. A. Arias, M. Jones, S. Lefort, L. K. Borysiewicz, P. Ferrara & M. O. Labeta (1999) Soluble CD14 acts as a negative regulator of human T cell activation and function. *Eur J Immunol,* 29**,** 265-76.

Riederer, P., M. B. Youdim, S. Mandel, M. Gerlach & E. Grunblatt (2008) Genomic aspects of sporadic Parkinson's disease. *Parkinsonism Relat Disord,* 14 Suppl 2**,** S88-91.

Ring, S., S. W. Weyer, S. B. Kilian, E. Waldron, C. U. Pietrzik, M. A. Filippov, J. Herms, C. Buchholz, C. B. Eckman, M. Korte, D. P. Wolfer & U. C. Muller (2007) The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. *J Neurosci,* 27**,** 7817-26.

Rite, I., A. Machado, J. Cano & J. L. Venero (2007) Blood-brain barrier disruption induces in vivo degeneration of nigral dopaminergic neurons. *J Neurochem,* 101**,** 1567-82.

Ritter, M., C. Buechler, M. Kapinsky & G. Schmitz (2001) Interaction of CD163 with the regulatory subunit of casein kinase II (CKII) and dependence of CD163 signaling on CKII and protein kinase C. *Eur J Immunol,* 31**,** 999-1009.

Ritter, M., C. Buechler, T. Langmann & G. Schmitz (1999) Genomic organization and chromosomal localization of the human CD163 (M130) gene: a member of the scavenger receptor cysteine-rich superfamily. *Biochem Biophys Res Commun,* 260**,** 466-74.

Roberson, E. D., K. Scearce-Levie, J. J. Palop, F. Yan, I. H. Cheng, T. Wu, H. Gerstein, G. Q. Yu & L. Mucke (2007) Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science,* 316**,** 750-4.

Roberts, E. S., E. Masliah & H. S. Fox (2004) CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). *J Neuropathol Exp Neurol,* 63**,** 1255-64.

Roberts, G. W., S. M. Gentleman, A. Lynch, L. Murray, M. Landon & D. I. Graham (1994) Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiatry,* 57**,** 419-25.

Rocchi, A., S. Pellegrini, G. Siciliano & L. Murri (2003) Causative and susceptibility genes for Alzheimer's disease: a review. *Brain Res Bull,* 61**,** 1-24.

Rosenfeld, J. V., A. I. Maas, P. Bragge, M. C. Morganti-Kossmann, G. T. Manley & R. L. Gruen (2012) Early management of severe traumatic brain injury. *Lancet,* 380**,** 1088-98.

Rossler, M., R. Zarski, J. Bohl & T. G. Ohm (2002) Stage-dependent and sector-specific neuronal loss in hippocampus during Alzheimer's disease. *Acta Neuropathol,* 103**,** 363-9.

Rozemuller, A. J., P. Eikelenboom, J. W. Theeuwes, E. N. Jansen Steur & R. A. de Vos (2000) Activated microglial cells and complement factors are unrelated to cortical Lewy bodies. *Acta Neuropathol,* 100**,** 701-8.

Rubartelli, A. & M. T. Lotze (2007) Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol,* 28**,** 429-36.

Ruocco, A., O. Nicole, F. Docagne, C. Ali, L. Chazalviel, S. Komesli, F. Yablonsky, S. Roussel, E. T. MacKenzie, D. Vivien & A. Buisson (1999) A transforming growth factor-beta antagonist unmasks the neuroprotective role of this endogenous

cytokine in excitotoxic and ischemic brain injury. *J Cereb Blood Flow Metab,* 19**,** 1345-53.

Ryu, J. K. & J. G. McLarnon (2009) A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. *J Cell Mol Med,* 13**,** 2911-25.

Sabo, S. L., A. F. Ikin, J. D. Buxbaum & P. Greengard (2001) The Alzheimer amyloid precursor protein (APP) and FE65, an APP-binding protein, regulate cell movement. *J Cell Biol,* 153**,** 1403-14.

Sagare, A., R. Deane, R. D. Bell, B. Johnson, K. Hamm, R. Pendu, A. Marky, P. J. Lenting, Z. Wu, T. Zarcone, A. Goate, K. Mayo, D. Perlmutter, M. Coma, Z. Zhong & B. V. Zlokovic (2007) Clearance of amyloid-beta by circulating lipoprotein receptors. *Nat Med,* 13**,** 1029-31.

Salminen, A., J. Ojala, A. Kauppinen, K. Kaarniranta & T. Suuronen (2009) Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence via pattern recognition receptors. *Prog Neurobiol,* 87**,** 181-94.

Samii, A., M. Etminan, M. O. Wiens & S. Jafari (2009) NSAID use and the risk of Parkinson's disease: systematic review and meta-analysis of observational studies. *Drugs Aging,* 26**,** 769-79.

Sanchez, C., N. Domenech, J. Vazquez, F. Alonso, A. Ezquerra & J. Dominguez (1999) The porcine 2A10 antigen is homologous to human CD163 and related to macrophage differentiation. *J Immunol,* 162**,** 5230-7.

Sareen, J., D. W. Campbell, W. D. Leslie, K. L. Malisza, M. B. Stein, M. P. Paulus, L. B. Kravetsky, K. D. Kjernisted, J. R. Walker & J. P. Reiss (2007) Striatal function in generalized social phobia: a functional magnetic resonance imaging study. *Biol Psychiatry,* 61**,** 396-404.

Schaer, D. J., A. I. Alayash & P. W. Buehler (2007) Gating the radical hemoglobin to macrophages: the anti-inflammatory role of CD163, a scavenger receptor. *Antioxid Redox Signal,* 9**,** 991-9.

Schaer, D. J., F. S. Boretti, G. Schoedon & A. Schaffner (2002) Induction of the CD163-dependent haemoglobin uptake by macrophages as a novel anti-inflammatory action of glucocorticoids. *Br J Haematol,* 119**,** 239-43.

Schapira, A. H. (2008) Progress in neuroprotection in Parkinson's disease. *Eur J Neurol,* 15 Suppl 1**,** 5-13.

--- (2009) Neurobiology and treatment of Parkinson's disease. *Trends Pharmacol Sci,* 30**,** 41-7.

Schapira, A. H. & J. Obeso (2006) Timing of treatment initiation in Parkinson's disease: a need for reappraisal? *Ann Neurol,* 59**,** 559-62.

Scheff, S. W. & D. A. Price (1993) Synapse loss in the temporal lobe in Alzheimer's disease. *Ann Neurol,* 33**,** 190-9.

--- (1998) Synaptic density in the inner molecular layer of the hippocampal dentate gyrus in Alzheimer disease. *J Neuropathol Exp Neurol,* 57**,** 1146-53.

Scheff, S. W., D. L. Sparks & D. A. Price (1996) Quantitative assessment of synaptic density in the outer molecular layer of the hippocampal dentate gyrus in Alzheimer's disease. *Dementia,* 7**,** 226-32.

Schenk, D., M. Hagen & P. Seubert (2004) Current progress in beta-amyloid immunotherapy. *Curr Opin Immunol,* 16**,** 599-606.

Schlegel, R. A., S. Krahling, M. K. Callahan & P. Williamson (1999) CD14 is a component of multiple recognition systems used by macrophages to phagocytose apoptotic lymphocytes. *Cell Death Differ,* 6**,** 583-92.

Schmidt, M. L., V. Zhukareva, K. L. Newell, V. M. Lee & J. Q. Trojanowski (2001) Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathol,* 101**,** 518-24.

Schmitz, C. & P. R. Hof (2005) Design-based stereology in neuroscience. *Neuroscience,* 130**,** 813-31.

Schneider, J. A., J. L. Li, Y. Li, R. S. Wilson, J. H. Kordower & D. A. Bennett (2006) Substantia nigra tangles are related to gait impairment in older persons. *Ann Neurol,* 59**,** 166-73.

Schor, N. F. (2011) What the halted phase III γ-secretase inhibitor trial may (or may not) be telling us. *Ann Neurol,* 69**,** 237-9.

Schrag, A. (2006) Quality of life and depression in Parkinson's disease. *J Neurol Sci,* 248**,** 151-7.

Schulz-Schaeffer, W. J. (2010) The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta Neuropathol,* 120**,** 131-43.

Scotton, C. J., F. O. Martinez, M. J. Smelt, M. Sironi, M. Locati, A. Mantovani & S. Sozzani (2005) Transcriptional profiling reveals complex regulation of the monocyte IL-1 beta system by IL-13. *J Immunol,* 174**,** 834-45.

Serhan, C. N. & J. Savill (2005) Resolution of inflammation: the beginning programs the end. *Nat Immunol,* 6**,** 1191-7.

Sheffield, L. G. & N. E. Berman (1998) Microglial expression of MHC class II increases in normal aging of nonhuman primates. *Neurobiol Aging,* 19**,** 47-55.

Sheffield, L. G., J. G. Marquis & N. E. Berman (2000) Regional distribution of cortical microglia parallels that of neurofibrillary tangles in Alzheimer's disease. *Neurosci Lett,* 285**,** 165-8.

Shen, L., A. Figurov & B. Lu (1997) Recent progress in studies of neurotrophic factors and their clinical implications. *J Mol Med (Berl),* 75**,** 637-44.

Sheng, J. G., S. H. Bora, G. Xu, D. R. Borchelt, D. L. Price & V. E. Koliatsos (2003) Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice. *Neurobiol Dis,* 14**,** 133-45.

Sheng, J. G., R. E. Mrak & W. S. Griffin (1997a) Glial-neuronal interactions in Alzheimer disease: progressive association of IL-1alpha+ microglia and S100beta+ astrocytes with neurofibrillary tangle stages. *J Neuropathol Exp Neurol,* 56**,** 285-90.

--- (1997b) Neuritic plaque evolution in Alzheimer's disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms. *Acta Neuropathol,* 94**,** 1-5.

Sherrington, R., E. I. Rogaev, Y. Liang, E. A. Rogaeva, G. Levesque, M. Ikeda, H. Chi, C. Lin, G. Li, K. Holman, T. Tsuda, L. Mar, J. F. Foncin, A. C. Bruni, M. P. Montesi, S. Sorbi, I. Rainero, L. Pinessi, L. Nee, I. Chumakov, D. Pollen, A. Brookes, P. Sanseau, R. J. Polinsky, W. Wasco, H. A. Da Silva, J. L. Haines, M. A. Perkicak-Vance, R. E. Tanzi, A. D. Roses, P. E. Fraser, J. M. Rommens & P. H. St George-Hyslop (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature,* 375**,** 754-60.

Shin, W. H., D. Y. Lee, K. W. Park, S. U. Kim, M. S. Yang, E. H. Joe & B. K. Jin (2004) Microglia expressing interleukin-13 undergo cell death and contribute to neuronal survival in vivo. *Glia,* 46**,** 142-52.

Sian, J., D. T. Dexter, A. J. Lees, S. Daniel, P. Jenner & C. D. Marsden (1994) Glutathione-related enzymes in brain in Parkinson's disease. *Ann Neurol,* 36**,** 356-61.

Siest, G., T. Pillot, A. Regis-Bailly, B. Leininger-Muller, J. Steinmetz, M. M. Galteau & S. Visvikis (1995) Apolipoprotein E: an important gene and protein to follow in laboratory medicine. *Clin Chem,* 41**,** 1068-86.

Simard, A. R. & S. Rivest (2004) Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J,* 18**,** 998-1000.

Simard, A. R., D. Soulet, G. Gowing, J. P. Julien & S. Rivest (2006) Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron,* 49**,** 489-502.

Simpson, J. E., M. S. Fernando, L. Clark, P. G. Ince, F. Matthews, G. Forster, J. T. O'Brien, R. Barber, R. N. Kalaria, C. Brayne, P. J. Shaw, C. E. Lewis, S. B.

Wharton, M. R. C. C. Function & G. Ageing Neuropathology Study (2007) White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. *Neuropathol Appl Neurobiol,* 33**,** 410-9.

Smith, C. (2013) Review: the long-term consequences of microglial activation following acute traumatic brain injury. *Neuropathol Appl Neurobiol,* 39**,** 35-44.

Smith, C., S. M. Gentleman, P. D. Leclercq, L. S. Murray, W. S. Griffin, D. I. Graham & J. A. Nicoll (2012) The neuroinflammatory response in humans after traumatic brain injury. *Neuropathol Appl Neurobiol*.

Smith, D. H., R. Hicks & J. T. Povlishock (2013) Therapy development for diffuse axonal injury. *J Neurotrauma,* 30**,** 307-23.

Smith, D. H., D. F. Meaney & W. H. Shull (2003) Diffuse axonal injury in head trauma. *J Head Trauma Rehabil,* 18**,** 307-16.

Soba, P., S. Eggert, K. Wagner, H. Zentgraf, K. Siehl, S. Kreger, A. Lower, A. Langer, G. Merdes, R. Paro, C. L. Masters, U. Muller, S. Kins & K. Beyreuther (2005) Homo- and heterodimerization of APP family members promotes intercellular adhesion. *EMBO J,* 24**,** 3624-34.

Sofic, E., W. Paulus, K. Jellinger, P. Riederer & M. B. Youdim (1991) Selective increase of iron in substantia nigra zona compacta of parkinsonian brains. *J Neurochem,* 56**,** 978-82.

Sommer, B. (2002) Alzheimer's disease and the amyloid cascade hypothesis: ten years on. *Curr Opin Pharmacol,* 2**,** 87-92.

Soulet, D. & S. Rivest (2008) Bone-marrow-derived microglia: myth or reality? *Curr Opin Pharmacol,* 8**,** 508-18.

Spillantini, M. G. (1999) Parkinson's disease, dementia with Lewy bodies and multiple system atrophy are alpha-synucleinopathies. *Parkinsonism Relat Disord,* 5**,** 157-62.

Spillantini, M. G. & M. Goedert (2000) The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. *Ann N Y Acad Sci,* 920**,** 16-27.

Spires-Jones, T. L., K. J. Kopeikina, R. M. Koffie, A. de Calignon & B. T. Hyman (2011) Are tangles as toxic as they look? *J Mol Neurosci,* 45**,** 438-44.

Sterka, D., Jr. & I. Marriott (2006) Characterization of nucleotide-binding oligomerization domain (NOD) protein expression in primary murine microglia. *J Neuroimmunol,* 179**,** 65-75.

Stolzing, A. & T. Grune (2004) Neuronal apoptotic bodies: phagocytosis and degradation by primary microglial cells. *FASEB J,* 18**,** 743-5.

Stoothoff, W. H. & G. V. Johnson (2005) Tau phosphorylation: physiological and pathological consequences. *Biochim Biophys Acta,* 1739**,** 280-97.

Stout, R. D. & J. Suttles (2004) Functional plasticity of macrophages: reversible adaptation to changing microenvironments. *J Leukoc Biol,* 76**,** 509-13.

Streit, W. J., H. Braak, Q. S. Xue & I. Bechmann (2009) Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. *Acta Neuropathol,* 118**,** 475-85.

Strittmatter, W. J., K. H. Weisgraber, M. Goedert, A. M. Saunders, D. Huang, E. H. Corder, L. M. Dong, R. Jakes, M. J. Alberts, J. R. Gilbert & et al. (1994) Hypothesis: microtubule instability and paired helical filament formation in the Alzheimer disease brain are related to apolipoprotein E genotype. *Exp Neurol,* 125**,** 163-71; discussion 172-4.

Sulahian, T. H., P. Hogger, A. E. Wahner, K. Wardwell, N. J. Goulding, C. Sorg, A. Droste, M. Stehling, P. K. Wallace, P. M. Morganelli & P. M. Guyre (2000) Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. *Cytokine,* 12**,** 1312-21.

Sulzer, D., J. Bogulavsky, K. E. Larsen, G. Behr, E. Karatekin, M. H. Kleinman, N. Turro, D. Krantz, R. H. Edwards, L. A. Greene & L. Zecca (2000) Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc Natl Acad Sci U S A,* 97**,** 11869-74.

Suzuki, N., T. T. Cheung, X. D. Cai, A. Odaka, L. Otvos, Jr., C. Eckman, T. E. Golde & S. G. Younkin (1994) An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. *Science,* 264**,** 1336-40.

Takasugi, N., T. Tomita, I. Hayashi, M. Tsuruoka, M. Niimura, Y. Takahashi, G. Thinakaran & T. Iwatsubo (2003) The role of presenilin cofactors in the gamma-secretase complex. *Nature,* 422**,** 438-41.

Tamo, W., T. Imaizumi, K. Tanji, H. Yoshida, F. Mori, M. Yoshimoto, H. Takahashi, I. Fukuda, K. Wakabayashi & K. Satoh (2002) Expression of alpha-synuclein, the precursor of non-amyloid beta component of Alzheimer's disease amyloid, in human cerebral blood vessels. *Neurosci Lett,* 326**,** 5-8.

Tang-Schomer, M. D., V. E. Johnson, P. W. Baas, W. Stewart & D. H. Smith (2012) Partial interruption of axonal transport due to microtubule breakage accounts for the formation of periodic varicosities after traumatic axonal injury. *Exp Neurol,* 233**,** 364-72.

Tansey, M. G. & M. S. Goldberg (2010) Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. *Neurobiol Dis,* 37**,** 510-8.

Tasker, R. C., C. H. Salmond, A. G. Westland, A. Pena, J. H. Gillard, B. J. Sahakian & J. D. Pickard (2005) Head circumference and brain and hippocampal volume after severe traumatic brain injury in childhood. *Pediatr Res,* 58**,** 302-8.

Taylor, P. R., S. Gordon & L. Martinez-Pomares (2005a) The mannose receptor: linking homeostasis and immunity through sugar recognition. *Trends Immunol,* 26**,** 104-10.

Taylor, P. R., L. Martinez-Pomares, M. Stacey, H. H. Lin, G. D. Brown & S. Gordon (2005b) Macrophage receptors and immune recognition. *Annu Rev Immunol,* 23**,** 901-44.

Teismann, P. & J. B. Schulz (2004) Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation. *Cell Tissue Res,* 318**,** 149-61.

Teismann, P., K. Tieu, D. K. Choi, D. C. Wu, A. Naini, S. Hunot, M. Vila, V. Jackson-Lewis & S. Przedborski (2003) Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc Natl Acad Sci U S A,* 100**,** 5473-8.

Tennent, G. A., L. B. Lovat & M. B. Pepys (1995) Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. *Proc Natl Acad Sci U S A,* 92**,** 4299-303.

Thal, D. R., E. Capetillo-Zarate, S. Larionov, M. Staufenbiel, S. Zurbruegg & N. Beckmann (2009) Capillary cerebral amyloid angiopathy is associated with vessel occlusion and cerebral blood flow disturbances. *Neurobiol Aging,* 30**,** 1936-48.

Thal, D. R., E. Ghebremedhin, U. Rub, H. Yamaguchi, K. Del Tredici & H. Braak (2002) Two types of sporadic cerebral amyloid angiopathy. *J Neuropathol Exp Neurol,* 61**,** 282-93.

Theodore, S., S. Cao, P. J. McLean & D. G. Standaert (2008) Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *J Neuropathol Exp Neurol,* 67**,** 1149-58.

Thomas, W. E. (1999) Brain macrophages: on the role of pericytes and perivascular cells. *Brain Res Brain Res Rev,* 31**,** 42-57.

Thornton, E., R. Vink, P. C. Blumbergs & C. Van Den Heuvel (2006) Soluble amyloid precursor protein alpha reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Res,* 1094**,** 38-46.

Tikka, T. M. & J. E. Koistinaho (2001) Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol,* 166**,** 7527-33.

Tofaris, G. K. & M. G. Spillantini (2005) Alpha-synuclein dysfunction in Lewy body diseases. *Mov Disord,* 20 Suppl 12**,** S37-44.

Tompkins, M. M. & W. D. Hill (1997) Contribution of somal Lewy bodies to neuronal death. *Brain Res,* 775**,** 24-9.

Topoll, H. H., G. Zwadlo, D. E. Lange & C. Sorg (1989) Phenotypic dynamics of macrophage subpopulations during human experimental gingivitis. *J Periodontal Res,* 24**,** 106-12.

Town, T., Y. Laouar, C. Pittenger, T. Mori, C. A. Szekely, J. Tan, R. S. Duman & R. A. Flavell (2008) Blocking TGF-beta-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. *Nat Med,* 14**,** 681-7.

Tran, H. T., F. M. LaFerla, D. M. Holtzman & D. L. Brody (2011) Controlled cortical impact traumatic brain injury in 3xTg-AD mice causes acute intra-axonal amyloid-beta accumulation and independently accelerates the development of tau abnormalities. *J Neurosci,* 31**,** 9513-25.

Tran, H. T., L. Sanchez & D. L. Brody (2012) Inhibition of JNK by a peptide inhibitor reduces traumatic brain injury-induced tauopathy in transgenic mice. *J Neuropathol Exp Neurol,* 71**,** 116-29.

Tseng, B. P., K. N. Green, J. L. Chan, M. Blurton-Jones & F. M. LaFerla (2008) Abeta inhibits the proteasome and enhances amyloid and tau accumulation. *Neurobiol Aging,* 29**,** 1607-18.

Tsuboi, Y., H. Uchikado & D. W. Dickson (2007) Neuropathology of Parkinson's disease dementia and dementia with Lewy bodies with reference to striatal pathology. *Parkinsonism Relat Disord,* 13 Suppl 3**,** S221-4.

Turner, P. R., K. O'Connor, W. P. Tate & W. C. Abraham (2003) Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol,* 70**,** 1-32.

Ulevitch, R. J. & P. S. Tobias (1999) Recognition of gram-negative bacteria and endotoxin by the innate immune system. *Curr Opin Immunol,* 11**,** 19-22.

Ulvestad, E., K. Williams, S. Mork, J. Antel & H. Nyland (1994) Phenotypic differences between human monocytes/macrophages and microglial cells studied in situ and in vitro. *J Neuropathol Exp Neurol,* 53**,** 492-501.

Vagnozzi, R., S. Signoretti, B. Tavazzi, R. Floris, A. Ludovici, S. Marziali, G. Tarascio, A. M. Amorini, V. Di Pietro, R. Delfini & G. Lazzarino (2008) Temporal window of metabolic brain vulnerability to concussion: a pilot 1H-magnetic resonance

spectroscopic study in concussed athletes--part III. *Neurosurgery,* 62**,** 1286-95; discussion 1295-6.

Valente, E. M., P. M. Abou-Sleiman, V. Caputo, M. M. Muqit, K. Harvey, S. Gispert, Z. Ali, D. Del Turco, A. R. Bentivoglio, D. G. Healy, A. Albanese, R. Nussbaum, R. Gonzalez-Maldonado, T. Deller, S. Salvi, P. Cortelli, W. P. Gilks, D. S. Latchman, R. J. Harvey, B. Dallapiccola, G. Auburger & N. W. Wood (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science,* 304**,** 1158-60.

Valente, T., A. Gella, X. Fernandez-Busquets, M. Unzeta & N. Durany (2010) Immunohistochemical analysis of human brain suggests pathological synergism of Alzheimer's disease and diabetes mellitus. *Neurobiol Dis,* 37**,** 67-76.

Valko, M., H. Morris & M. T. Cronin (2005) Metals, toxicity and oxidative stress. *Curr Med Chem,* 12**,** 1161-208.

Vallieres, L. & P. E. Sawchenko (2003) Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. *J Neurosci,* 23**,** 5197-207.

Van Den Eeden, S. K., C. M. Tanner, A. L. Bernstein, R. D. Fross, A. Leimpeter, D. A. Bloch & L. M. Nelson (2003) Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. *Am J Epidemiol,* 157**,** 1015-22.

Van den Heuvel, M. M., C. P. Tensen, J. H. van As, T. K. Van den Berg, D. M. Fluitsma, C. D. Dijkstra, E. A. Dopp, A. Droste, F. A. Van Gaalen, C. Sorg, P. Hogger & R. H. Beelen (1999) Regulation of CD 163 on human macrophages: cross-linking of CD163 induces signaling and activation. *J Leukoc Biol,* 66**,** 858-66.

Vanitallie, T. B. (2008) Parkinson disease: primacy of age as a risk factor for mitochondrial dysfunction. *Metabolism,* 57 Suppl 2**,** S50-5.

Vassar, R., B. D. Bennett, S. Babu-Khan, S. Kahn, E. A. Mendiaz, P. Denis, D. B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M. A. Jarosinski, A. L. Biere, E. Curran, T. Burgess, J. C. Louis, F. Collins, J. Treanor, G. Rogers & M. Citron (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science,* 286**,** 735-41.

Vivien, D. & C. Ali (2006) Transforming growth factor-beta signalling in brain disorders. *Cytokine Growth Factor Rev,* 17**,** 121-8.

Vlad, S. C., D. R. Miller, N. W. Kowall & D. T. Felson (2008) Protective effects of NSAIDs on the development of Alzheimer disease. *Neurology,* 70**,** 1672-7.

Volles, M. J. & P. T. Lansbury, Jr. (2002) Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. *Biochemistry,* 41**,** 4595-602.

Volpicelli-Daley, L. A., K. C. Luk, T. P. Patel, S. A. Tanik, D. M. Riddle, A. Stieber, D. F. Meaney, J. Q. Trojanowski & V. M. Lee (2011) Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron,* 72**,** 57-71.

von Bohlen und Halbach, O., L. Minichiello & K. Unsicker (2005) Haploinsufficiency for trkB and trkC receptors induces cell loss and accumulation of alpha-synuclein in the substantia nigra. *FASEB J,* 19**,** 1740-2.

von Reyn, C. R., J. M. Spaethling, M. N. Mesfin, M. Ma, R. W. Neumar, D. H. Smith, R. Siman & D. F. Meaney (2009) Calpain mediates proteolysis of the voltage-gated sodium channel alpha-subunit. *J Neurosci,* 29**,** 10350-6.

Walker, D. G., J. Link, L. F. Lue, J. E. Dalsing-Hernandez & B. E. Boyes (2006) Gene expression changes by amyloid beta peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes. *J Leukoc Biol,* 79**,** 596-610.

Waller, R., M. N. Woodroofe, S. Francese, P. R. Heath, S. B. Wharton, P. G. Ince, B. Sharrack & J. E. Simpson (2012) Isolation of enriched glial populations from post-mortem human CNS material by immuno-laser capture microdissection. *J Neurosci Methods,* 208**,** 108-13.

Walsh, D. M., D. M. Hartley, Y. Kusumoto, Y. Fezoui, M. M. Condron, A. Lomakin, G. B. Benedek, D. J. Selkoe & D. B. Teplow (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem,* 274**,** 25945-52.

Walsh, D. M., I. Klyubin, J. V. Fadeeva, W. K. Cullen, R. Anwyl, M. S. Wolfe, M. J. Rowan & D. J. Selkoe (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature,* 416**,** 535-9.

Walsh, D. M., A. Lomakin, G. B. Benedek, M. M. Condron & D. B. Teplow (1997) Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem,* 272**,** 22364-72.

Walsh, D. M., B. P. Tseng, R. E. Rydel, M. B. Podlisny & D. J. Selkoe (2000) The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry,* 39**,** 10831-9.

Weaver, L. K., P. A. Pioli, K. Wardwell, S. N. Vogel & P. M. Guyre (2007) Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. *J Leukoc Biol,* 81**,** 663-71.

Weber-Nordt, R. M., R. Henschler, E. Schott, J. Wehinger, D. Behringer, R. Mertelsmann & J. Finke (1996) Interleukin-10 increases Bcl-2 expression and survival in primary human CD34+ hematopoietic progenitor cells. *Blood,* 88**,** 2549-58.

Weis, N., A. Weigert, A. von Knethen & B. Brune (2009) Heme oxygenase-1 contributes to an alternative macrophage activation profile induced by apoptotic cell supernatants. *Mol Biol Cell,* 20**,** 1280-8.

Weksler, M. E., N. Relkin, R. Turkenich, S. LaRusse, L. Zhou & P. Szabo (2002) Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol,* 37**,** 943-8.

Weller, R. O., E. Djuanda, H. Y. Yow & R. O. Carare (2009) Lymphatic drainage of the brain and the pathophysiology of neurological disease. *Acta Neuropathol,* 117**,** 1-14.

Wenkel, H., J. W. Streilein & M. J. Young (2000) Systemic immune deviation in the brain that does not depend on the integrity of the blood-brain barrier. *J Immunol,* 164**,** 5125-31.

Wenning, G. K., G. Ebersbach, M. Verny, K. R. Chaudhuri, K. Jellinger, A. McKee, W. Poewe & I. Litvan (1999) Progression of falls in postmortem-confirmed parkinsonian disorders. *Mov Disord,* 14**,** 947-50.

Wenzel, I., J. Roth & C. Sorg (1996) Identification of a novel surface molecule, RM3/1, that contributes to the adhesion of glucocorticoid-induced human monocytes to endothelial cells. *Eur J Immunol,* 26**,** 2758-63.

White, J. A., A. M. Manelli, K. H. Holmberg, L. J. Van Eldik & M. J. Ladu (2005) Differential effects of oligomeric and fibrillar amyloid-beta 1-42 on astrocyte-mediated inflammation. *Neurobiol Dis,* 18**,** 459-65.

Whitehouse, P. J., J. C. Hedreen, C. L. White, 3rd & D. L. Price (1983) Basal forebrain neurons in the dementia of Parkinson disease. *Ann Neurol,* 13**,** 243-8.

Whitmer, R. A., D. R. Gustafson, E. Barrett-Connor, M. N. Haan, E. P. Gunderson & K. Yaffe (2008) Central obesity and increased risk of dementia more than three decades later. *Neurology,* 71**,** 1057-64.

Whitmer, R. A., S. Sidney, J. Selby, S. C. Johnston & K. Yaffe (2005) Midlife cardiovascular risk factors and risk of dementia in late life. *Neurology,* 64**,** 277-81.

Whitton, P. S. (2007) Inflammation as a causative factor in the aetiology of Parkinson's disease. *Br J Pharmacol,* 150**,** 963-76.

Wilcock, D. M., G. DiCarlo, D. Henderson, J. Jackson, K. Clarke, K. E. Ugen, M. N. Gordon & D. Morgan (2003) Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. *J Neurosci,* 23**,** 3745-51.

Wilde, E. A., J. V. Hunter, M. R. Newsome, R. S. Scheibel, E. D. Bigler, J. L. Johnson, M. A. Fearing, H. B. Cleavinger, X. Li, P. R. Swank, C. Pedroza, G. S. Roberson, J. Bachevalier & H. S. Levin (2005) Frontal and temporal morphometric findings on MRI in children after moderate to severe traumatic brain injury. *J Neurotrauma,* 22**,** 333-44.

Williams, D. R., H. C. Watt & A. J. Lees (2006) Predictors of falls and fractures in bradykinetic rigid syndromes: a retrospective study. *J Neurol Neurosurg Psychiatry,* 77**,** 468-73.

Williams, K. C., S. Corey, S. V. Westmoreland, D. Pauley, H. Knight, C. deBakker, X. Alvarez & A. A. Lackner (2001) Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. *J Exp Med,* 193**,** 905-15.

Williams, L., G. Jarai, A. Smith & P. Finan (2002) IL-10 expression profiling in human monocytes. *J Leukoc Biol,* 72**,** 800-9.

Williams-Gray, C. H., T. Foltynie, C. E. Brayne, T. W. Robbins & R. A. Barker (2007) Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. *Brain,* 130**,** 1787-98.

Williamson, J., J. Goldman & K. S. Marder (2009) Genetic aspects of Alzheimer disease. *Neurologist,* 15**,** 80-6.

Willment, J. A., H. H. Lin, D. M. Reid, P. R. Taylor, D. L. Williams, S. Y. Wong, S. Gordon & G. D. Brown (2003) Dectin-1 expression and function are enhanced on alternatively activated and GM-CSF-treated macrophages and are negatively regulated by IL-10, dexamethasone, and lipopolysaccharide. *J Immunol,* 171**,** 4569-73.

Wilms, H., P. Rosenstiel, J. Sievers, G. Deuschl, L. Zecca & R. Lucius (2003) Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease. *FASEB J,* 17**,** 500-2.

Wirdefeldt, K., H. O. Adami, P. Cole, D. Trichopoulos & J. Mandel (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur J Epidemiol,* 26 Suppl 1**,** S1-58.

Wisniewski, H. M., M. Barcikowska & E. Kida (1991) Phagocytosis of beta/A4 amyloid fibrils of the neuritic neocortical plaques. *Acta Neuropathol,* 81**,** 588-90.

Witte, M. B. & A. Barbul (2003) Arginine physiology and its implication for wound healing. *Wound Repair Regen,* 11**,** 419-23.

Wolters, E. & H. Braak (2006) Parkinson's disease: premotor clinico-pathological correlations. *J Neural Transm Suppl***,** 309-19.

Wolters, E. C. (1999) Dopaminomimetic psychosis in Parkinson's disease patients: diagnosis and treatment. *Neurology,* 52**,** S10-3.

--- (2001) Intrinsic and extrinsic psychosis in Parkinson's disease. *J Neurol,* 248 Suppl 3**,** III22-7.

Wu, X. F., M. L. Block, W. Zhang, L. Qin, B. Wilson, W. Q. Zhang, B. Veronesi & J. S. Hong (2005) The role of microglia in paraquat-induced dopaminergic neurotoxicity. *Antioxid Redox Signal,* 7**,** 654-61.

Wu, Z., Y. Tokuda, X. W. Zhang & H. Nakanishi (2008) Age-dependent responses of glial cells and leptomeninges during systemic inflammation. *Neurobiol Dis,* 32**,** 543-51.

Xia, Y., T. Saitoh, K. Ueda, S. Tanaka, X. Chen, M. Hashimoto, L. Hsu, C. Conrad, M. Sundsmo, M. Yoshimoto, L. Thal, R. Katzman & E. Masliah (2001) Characterization of the human alpha-synuclein gene: Genomic structure, transcription start site, promoter region and polymorphisms. *J Alzheimers Dis,* 3**,** 485-494.

Xie, R., J. Y. Chung, K. Ylaya, R. L. Williams, N. Guerrero, N. Nakatsuka, C. Badie & S. M. Hewitt (2011) Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. *J Histochem Cytochem,* 59**,** 356-65.

Xu, H., M. Chen, E. J. Mayer, J. V. Forrester & A. D. Dick (2007) Turnover of resident retinal microglia in the normal adult mouse. *Glia,* 55**,** 1189-98.

Yaguchi, T., Y. Goto, K. Kido, H. Mochimaru, T. Sakurai, N. Tsukamoto, C. Kudo-Saito, T. Fujita, H. Sumimoto & Y. Kawakami (2012) Immune suppression and resistance mediated by constitutive activation of Wnt/beta-catenin signaling in human melanoma cells. *J Immunol,* 189**,** 2110-7.

Yan, B. C., C. Gong, J. Song, T. Krausz, M. Tretiakova, E. Hyjek, H. Al-Ahmadie, V. Alves, S. Y. Xiao, R. A. Anders & J. A. Hart (2010) Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. *Am J Surg Pathol,* 34**,** 1147-54.

Yan, Q., J. Zhang, H. Liu, S. Babu-Khan, R. Vassar, A. L. Biere, M. Citron & G. Landreth (2003) Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer's disease. *J Neurosci,* 23**,** 7504-9.

Yan, Y. & C. Wang (2007) Abeta40 protects non-toxic Abeta42 monomer from aggregation. *J Mol Biol,* 369**,** 909-16.

Zaghi, J., B. Goldenson, M. Inayathullah, A. S. Lossinsky, A. Masoumi, H. Avagyan, M. Mahanian, M. Bernas, M. Weinand, M. J. Rosenthal, A. Espinosa-Jeffrey, J. de Vellis, D. B. Teplow & M. Fiala (2009) Alzheimer disease macrophages shuttle amyloid-beta from neurons to vessels, contributing to amyloid angiopathy. *Acta Neuropathol,* 117**,** 111-24.

Zecca, L., H. Wilms, S. Geick, J. H. Claasen, L. O. Brandenburg, C. Holzknecht, M. L. Panizza, F. A. Zucca, G. Deuschl, J. Sievers & R. Lucius (2008) Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: implications for Parkinson's disease. *Acta Neuropathol,* 116**,** 47-55.

Zhang, H. T., L. Y. Li, X. L. Zou, X. B. Song, Y. L. Hu, Z. T. Feng & T. T. Wang (2007a) Immunohistochemical distribution of NGF, BDNF, NT-3, and NT-4 in adult rhesus monkey brains. *J Histochem Cytochem,* 55**,** 1-19.

Zhang, W., K. Phillips, A. R. Wielgus, J. Liu, A. Albertini, F. A. Zucca, R. Faust, S. Y. Qian, D. S. Miller, C. F. Chignell, B. Wilson, V. Jackson-Lewis, S. Przedborski, D. Joset, J. Loike, J. S. Hong, D. Sulzer & L. Zecca (2011) Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: implications for progression of Parkinson's disease. *Neurotox Res,* 19**,** 63-72.

Zhang, W., T. Wang, Z. Pei, D. S. Miller, X. Wu, M. L. Block, B. Wilson, Y. Zhou, J. S. Hong & J. Zhang (2005) Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J,* 19**,** 533-42.

Zhang, X., J. A. Winkles, M. C. Gongora, R. Polavarapu, J. S. Michaelson, K. Hahm, L. Burkly, M. Friedman, X. J. Li & M. Yepes (2007b) TWEAK-Fn14 pathway inhibition protects the integrity of the neurovascular unit during cerebral ischemia. *J Cereb Blood Flow Metab,* 27**,** 534-44.

Zhang, Y., J. Gao, K. K. Chung, H. Huang, V. L. Dawson & T. M. Dawson (2000) Parkin functions as an E2-dependent ubiquitin- protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc Natl Acad Sci U S A,* 97**,** 13354-9.

Zhang, Z., Z. Y. Zhang, Y. Wu & H. J. Schluesener (2012) Lesional accumulation of CD163+ macrophages/microglia in rat traumatic brain injury. *Brain Res,* 1461**,** 102-10.

Zhou, J., H. F. Bradford & G. M. Stern (1994) The response of human and rat fetal ventral mesencephalon in culture to the brain-derived neurotrophic factor treatment. *Brain Res,* 656**,** 147-56.

Ziebell, J. M. & M. C. Morganti-Kossmann (2010) Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics,* 7**,** 22-30.

Zilka, N., Z. Stozicka, A. Kovac, E. Pilipcinec, O. Bugos & M. Novak (2009) Human misfolded truncated tau protein promotes activation of microglia and leukocyte infiltration in the transgenic rat model of tauopathy. *J Neuroimmunol,* 209**,** 16-25.

Zotova, E., C. Holmes, D. Johnston, J. W. Neal, J. A. Nicoll & D. Boche (2011) Microglial alterations in human Alzheimer's disease following Abeta42 immunization. *Neuropathol Appl Neurobiol,* 37**,** 513-24.

Zwadlo, G., R. Voegeli, K. Schulze Osthoff & C. Sorg (1987) A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. *Exp Cell Biol,* 55**,** 295-304.

Zwadlo-Klarwasser, G., S. Bent, H. D. Haubeck, C. Sorg & W. Schmutzler (1990) Glucocorticoid-induced appearance of the macrophage subtype RM 3/1 in peripheral blood of man. *Int Arch Allergy Appl Immunol,* 91**,** 175-80.

Appendix I

Buffers and Solutions

2.6.1 Protein extraction

- Lysis buffer

  PBS

  0.1% Triton X

  Roche complete protease inhibitor cocktail

2.7.1 Gel preparation

- 1 x 8% running gel

  2ml of acrylamide

  2.5ml of 1.5M Tris-HCl pH 8.8

  100μl of 10% SDS

  10μl TEMED (Tetramethylethylenediamine)

  50μl APS (10% Ammonium persulfate)

  5.34ml of ddH_2O (double distilled)

- Stacking gel

  250μl of acrylamide

  315μl of 1.5M Tris-HCl pH 6.8

  25μl of 10% SDS

  2.5ml TEMED (Tetramethylethylenediamine)

  12.5μl APS (10% Ammonium persulfate)

  1.9ml of ddH_2O

2.7.2 SDS-PAGE gel electrophoresis

- Sample buffer

  3ml of 20% SDS

  3ml of glycerol

  2.4ml of 1M Tris pH 6.8

  1.5ml of β-mercaptoethanol

  a pinch of Bromo-phonol-blue

- Running buffer

  14.3g of glycine

  3g of Tris base

  1g of SDS

  1 litre of ddH_2O

2.7.3 Transfer and Detection

- Tris-Buffered Saline with Tween 20 (TBST)

  6.05g of Tris base

  8.76g of NaCl

  1ml of Tween 20

  1 liter of ddH_2O

  pH adjusted to 7.5 with HCl

- Transfer buffer

  200ml of methanol

  28.8g of glycine

  6.05g of Tris base

  1.6 liter of ddH_2O

3.3.3 Pretreatments

- Proteinase K solution

  50mM Tris-HCl buffer pH 8

  20 ug/ml Proteinase K

- 4M Guanidine thiocyanate solution

  472.64 g of guanidine thiocyanate

  1 litre of ddH_2O

- 0.4% Pepsin solution

  4mg of pepsin

  1ml of 10mM HCl at pH 2.0

Appendix II

CD163 %area assessment in AD cases

AD cases	Frontal cortex	Occipital cortex	Hippocampus					
			CA1	CA2	CA3	CA4	Subiculum	Entorhinal cortex
1	0.925	1.126	-	-	-	-	-	-
2	2.946	1.466	0.548	0.238	0	0.22	0.181	0
3	1.049	8.015	-	-	-	-	-	-
4	8.562	6.429	3.128	1.154	0.863	2.978	3.128	3.433
5	5.107	3.245	0.701	0	0.288	1.085	2.478	4.93
6	4.068	1.624	0.663	0.384	0	0.06	0.052	0.063
7	3.279	1.243	0	0	0.011	0.032	0.512	0.115
8	1.143	1.8	0.204	0.055	0	0.054	0.095	0.376
9	0.7	1.911	0.749	0.659	0.167	0.715	1.565	2.384
10	1.135	1.439	0.557	0.254	0.455	0.497	2.203	2.194
11	3.538	1.308	-	-	-	-	-	-
12	1.227	1.331	0.318	0	0.089	0.207	1.041	4.186
13	2.575	3.488	-	-	-	-	-	-
14	2.771	2.071	1.741	0.205	0.187	0.11	1.47	1.479
15	0.692	1.141	1.891	0	0.456	0.191	1.335	0.43
16	2.623	5.077	-	-	-	-	-	-
17	0.55	0.231	0.094	0	0	0	0.042	0
18	1.021	1.676	0.455	0	0.198	0.256	0.528	0.808
19	2.711	1.68	-	-	-	-	-	-
20	4.037	4.048	1.265	0.459	0.309	0.433	1.181	0.719
21	0.38	0.176	0.203	0.294	0.337	0.247	1.634	0.3
22	0.437	0.184	0.242	0	0	0.388	0.194	0.67
23	1.893	1.602	0.094	0	0.151	0.158	0.206	2.133
24	1.192	1.056	-	-	-	-	-	-
25	1.323	2.143	0.674	0.129	0.257	0.73	1.075	0.573
26	0.866	1.911	-	-	-	-	-	-
27	0.406	0.973	-	-	-	-	-	-
28	0.177	0.355	-	-	-	-	-	-
29	0.067	0.374	0	2.231	0	0	0.305	2.238
30	1.615	2.099	0.658	0.437	0.38	0.421	1.025	2.219
31	0.943	2.03	0.192	0	0	0.217	0.588	0.156

CD14+ microglia load assessment in AD cases

AD cases	Frontal cortex	Occipital cortex	Hippocampus					
			CA1	CA2	CA3	CA4	Subiculum	Entorhinal cortex
1	1	2	-	-	-	-	-	-
2	2	2	2	0	0	0	1	2
3	2	3	-	-	-	-	-	-
4	1	3	3	2	3	3	3	3
5	2	2	2	0	0	0	1	1
6	1	2	0	0	0	0	0	1
7	2	2	1	0	0	0	1	1
8	1	1	1	0	0	0	1	1
9	3	3	3	2	0	2	2	3
10	1	2	1	0	0	0	1	-
11	2	1	-	-	-	-	-	-
12	2	3	1	0	0	0	1	2
13	2	3	-	-	-	-	-	-
14	3	3	3	1	0	1	2	3
15	1	2	1	0	0	0	1	0
16	1	2	-	-	-	-	-	-
17	1	1	1	0	0	0	1	1
18	2	2	1	0	0	1	1	1
19	2	2	-	-	-	-	-	-
20	2	3	1	0	0	1	1	3
21	1	2	2	1	1	2	2	2
22	1	1	0	0	0	0	0	0
23	2	2	1	0	0	1	1	2
24	1	2	-	-	-	-	-	-
25	3	3	3	2	2	2	2	2
26	2	2	-	-	-	-	-	-
27	1	1	-	-	-	-	-	-
28	0	2	-	-	-	-	-	-
29	0	1	0	2	1	0	0	1
30	2	3	1	1	1	1	0	2
31	1	2	1	0	0	1	0	1

Appendix III

Clinical data for LBD patients

PD case	Gender	Clinical Diagnosis	α-syn Braak staging	Age of disease onset	Age at death	Disease duration	Clinical symptoms				Motor onset symptoms		
							Anxiety	Depression	Visual Hallucinations	Psychosis	Tremor	Rigidity	Gait/ Balance
1	F	PD	4	65	75	10	N	N	N	N	Y	N	N
2	M	PD	4	67	81	15	N	N	Y	N	Y	N	N
3	F	PD	4	67	80	13	N	N	Y	N	Y	Y	N
4	F	PD	4	77	87	9	N	N	N	N	Y	Y	N
5	M	PD	5	48	49	1	Y	Y	N	N	Y	Y	Y
6	M	PD	5	67	75	8	N	N	N	N	N	Y	N
7	M	PD	4	66	72	6	Y	Y	Y	N	Y	Y	N
8	F	PD	5	61	77	16	N	Y	Y	N	N	Y	Y
9	M	PD	5	69	79	10	N	Y	Y	N	Y	Y	N
10	M	PD	4	40	66	26	Y	N	Y	N	Y	N	N
11	M	PD	3	52	61	9	N	N	Y	N	Y	N	N
12	M	PD	6	80	86	6	N	N	Y	N	Y	N	Y
13	F	PD	3	78	96	18	Y	Y	Y	N	Y	N	N
14	M	PD	6	72	74	3	N	N	N	N	Y	N	N
15	F	PD	3	67	73	6	9	9	9	9	N	Y	N
16	M	PD	3	86	89	3	N	N	N	N	N	N	Y
17	M	PD	5	43	57	14	Y	Y	N	N	Y	N	N
18	F	PDD	6	66	80	14	Y	Y	Y	Y	Y	N	N
19	M	PDD	6	46	72	26	N	N	N	N	Y	N	N
20	M	PDD	6	58	66	8	N	N	Y	Y	Y	Y	Y
21	M	PDD	3	72	80	8	N	N	Y	9	N	N	Y
22	F	PDD	6	64	84	20	N	N	Y	N	N	Y	N
23	F	PDD	6	68	78	10	N	Y	Y	N	Y	N	N
24	M	DLB	6	47	61	14	N	Y	Y	Y	Y	Y	N
25	M	DLB	5	69	70	1	Y	Y	Y	Y	Y	N	Y
26	M	DLB	6	61	69	8	N	N	Y	N	Y	N	N
27	M	DLB	6	64	74	10	Y	Y	Y	N	Y	N	N

CD163 %area assessment in LBD cases

PD cases	Frontal cortex	Cingulate cortex	Caudate nucleus	Internal capsule	Putamen	NBM	Hippocampus						SN	LC	DMV
							CA1	CA2	CA3	CA4	Subiculum	Entorhinal cortex			
1	0.02	0.04	0.09	0.04	0.24	0	0	0	0	0.34	0	0.02	0.52	1.07	0.61
2	0.21	0.09	0.08	1.41	0.16	0.38	0.08	0	0.01	0.09	0	1.09	1.09	0.14	1.43
3	0.1	0.05	0.13	0.04	0.08	0.23	0	0	0	0	0.03	0.3	0.84	0.49	0.9
4	0	0	0	0	0.05	0.34	1.82	0	0	0	0	0.2	0.78	0.2	0.53
5	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0.1	0.06
6	0.19	1.06	0.18	3.65	2.31	1.26	0.93	0	0	0.05	0.32	1.03	0.76	0.91	2.78
7	0.19	0	0	0	0	0.17	0	0.16	0	0.04	0	0.12	0.45	0.24	0.42
8	0.07	0	0	0	0.67	0.16	0	0	0	0	0	0.26	0.46	0.14	0.28
9	1.55	0.37	0	1.07	1.55	0.23	0.81	0.34	0.56	0.2	1.57	0.53	1.57	0.19	0.16
10	0	0	0	0	0	0.12	0	0	0	0	0	0	0	0.07	0
11	0	0	0	0	0	1.9	0	0.24	0.12	0.33	0	0	0.12	-	0
12	1.2	1.8	0.27	0.95	0.49	2.48	0.71	0.63	1.14	1.57	0.89	1.43	1.86	3.86	2.31
13	0.27	0	0	0	0	0.04	0	0	0.02	0.1	0.12	0	0.58	0.44	1.5
14	0.42	1.26	1.65	2.8	3.87	0.41	0.84	0.49	0	0.09	0.41	0.67	2.27	1.84	3.69
15	0.47	0.14	0	0.65	0	0.57	0.18	0.03	0.07	0.69	0.26	0.22	1.87	0	1.04
16	0.28	0.8	0.54	0.27	0.13	0.38	0	0	0	0.1	0	0	1.27	0.46	0.08
17	0	0	0	0	0.04	0.5	0	0	0	0.06	0.05	0	0.32	0.13	0.21
18	0.06	0	0.37	0	0.8	0.28	0	0	0	0	0	0.17	1.43	0.23	0.27
19	0.08	0	0	0	0	-	0	0.38	0	0.02	0	0.35	0.87	0.76	2.81
20	0.03	0.08	0	0	0	0	0	0	0	0	0.05	0.62	0.63	0.17	0.22
21	0.21	0.39	0	0	0	0	0	0	0	0.12	0	0.06	0.22	0.63	0.7
22	0.65	0.37	0.26	0.12	0.11	0.34	0.19	0.56	0.02	0.27	0.23	0.17	0.21	0.52	0.68
23	1.83	1.41	0.57	2.26	1.08	1.87	-	-	-	-	-	-	2.83	2.76	1.12
24	0.05	0.1	0	0	0	0	0	0	0	0	0	0.06	0	0.26	0.14
25	0.27	0	0.08	0.5	0.25	0.4	0	0	0	0	0.04	0.07	0.65	0.42	0.44
26	0.13	0.22	0.21	0	0.14	-	0.62	0.26	0.32	0.28	2.8	0.65	0.87	0	0.29
27	1.41	0.4	0.2	0.32	0.13	0.19	0.54	1.94	0.4	0.71	1.32	0.5	1.55	0.11	0

CD14+ microglia load assessment in LBD cases

PD cases	Frontal cortex	Cingulate cortex	Caudate nucleus	Internal capsule	Putamen	NBM	Hippocampus						SN	LC	DMV
							CA1	CA2	CA3	CA4	Subiculum	Entorhinal cortex			
1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0
2	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	2	2	2	3	3	2	2	0	1	2	3	2	2	1	2
7	1	1	2	0	1	1	2	1	0	1	1	1	1	0	0
8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	2	2	3	3	3	0	2	1	0	2	2	2	2	2	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	1	1	1	0	0	0	0	0	0	0	0	-	0
12	1	1	1	1	1	2	0	0	0	0	0	0	1	1	0
13	1	1	1	0	2	1	0	0	0	1	0	0	1	1	2
14	2	2	3	2	3	2	2	2	2	2	2	2	3	2	3
15	1	0	1	1	1	0	1	0	0	0	1	1	0	0	0
16	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
18	0	1	0	0	1	0	1	0	0	0	0	0	2	0	0
19	0	0	0	0	0	-	0	0	0	0	0	0	1	0	1
20	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
22	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	2	1	2	1	2	2	-	-	-	-	-	-	2	1	2
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
26	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0
27	2	2	2	2	2	0	3	3	1	2	3	2	1	2	1

Appendix IV

CD163+ microglia load assessment in TBI cases

TBI case	Age at death	Survival time (days)	Presence of Aβ plaques	CD163 microglia load	
				Cerebral cortex	Cingulate cortex
1	47	0	0	0	-
2	1	0	0	0	-
3	25	0	0	0	-
4	14	0	0	0	-
5	47	1	0	3	-
6	65	1	1	0	0
7	21	1	0	0	-
8	26	1	0	0	-
9	27	1	0	0	-
10	62	1	1	0	-
11	28	1	1	1	0
12	20	2	0	0	-
13	18	2	0	0	-
14	37	2	0	0	-
15	9	2	0	0	-
16	16	2	1	0	2
17	16	2	0	0	-
18	56	3	0	3	-
19	26	3	0	0	-
20	32	3	0	2	-
21	75	3	0	1	-
22	24	4	0	3	-
23	52	4	0	2	-
24	17	4	0	0	-
25	56	4	1	2	1
26	59	4	1	1	1
27	20	5	0	2	-
28	20	5	0	3	-
29	9	5	0	0	-
30	32	5	0	3	-
31	20	7	0	2	-
32	17	7	0	2	-
33	21	8	0	3	-
34	5	9	0	0	0
35	46	10	1	1	1
36	46	10	0	0	-
37	62	11	0	1	-
38	43	11	0	3	-
39	29	12	0	2	-
40	45	13	1	3	2
41	22	13	0	1	-

Publication arising from this work

Pey, P., Pearce, R.K.B., Kalaitzakis, M.E., Griffin, W.S.T., Gentleman, S.M. Differential parenchymal distribution of the perivascular macrophage marker CD163 in Alzheimer's and Parkinson's disease (in submission)

Conferences

Abstract, poster and presentation at the XVIIth International Congress of Neuropathology, Salzburg, Austria. CD163: a marker of alternative microglial activation in Alzheimer's disease.