

# Antibody repertoire analysis in polygenic autoimmune diseases

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## Summary

High-throughput sequencing of the DNA/RNA encoding antibody heavy- and light-chains is rapidly transforming the field of adaptive immunity. It can address key questions, including: (i) how the B-cell repertoire differs in health and disease; and (ii) if it does differ, the point(s) in B-cell development at which this occurs. The advent of technologies, such as whole-genome sequencing, offers the chance to link abnormalities in the B-cell antibody repertoire to specific genomic variants and polymorphisms. Here, we discuss the current research using B-cell antibody repertoire sequencing in three polygenic autoimmune diseases where there is good evidence for a pathological role for B-cells, namely systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis. These autoimmune diseases exhibit significantly skewed B-cell receptor repertoires compared with healthy controls. Interestingly, some common repertoire defects are shared between diseases, such as elevated IGHV4-34 gene usage. B-cell clones have effectively been characterized and tracked between different tissues and blood in autoimmune disease. It has been hypothesized that these differences may signify differences in B-cell tolerance; however, the mechanisms and implications of these defects are not clear.

**Keywords:** antibodies; autoantibodies; autoimmunity; B-cell; B-cell receptors.

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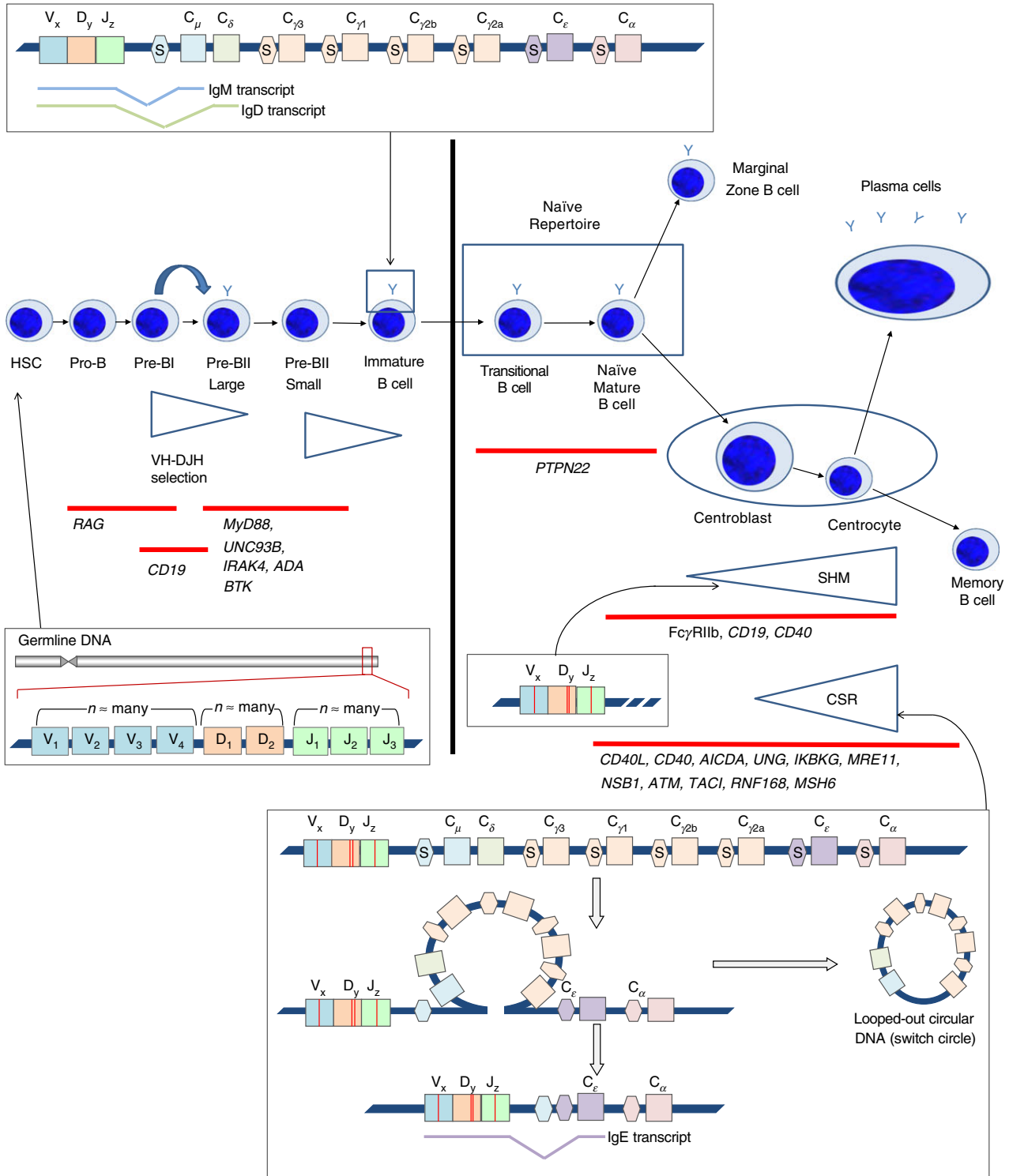
## Introduction

B-cells produce antibodies and are crucial for effective immunity. B-cell clones selectively expand following antigen recognition by B-cell receptors (BCRs). BCRs are the membrane-form of antibodies and are generated through DNA recombination. B-cells have the potential to recognize a vast array of pathogens, but diversity in the B-cell repertoire comes at a price, namely that there is a potential for autoreactivity in a subset of B-cells. Defects in B-cell development and function can lead to a breakdown of immunological tolerance and therefore autoimmune diseases, which affect approximately 1 in 12 people worldwide.<sup>1</sup>

B-cells develop from haematopoietic stem cells and differentiate through several maturation stages in the bone marrow. The germline immunoglobulin heavy-chain (IgH) gene locus encodes multiple distinct copies of the variable (V), diversity (D) and joining (J) genes, which are separated by over 100 kbp from a much smaller number of DNA segments encoding the constant genes.<sup>2</sup> During B-cell development, functional immunoglobulin genes

are generated through the deletion of intervening DNA,<sup>3</sup> creating a IgH gene containing one V, one D and one J gene (VDJ). This encodes the protein sequence for the antigen-binding region of the IgH protein<sup>2,4</sup> (Fig. 1). This process of site-specific recombination is highly orchestrated and mediated by recombination activating genes 1 (RAG1) and 2 (RAG2).<sup>5,6</sup> The imprecise joining of the V, D and J gene segments leads to the introduction of random deletions and insertions of nucleotides through exonucleases and terminal deoxynucleotidyl transferase (TdT), respectively. This results in sequence diversification at the junctional regions.<sup>7</sup> Further mechanisms that contribute to the generation of diversity include alternative IgHD reading frames and IgHD-IgHD fusions.<sup>8</sup> These pre-B-cells are selected for functional heavy-chain by IgV-D-J expression and IgH assembly by pairing.<sup>9,10</sup>

Likewise, each IgL chain locus encodes multiple distinct copies of  $\lambda$  chain and  $\kappa$  chain variable (V) gene segments and joining (J) gene segments.<sup>11</sup> When a cell has successfully rearranged a IgH gene, the B-cell begins to rearrange the light-chain genes. Therefore, each mature antigen-naïve



**Figure 1.** Schematic diagram of the processes of B-cell differentiation and selection, annotated with known human genetic modifiers of the process and the point in selection at which they are thought to act. B-cells are generated from haematopoietic stem cells. During B-cell development, V-D-J recombination occurs to produce a functional heavy-chain. Similarly, V-J recombination occurs in the light-chain. The resulting B-cell receptor (BCR) may be expressed as both surface IgD and IgM on naïve B-cells through alternative splicing. Somatic hypermutation (SHM) can occur during B-cell activation, in which mutations are introduced into the V-(D)-J region of the BCR. Class-switch recombination (CSR) can also occur during B-cell activation, which is a chromosomal deletion process leading to the expression of a different antibody isotype.

B-cell typically expresses BCR sequences encoding the heavy- and light-chains. After functional V-(D)-J recombination of IgH and IgL chain genes, the resulting immature naïve B-cells transcribe the IgH and IgL genes, and are able to produce IgD and IgM immunoglobulin isotype by alternative splicing of the transcript to fuse the  $\mu$  and  $\delta$  exon to the IgHJ exon, respectively.<sup>12</sup> At this immature B-cell stage, the cells are first tested for tolerance to self-antigens.

### Central tolerance

Immature B-cells that have no reactivity for self-antigen leave the bone marrow. For self-reactive B-cells, there are four possible fates.

- (1) Deletion – this predominates when the self-antigen is multivalent.
- (2) Receptor editing – the continued expression of RAG means that the light-chain of any self-reactive B-cell can be deleted and replaced with another sequence. Receptor editing is particularly amenable to analysis by BCR sequencing (Box 1).
- (3) Anergy – a state of unresponsiveness that occurs when B-cells encounter weakly cross-linking self-antigens of low valence.
- (4) Ignorance – the B-cell does not encounter the self-antigen for which it is specific in the bone marrow. It might yet do so in the periphery.

Some autoreactive B-cells escape central tolerance. In humans, approximately 55%–75% of the early immature B-cells in humans are autoreactive. This decreases to 40% for bone marrow immature B-cells and transitional B-cells, and to approximately 20% for mature naïve B-cells.<sup>13,14</sup> Autoreactive B-cells therefore remain present in the peripheral repertoire, and autoantibodies can be found in healthy humans and mice (reviewed in<sup>15</sup>). Therefore, within a human B-cell population, there will be a continuum of autoreactivity.

### Diversification of the repertoire in the periphery and peripheral tolerance

Through B-cell activation through antigen binding by the BCR in addition to auxiliary signals, BCR genes can be further diversified through somatic hypermutation (SHM) in the germinal centre<sup>16</sup> (Box 2) and/or undergo class-switch recombination (CSR; Box 3). In some species, gene conversion can also take place. All three processes are dependent on the activation-induced deaminase (AID) enzyme. SHM diversifies the variable region by introducing point mutations and this leads to affinity maturation. Clearly, the process of SHM also has the potential to drive the formation of newly autoreactive B-cells in the periphery. For autoreactive cells that escape central tolerance, peripheral tolerance mechanisms can prevent overt autoreactivity.

#### Box 1 Receptor editing

If a maturing naïve B-cell has high affinity for self-antigens or does not form a functional BCR, the cells may be removed by developmental arrest and/or induced programmed cell death in the bone marrow. These B-cells can be rescued by modifying the V-J light-chain recombination so that the B-cell receptor no longer recognizes self-antigens or creates a new functional reading frame.<sup>52,116</sup> This occurs by the process of secondary rearrangement, where renewed IgHV-D-J or light-chain rearrangement can result in expression of a functional and non-autoreactive BCR. Receptor editing is again mediated by RAG1/2.<sup>117–119</sup> An upstream V gene recombines with the original V-D-J rearrangement to generate a BCR with a new BCR specificity. This occurs between a cryptic recombination signal sequence within the original rearrangement leaving behind a ~5 nucleotide footprint from the original V gene within the secondary rearranged BCR.<sup>120</sup> As IgHD-J recombinations (including junctional regions), known as ‘stem sequences’ are stable in instances of secondary rearrangements,<sup>121</sup> these can be identified in BCR repertoire sequencing. However, exonucleases and TdT may introduce random deletions and insertions at this new junctional region, which may obscure the V replacement footprint.<sup>122</sup> Although this can confound inference of secondary rearrangements events from BCR sequencing, secondary rearrangements result in longer CDR3 regions, which is a feature that can be tracked using BCR repertoire sequencing data.

#### Box 2 Somatic hypermutation (SHM)

SHM is a process that introduces point mutations and, occasionally, insertions and deletions into the variable regions of the heavy-chain immunoglobulin, where some of the resulting populations are expanded through positive selection for higher affinity antigen binding.<sup>123</sup> These lead to some B-cells improving their antigen specificity and affinity to the antigen, often by several orders of magnitude.<sup>124,125</sup> This process is mediated through the action of cell type-specific expression activation-induced cytosine deaminase enzyme (AID). SHM can be determined from BCR sequencing data on comparison with reference germline genes. However, SHM needs to be distinguished from germline alleles present in an individual that are not recorded in a reference database. Germline genes may be inferred from sequencing data (through programmes such as IMPRe<sup>126</sup>), against which SHM may be determined.

These include: (i) deletion of autoreactive B-cell clones in the periphery; (ii) the functional suppression of B-cells through anergy; and (iii) immunomodulation through regulatory T-cell and B-cell subsets; and (iv) a lack of T-cell help from a cognate autoreactive T-cell.

Some individuals will generate and retain a greater number of autoreactive clones or clones with higher affinity for particular self-antigens arising at any of these

**Box 3** Class-switch recombination (CSR)

There are five major classes of antibody isotype, namely IgM, IgD, IgG1/2/3/4, IgA1/2 and IgE, each with distinct functions through engagement of different activating or inhibitory Fc receptors, and leading to the activation of different immune cells. Through varying the isotype, an antibody can exhibit significant differences in antigen avidity, through dimerization or polymerization of IgA1 and IgM, respectively,<sup>127,128</sup> as well as binding distinct sets of antibody (Fc) receptors, and ultimately inducing distinct immune responses.<sup>129</sup> B-cell activation can lead to isotype class-switching from IgM to IgG, IgA, IgD or IgE through recombination and deletion processes, known as CSR. This process is coordinated by activation-induced cytosine deaminase enzyme (AID) and occurs primarily within the germinal centre through close cooperation between B-cells and T-helper cells via the interaction of B-cell surface CD40 protein and CD40L, which is expressed by activated T-helper cells.<sup>130</sup>

stages of diversification. Such individuals may be at higher risk of developing an autoimmune disease.

B-cell receptor sequencing is helping us address key questions in the field of B-cell repertoire development, and how B-cell populations differ between health and disease. B-cells play important roles in a range of autoimmune diseases. Here, we discuss the current research on B-cell repertoire in the most well-studied of these polygenic autoimmune diseases with evidence for the role of B-cells in pathology, namely systemic lupus erythematosus (SLE), multiple sclerosis (MS) and rheumatoid arthritis (RA).

**SLE**

Systemic lupus erythematosus is an autoimmune disease that is characterized by immune complex deposition with concomitant inflammation in almost any organ. Renal involvement, in the form of glomerulonephritis, occurs in about 40% of cases and is associated with a higher mortality. Over 100 autoantibody targets are associated with SLE, and antinuclear antibodies are present in more than 95% of patients. Autoantibodies can be present years before the onset of clinical symptoms.<sup>17</sup> Transfer of human autoantibodies from patients with SLE can cause glomerulonephritis and proteinuria in murine experimental systems,<sup>18,19</sup> emphasizing that they can be pathogenic. Genome-wide association studies (GWAS) have shown that over 80 loci can contribute to the risk of developing SLE.<sup>20</sup> The pathogenesis of the disease is complex, but several broad themes emerge. Multiple genes involved in: (i) the clearance of immune complexes and apoptotic cells; (ii) the regulation of innate immunity, such as the type 1 interferon and complement pathways; and (iii) the regulation of lymphocyte activation and function have been implicated.

The pathogenicity of autoantibodies and the large number of GWAS risk loci that are involved in B-cell development and function suggest B-cells are a key driver of disease.<sup>21,22</sup> A central question, then, is how the B-cell repertoire is altered in SLE and whether this can be related to specific polygenic or monogenic drivers of disease. Here we discuss the current studies performed on B-cell repertoire in SLE (summarized in Table 1).

**B-cell population differences in SLE**

Patients with SLE exhibit significantly different B-cell subpopulation frequencies in peripheral blood. These may be a cause or consequence of autoimmunity, but they should be considered when discussing how the BCR repertoire differs from healthy controls. The abnormalities include the following.

- Significantly decreased numbers of naïve B-cells.<sup>23</sup>
- More self-reactive B-cells than healthy individuals.<sup>24,25</sup>
- Increased frequencies of double-negative (CD27-class-switched) B-cells,<sup>26–28</sup> which has been shown to correlate with increased autoantibody titres.<sup>26</sup>
- Increased levels of *CD19<sup>low</sup>CD27<sup>high</sup>* plasma cells,<sup>23</sup> which may be a consequence of systemic inflammation.<sup>29</sup>

**Germline repertoire variation in the Ig locus in SLE**

One reason for studying the BCR repertoire is that variation in germline immunoglobulin heavy-chain (IGHV) genes has been associated with disease susceptibility. Homozygous deletions of IGHV3-30\*01 and IGHV3-30-3 were found to be enriched 2.8-fold in SLE patients with nephritis compared with ethnically matched healthy individuals, and SLE patients with these deletions exhibited higher titres of anti-DNA antibodies.<sup>30,31</sup> This deletion has also been shown to be associated with susceptibility to chronic idiopathic thrombocytopenic purpura<sup>32</sup> and Kawasaki disease<sup>33</sup> (reviewed in Watson *et al.*<sup>34</sup>).

**Peripheral B-cell repertoire analysis in SLE**

Analysis of peripheral blood B-cells using BCR sequencing can provide important information in four key areas, summarized in Table 1. These are as follows.

- Variable gene usage.
- Clonality and CDR3 length.
- SHM.
- CSR.

*Variable gene usages in SLE*

B-cell receptor sequencing of antibody repertoires in patients with SLE have shown significant changes in

Table 1. BCR repertoire sequencing analyses in SLE

References	Sample number	Peripheral blood B-cells <sup>1</sup>			Other
		IGHV4 family usage increased	Clonal expansions observed	Shorter CDR3 lengths than healthy controls	
(Fraser <i>et al.</i> 2003) <sup>45</sup>	1 SLE patient	No (GC B-cells observed only)			Increased IGHV5 family usage and underrepresentation of IGHV1 family usage.
(Arbuckle <i>et al.</i> 2003) <sup>17</sup>	8 SLE patients	IGHV4-34 increased		Increased SHM than healthy controls	A fraction of antibody-secreting cell clones contained autoantibodies without mutation.
(Demaison <i>et al.</i> 1996) <sup>35</sup>	4 SLE and 5 healthy individuals	IGHV4 family variable dependent on stage of disease		Mixed	
(Oden Dahl <i>et al.</i> 2000) <sup>23</sup>	6 active SLE, 7 SLE under therapy, and 14 healthy controls	IGHV4 family usage increased in CD27 <sup>high</sup> plasma cells			Preferential usage of IGHV3 family in CD27 + IgD+ memory B-cells.
(Tipton <i>et al.</i> 2015) <sup>36</sup>	8 SLE patients and 8 vaccinated healthy controls	IGHV4-34 increased in ASCs	Yes: polyclonal expansions	No: lower SHM in ASCs	
(Liu <i>et al.</i> 2017) <sup>46</sup>	10 SLE and 6 healthy individuals		Yes	Yes	Significantly higher levels of percentage of charged amino acids, namely arginine, within CDR3 regions than healthy controls.
(Yin <i>et al.</i> 2013) <sup>47</sup>	4 SLE and 4 healthy individuals		Yes		From light-chain BCR sequencing. Increased receptor editing in SLE also.
(Dorner <i>et al.</i> 1998) <sup>52</sup>	1 untreated SLE patient			Yes	SLE patients exhibited increased levels of SHM.
(Sfikakis <i>et al.</i> 2009) <sup>53</sup>	7 SLE and 4 healthy individuals			Yes	

<sup>1</sup>Unless otherwise stated. ASC, Antibody secreting cell; BCR, B-cell receptor; CDR3, complementarity determining region3; SHM, somatic hypermutation; SLE, systemic lupus erythematosus.



IGHV gene usages compared with healthy individuals. This has been observed in multiple studies, suggesting differences in B-cell selection and antigen-specific B-cell clonal expansion. All the BCR sequencing studies to date have shown an enrichment of IGHV4 gene family usages,<sup>17,23,35,36</sup> specifically IGHV4-34.<sup>17,36</sup> IGHV4-34 was the dominant serum autoantibody IGHV gene.<sup>17</sup> Notably, IGHV4-34 is strongly associated with autoreactivity, with unmutated IGHV4-34 genes containing the AVY motifs in the framework region 1 known to recognize I/i self-antigen against red blood cell antigens.<sup>37,38</sup> IGHV4-34 gene-containing antibodies have also been shown to recognize other autoantigens and include anti-DNA antibodies,<sup>39–42</sup> rheumatoid factors (antibodies against the Fc portion of IgG),<sup>43</sup> as well as commensal bacteria<sup>44</sup>. Some other IGHV families have also been found to be enriched in peripheral blood B-cells SLE, including IGHV1 and IGHV3.<sup>35,45</sup> These data are therefore consistent with the idea that the peripheral B-cell repertoire may be skewed towards autoreactivity in patients with SLE.

#### *Clonality and CDR3 region composition of antibodies in SLE*

High-throughput sequencing of BCR repertoires from peripheral blood has shown that patients with SLE exhibit increased B-cell clonality compared with healthy individuals.<sup>46,47</sup> This is characterized by polyclonal (multiple) B-cell expansions.<sup>36</sup> This is possibly secondary to increased numbers of plasmablasts. In a patient with active SLE, it is likely that plasmablasts generated by the ongoing immune response will be more numerous in peripheral blood. As these plasmablasts have higher levels of BCR RNA per cell, the apparent clonality of the peripheral B-cell population may increase when sequencing BCR repertoires are sourced from B-cell RNA.

The complementarity determining region 3 (CDR3) is the most variable region of the antibody sequence (Fig. 1). Longer CDR3 lengths have been associated with both auto- and polyreactivity.<sup>48</sup> Interestingly, patients with SLE display significantly shorter CDR3 lengths in B-cells from peripheral blood<sup>46</sup> than controls. Again though, this might be due to increased proportions of plasmablasts in peripheral blood in SLE as naïve B-cell BCRs tend to have longer CDR3 lengths than antigen-experienced B-cells.<sup>49</sup> Some of the difficulties interpreting such data could be resolved through isotype-specific BCR sequencing or through investigation of cell-sorted B-cell populations, including naïve, memory and plasma cells. As well as changes in CDR3 length, patients with SLE also appear to have qualitative differences in the CDR3 region compared with controls. For instance, CDR3s from B-cells from patients with SLE code for significantly higher proportions of charged amino acids, such as arginine, but the functional significance of such changes is unclear.

#### *SHM in SLE*

There are numerous reports suggesting that patients with SLE exhibit increased levels of SHM compared with healthy controls. This provides potential mechanistic insight into the pathogenesis of SLE. If SHM is not stringently controlled and/or B-cells in the germinal centre receive inappropriate help from autoreactive T-cells, then autoimmunity might ensue. Accordingly, Dorner and colleagues described increased levels of SHM in SLE from CD19 + B-cells<sup>23,50,51</sup> as well as CD27<sup>hi</sup> plasma cells.<sup>23</sup> These authors also showed that the peripheral memory BCR repertoire in SLE is shaped by abnormal selection, increased SHM and increased receptor editing.<sup>52</sup> In agreement with this, Sfrikakis *et al.*<sup>53</sup> showed increased levels of SHM in SLE.

#### *Isotype in SLE*

Certain isotypes are associated with autoreactivity, and potentially pathogenicity, in SLE. IgG anti-dsDNA antibodies have been found to be more closely associated with SLE disease activity and tissue damage than IgM antibodies.<sup>54</sup> Indeed, some studies suggest that IgM anti-DNA antibodies may be protective,<sup>55</sup> whereas other isotypes may also play a role in disease.<sup>56–58</sup> However, there are no systematic BCR sequencing studies in SLE that incorporate analysis of isotypes. Isotype-resolved BCR repertoire sequencing on peripheral blood or tissue B-cells subpopulations may be able to provide clues to the extent to which a particular clone has undergone CSR and the relative contributions of each isotype to a certain autoantibody specificity.

#### *B-cell repertoire during or after therapy in SLE*

Systemic lupus erythematosus is currently treated with anti-malarial drugs such as hydroxychloroquine. Some patients are escalated to anti-proliferative medication such as azathioprine, methotrexate or mycophenolate mofetil in more severe disease. Corticosteroids are frequently used during flares to gain control of disease activity. B-cell depletion therapies with, for example, rituximab (anti-CD20) can be used in refractory disease. There is wide variation in the treatment regime used in patients with SLE, and limited data on how the repertoire changes with therapy. An interesting and unresolved question is whether any particular features of the repertoire correlate with either sustained clinical remission or treatment resistance. It is known that certain B-cell subsets such as antigen-experienced CD27 + IgD-class-switched memory B-cells are not well targeted by therapy and that this may contribute to treatment resistance. A study of the BCR repertoires of two patients with SLE before and after high-dose glucocorticoid therapy showed that IGHV3

gene family usage decreased after treatment,<sup>59</sup> but CDR3 region composition was similar at all time points. While the titre of some anti-nuclear antibodies reduced after treatment, they did not find evidence of a reduction in clone sizes over this time. In a study of patients with active SLE given rituximab, clonally related B-cells were found to be persistent in all seven patients, but not found in any of the four age-matched healthy controls.<sup>53</sup> This is consistent with preferential depletion of naïve and CD20<sup>high</sup> B-cells, with the surviving B-cell population consisting of CD20<sup>low</sup> B-cells, including plasmablasts<sup>60</sup> and mucosal IgA + plasmablasts that are not removed by B-cell depletion therapy. As yet, however, there has been no large-scale analysis of BCR usage in a large number of patients on 'standard-of-care' non-B-cell depleting therapy.

### Conclusions in SLE

These studies have shown that the B-cell repertoire differs between healthy controls and patients in SLE. However, it is still unclear how these relate to disease pathology, either as a cause or consequence of chronic inflammation. It is possible that both defects in central and peripheral tolerance in inadequate removal of autoreactive B-cells in SLE and, to some extent, these could be dissected through the study of monogenic drivers of autoimmunity.

A good example of this is a paper by Menard *et al.* They used BCR sequencing and ELISA of recombinant antibodies to study the R620W polymorphism in *PTPN22* that is implicated in susceptibility to autoimmune disease. They showed that B-cells from carriers of this *PTPN22* risk allele contained high frequencies of autoreactive clones compared with those from non-carriers showing how a single polymorphism at one genetic locus can affect the B-cell repertoire.<sup>61</sup> This *PTPN22* polymorphism is a gain-of-function variant leading to reduced B- and T-cell receptor signalling,<sup>62,63</sup> and has been associated with a range of autoimmune diseases, including RA,<sup>64,65</sup> type 1 diabetes<sup>66</sup> and SLE.<sup>67</sup> Similar studies on variation in other genes are likely to provide further useful information on how specific biological pathways regulate the B-cell repertoire.

Unanswered BCR repertoire questions remaining in SLE include the following.

- What are the differences in B-cell repertoire in SLE patients on an isotype level and the differences between peripheral blood and affected tissues?
- How do specific genetic loci affect the B-cell repertoire?
- How does the repertoire change with disease course and with therapy?
- Can we use BCR sequencing to predict disease prognosis or outcome?

### MS

Multiple sclerosis is an autoimmune disorder characterized by inflammation in the central nervous system (CNS).<sup>68</sup> It is the most common chronic neurological disorder in young Caucasian adults.<sup>69</sup> MS initially presents as transient inflammation leading to demyelination of neurons, and is characterized initially by relapsing and remitting episodes of neurological dysfunction. Remyelination with functional recovery may occur between episodes in early disease. Over time, however, the pathology is dominated by extensive microglial activation and neurodegeneration. Functionally this leads to progressive disability.<sup>68</sup> As with any autoimmune disease, the pathogenesis of MS is driven by a combination of genetic susceptibility and environmental factors.<sup>70–72</sup> The presence of B-cell clonal expansions and oligoclonal immunoglobulin bands (i.e. an increased concentration of a restricted number of antibodies) in the cerebrospinal fluid (CSF) of the majority of patients suggests a role for B-cells in MS pathology.<sup>69</sup> Intrathecal plasma cells, B-cells, antibody and complement activation have all been observed in MS,<sup>73</sup> and intrathecal antibody production was noted in very early studies of the disease.<sup>74</sup>

The positive effects of B-cell depletion therapies and the observation that IGHV2 gene polymorphisms have been associated with susceptibility to MS<sup>75,76</sup> indicate that B-cells and antibodies play a significant role in MS. Many autoantigens have been proposed as pathogenic antibody targets in MS, including those derived from the following.

- Myelin-derived antigens [such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP),<sup>77</sup> proteolipid protein and myelin-associated glycoprotein].
- Oligodendrocytes (such as CNPase, transaldolase and transketolase).
- Axons (such as neurofilament light-chain, tubulin and neurofascin).
- Astrocytes (such as potassium channel KIR4.1).
- Ubiquitous autoantigens (such as heat-shock proteins and nuclear proteins).
- Microbial antigens.<sup>77,78</sup>

Given the evidence that B-cells contribute to de-myelination, there have been numerous studies of the B-cell repertoire in MS, typically focussing on B-cells in CSF and blood (summarised in Table 2). A particularly exciting aspect of repertoire analysis in MS is the ability to compare the repertoire of B-cells that have infiltrated the CNS with those in the peripheral blood. This may give vital clues to disease pathogenesis and how the immune response at the site of inflammation differs from the systemic response.

### B-cell repertoire analysis in MS

As before, however, the studies can be divided into those providing insight into variation in the following.

Table 2. BCR repertoire sequencing analyses in MS

References	Sample number	CSF infiltrating B-cells		Clonal expansions observed	Clones spanning CSF and peripheral blood	B-cells undergone SHM	B-cells undergone CSR
		IGHV4 family usage increased	IGHV4 family usage increased				
(Baranzini <i>et al.</i> 1999) <sup>82</sup>	10 MS and 4 non-MS controls	Yes: increased IGHV1-69, IGHV4-34 and IGHV4-39	Yes: increased IGHV1-69, IGHV4-34 and IGHV4-39				
(Qin <i>et al.</i> 2003) <sup>69</sup>	16 MS patients and 32 with other neurological diseases	Yes, as well as IGHV3 and IGHV1	Yes			Yes, and high replacement-to-silent ratios	
(Palanichamy <i>et al.</i> 2014) <sup>83</sup>	8 MS patients	Yes	Yes		Yes		
(Beltran <i>et al.</i> 2014) <sup>84</sup>	12 MS patients, 7 patients with other neurological diseases, and 8 healthy control subjects.	Yes	Yes		Yes	Yes	Yes
(Stern <i>et al.</i> 2014) <sup>79</sup>	5 MS patients			Yes	Yes	Yes	Yes
(Eggers <i>et al.</i> 2017) <sup>80</sup>	39 MS patients			Yes, diversity correlated with B-cell infiltration.	Yes	Yes	
(Colombo <i>et al.</i> 2000) <sup>85</sup>	10 MS patients and 10 patients with other neurological disorders	Yes, as well as IGHV3	Yes, as well as IGHV3	Yes: oligoclonal bands from PCR amplification products.		Yes	
(Lomakin <i>et al.</i> 2014) <sup>131</sup>	8 MS patients	Yes (after selection of B-cells reactive against MBP, Epstein-Barr virus LMP1 and MOG)	Yes (after selection of B-cells reactive against MBP, Epstein-Barr virus LMP1 and MOG)				

CSF, cerebral spinal fluid; CSR, class-switch recombination; LMP1, latent membrane protein 1; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PCR, polymerase chain reaction; SHM somatic hypermutation.



- Variable gene usage.
- Clonal expansion.
- SHM.
- CSR.

#### *Variable gene usage in MS*

The presence of oligoclonal immunoglobulin bands in the majority of patients with MS suggests that B-cell clonal expansion is a feature of the disease,<sup>69,79,80</sup> specifically as IgG and IgM.<sup>81</sup> However, there is no definitive evidence that these B-cells target myelin or other CNS components. Through both spectrotyping and high-throughput sequencing, increased usage of IGHV1 and IGHV4 families,<sup>69,82–84</sup> specifically IGHV1-69, IGHV4-34 and IGHV4-39, have all been found in the brains of patients with MS.<sup>82</sup> Overrepresentation of IGHD2, IGHD3 and IGHJ4 families has also been described.<sup>82</sup> More recent studies using high-throughput sequencing have shown B-cells in the CSF of patients with MS are not representative of peripheral blood lymphocytes, indicating tissue-specific clonal B-cell expansion and localization.<sup>85</sup>

The composition of the BCR repertoire was found to be distinct between peripheral blood and CSF, with markedly different frequencies of IGHV, D and J usages.<sup>79</sup> Compared with the periphery, the IGHV4 family was found to be overrepresented in the CNS of matched CSF samples, suggestive of enrichment of antigen-specific B-cells into this region. The difference between peripheral and CSF compartments may alternatively be due to differences in B-cell subpopulations: the majority of B-cells in MS patients in CSF have memory or short-lived plasmablast phenotypes,<sup>86</sup> which have been shown to have a distinct IGHV gene usage profile to that of naïve B-cells, which comprise the majority of peripheral blood B-cells.<sup>87</sup>

#### *Clonal expansion, SHM and CSR in MS*

B-cell receptor sequencing has shown that CSF-infiltrating B-cells exhibit evidence of somatic mutation<sup>69,79,80,84,85,88</sup> and CSR.<sup>79,80,83,84</sup> Indeed, ectopic lymphoid tissues have been found in the meninges of patients with MS and these exhibit germinal centre activity, and both IgM and class-switched B-cells have been found in the CSF. Beltran and colleagues found that IgM+ B-cells from CSF showed high degrees of SHM, whereas IgM+ B-cells from peripheral blood were primarily unmutated because they were naïve B-cells.<sup>84</sup>

Somatic hypermutation analysis may also be used to estimate the evolutionary relationship between B-cells of different phenotypes or from different anatomical locations. This allows direct inference of clonal tracking between peripheral blood and CSF. Indeed, clonally related B-cells have been identified spanning both the peripheral blood and CSF in several studies derived from

post-germinal centre B-cells, as evidenced by extensive levels of SHM.<sup>80,83</sup> These studies are suggestive of bi-directional exchange of B-cells across the blood–brain barrier. Interestingly, founding members of B-cell clones, defined as B-cells expressing BCRs most closely resembling germline, were more often found in draining cervical lymph nodes (CLNs), whereas more mature clone members, defined as B-cells expressing hypermutated BCRs, were observed in both draining CLNs and the CNS itself.<sup>79</sup> However, there is still controversy about where SHM and class-switching occurs in MS. For instance, Stern *et al.* suggest that the majority of B-cell maturation occurs outside the CNS in secondary lymphoid tissue.<sup>79</sup> By contrast, Palanichamy *et al.* suggest that SHM may occur in both the CNS tertiary lymphoid structures and in the CLN.<sup>83</sup> Indeed, a third study has shown that B-cell lineages with members in both the CNS and CLN are prone to undergo additional rounds of affinity maturation,<sup>86</sup> further suggesting ongoing and complex B-cell dynamics between sites of affinity maturation, circulation and lesions.<sup>86</sup> The differences in conclusions from these studies are likely to result from differences in sampling sites and depth. There is also likely to be significant variation between patients, including variation in their disease course or treatment.

#### *B-cell repertoire during or after therapy in MS*

There are few studies on the effect of treatment on antibody repertoire in MS. A study of three patients with CNS demyelination after a single dose of rituximab corresponded with significant loss of peripheral B-cells, including IgG memory B-cells.<sup>89</sup> Within the IgG memory B-cell population, there was no significant change in IGHV, D or J gene usage, CDR3 region length or charge before and after therapy. However, the frequencies of clonally expanded IgG memory B-cells significantly increased after therapy, corresponding to preferential depletion of naïve and CD20<sup>high</sup> B-cells, with the remainder low-level B-cell population consisting of CD20<sup>low</sup> B-cells, including plasmablasts.<sup>60</sup> However, further studies are required to determine the differences between therapies and if there are B-cell repertoire features associated with remission and long-term outcome.

#### **Conclusions in MS**

Whilst BCR sequencing has highlighted the potential clonal relationships between blood and brain in MS, it is still unknown how and where such B-cells differentiate and their contribution to pathogenesis. The emergence of high-throughput screening technologies alongside BCR sequencing may help elucidate the antigenic targets of CSF-infiltrating B-cells. However, determining whether

Table 3. BCR repertoire sequencing analyses in RA

References	Sample number	Synovial B-cells				
		IGHV4 family usage increased	Clonal expansions observed	Longer CDR3 regions	B-cells undergone SHM	B-cells undergone CSR
(Voswinkel <i>et al.</i> 1997) <sup>103</sup>	3 RA patients	Yes				
(Pascual and Capra 1992) <sup>104</sup>	Monoclonal antibodies from various diseases	Yes: IGHV4–34				
(Doorenspleet <i>et al.</i> 2014) <sup>105</sup>	12 RA patients	Yes: IGHV4–34	Yes. Peripheral blood dominant clones disappeared during active disease and appeared in the synovial tissue	Yes		Yes
(Samuels <i>et al.</i> 2005) <sup>92</sup>	9 RA patients		Yes	Yes		
(Lee <i>et al.</i> 1992) <sup>106</sup>	1 RA patient	NA: IGK2 light-chains enriched		Yes	Yes	
(Tak <i>et al.</i> 2017) <sup>108</sup>	21 individuals at risk for RA		Yes. Peripheral blood dominant clones disappeared during active disease and appeared in the synovial tissue			
(Kim <i>et al.</i> 1999) <sup>99</sup>	1 RA patient		Yes		Some SHM observed.	
(Morbach <i>et al.</i> 2011) <sup>110</sup>	31 juvenile idiopathic arthritis patients				Yes, and CSR	
(Amara <i>et al.</i> 2013) <sup>109</sup>	6 RA patients				Yes	
(Tan <i>et al.</i> 2014) <sup>94</sup>	16 RA patients		Yes			

CDR3, complementarity determining region 3; CSR, class-switch recombination; RA, rheumatoid arthritis; SHM somatic hypermutation.

B-cell infiltration is a result of reactivity against a common self-antigen or a result of infection or bystander activation is, as yet, unclear. Unanswered BCR repertoire questions remaining in MS are as follows.

- What are the differences in B-cell repertoire in MS between CSF and other (non-peripheral blood) tissue sites? A key question is the exact location where autoreactive clones arise and proliferate in MS.
- Can BCR sequencing be used to delineate whether there are differences in central and peripheral B-cell selection in MS patients compared with healthy individuals?

## RA

Rheumatoid arthritis is one of the most common chronic inflammatory disorders mainly targeting synovial

membrane of diarthrodial joints, but other systemic manifestations may also present in patients.<sup>90</sup> Again, the efficacy of B-cell depletion therapies, such as anti-CD20,<sup>91</sup> have pointed to a key role for B-cells. Alterations in B-cell tolerance have been posited to play a role in RA. In particular, BCR editing,<sup>92</sup> clonal deletion and anergy have been shown to be defective.<sup>93</sup>

The pathological nature of autoantibodies in RA remains controversial. Autoantibody production in patients with RA is well described. Rheumatoid factor and anti-citrullinated protein antibodies (ACPA)<sup>94,95</sup> are the most well studied. Infiltrating lymphocytes have been identified in the majority of synovial tissue samples in patients with RA.<sup>96,97</sup> In about 10% of patients, these manifest as large follicle-like structures, known as synovial germinal centres.<sup>98,99</sup> Furthermore, studies have shown

that the proportion of peripheral blood plasmablasts is positively correlated with disease activity in RA,<sup>94,100</sup> and these have been found to produce ACPAs.<sup>94,95</sup> In a manner redolent of SLE and MS, IGHV gene polymorphisms associate with susceptibility to RA, most notably in IGHV1-69.<sup>101,102</sup> Given the association of B-cells in RA pathology, we review the current research in BCR repertoire sequencing in RA (summarized in Table 3). The majority of studies in RA have focused on synovial tissue and peripheral blood repertoires.

## B-cell repertoire analysis in RA

### *Variable gene usages in RA*

Multiple studies have shown enrichment of IGHV4 genes in the BCR repertoires from synovial tissue from RA patients, specifically IGHV4-34.<sup>103–106</sup> This includes synovial plasma cells,<sup>103</sup> which are antibody-secreting cells. Studies of the light-chain BCR repertoire have also shown enrichment of the kappa IGK2 gene family segments in RA synovium B-cells,<sup>106</sup> which have been associated with anti-RF activity.<sup>107</sup> In agreement with this, Samuels *et al.*<sup>92</sup> showed evidence of different levels of receptor editing during central tolerance through analysis of kappa light-chain BCR repertoire. These data are consistent with, though not diagnostic of, a breakdown of B-cell tolerance in RA.

### *BCR clonality and CDR3 lengths in RA*

Several papers have shown evidence of B-cell clonal expansions in the synovial tissue of patients with RA.<sup>92,94,99,105,108</sup> Indeed, in a prospective study of 21 individuals at risk of RA, the risk of developing RA during follow-up was significantly associated with the presence of  $\geq 5$  dominant BCR clones, defined as clonally related BCRs representing  $> 0.5\%$  of the total repertoire.<sup>108</sup> This was validated in an independent prospective cohort of 50 at-risk individuals. Interestingly, when individuals developed RA, the clones seen in peripheral blood were no longer detectable there but could be found in synovial tissue, suggesting that activated B-cell clones migrate to target tissue during active disease. This is in agreement with a previous study showing that during active RA, there were multiple dominant clones within the inflamed synovial tissue that were absent from peripheral blood.<sup>105</sup> Furthermore, within individual patients, the same dominant B-cell clones were observed in different joints, suggesting that they migrated between sites of inflammation.<sup>108</sup> Together, this highlights that B-cell expansions are present at the sites of inflammation in RA; however, the role and antigenic specificities of these expanded clones is unknown.

Interestingly, expanded or dominant synovial B-cell clones also showed features of autoreactivity. B-cell

clones from patients with RA were enriched for longer CDR3 lengths in both the heavy-chain<sup>92,105,106</sup> and light-chain (kappa, Igk).<sup>92,106</sup> B-cells with BCRs containing long Igk CDR3 regions (of  $\geq 11$  amino acids) were found to be autoreactive or polyreactive.<sup>92</sup> Thus, a picture emerges in which B-cells from patients with RA and particularly B-cells from inflamed joints appear enriched for V gene usage that is associated with specificity for self-antigen.

### *SHM analysis in RA*

There is mounting evidence for antigen-driven B-cell affinity maturation and selection in patients with RA, particularly within joints. Using single-cell sequencing and monoclonal antibody expression of B-cells from the joints of ACPA+ RA patients with active disease, 25% of synovial IgG-expressing B-cells were specific for citrullinated autoantigens; however, these were not found in ACPA-negative RA patients.<sup>109</sup> While some of the ACPAs bound more than one citrullinated antigen, none was reactive to non-citrullinated antigen. Furthermore, when SHMs from ACPAs were reverted back to corresponding germline sequences, anti-citrullinated peptide reactivity was lost.

Despite evidence for SHM within synovial tissue CD20+ B-cells in RA,<sup>99,105</sup> the question of whether continued SHM occurs here has not been comprehensively addressed in RA. In a related disease, juvenile idiopathic arthritis, CD27+ IgD- and CD27- IgD- B-cells accumulate in the joints of patients, and express somatically hypermutated and class-switched BCRs.<sup>110</sup> Indeed, these cells displayed activated phenotypes, expressing co-stimulatory molecules CD80/CD86 and were able to activate allogeneic T-cells more potently than their peripheral blood B-cell counterparts, consistent with a role in driving disease.

### *CSR in RA*

Class-switched mature memory subsets were enriched in the synovial compartment compared with peripheral blood.<sup>111–113</sup> Switching occurred primarily to IgG and IgA.<sup>114</sup> Evidence for class-switched autoreactive B-cells in RA is also supported by other studies,<sup>105,109</sup> where  $\sim 25\%$  of synovial IgG+ B-cells were reactive to citrullinated autoantigens in ACPA-positive RA patients,<sup>109</sup> and production of ACPAs by RA peripheral blood IgG+ plasmablasts was found in ACPA+ RA patients, but not ACPA- RA or psoriatic arthritis patients.<sup>94</sup> Tan *et al.* examined B-cell specificities in RA using DNA barcoding and sequencing of heavy- and light-chains of antibodies expressed by plasmablasts. The antigen-specificities of antibodies encoded by IgG+ plasmablasts from patients with RA were tested using microarrays from

recombinantly expressed antibodies.<sup>94</sup> They showed that representative antibodies from patients with RA were able to bind cyclic citrullinated peptides from epitopes on  $\alpha$ -enolase, citrullinated fibrinogen and citrullinated histone H2B in an ongoing B-cell response in RA. Furthermore, these antibodies were not reactive to the non-citrullinated form of these peptides, suggesting that the activated B-cells that are present during active disease are selected for their ability to bind citrullinated antigens. This demonstrates that class-switched B-cells within the synovium are both autoreactive, class-switched and somatically hypermutated, suggesting a breakdown of peripheral tolerance. However, the sites of SHM and CSR for autoreactive B-cells are currently unknown.

#### *B-cell repertoire during or after therapy in RA*

There are only a small number of studies examining the effect of therapy on the BCR repertoire in RA. Rouziere *et al.*<sup>115</sup> evaluated the effect of rituximab on B-cell repertoires in two patients with active RA, and showed that B-cell depletion lasted between 5 and 7 months. B-cell reconstitution was characterized by a diverse BCR repertoire and IGHV gene usages similar to that of healthy adults. However, during the early phase of B-cell reconstitution (5–7 months post-rituximab initiation) there was an expansion and circulation of B-cells containing BCRs with significantly higher levels of somatic mutations. This corresponds to preferential depletion of naïve and CD20<sup>high</sup> B-cells,<sup>60,111</sup> and similar to the effects observed in SLE<sup>53</sup> and MS.<sup>89</sup> Indeed, it was shown that IgG serum concentrations were significantly reduced after a first infusion of rituximab in a study of 35 patients with RA, but IgA and IgM serum concentrations were stable until 3–12 months afterwards.<sup>111</sup> Consistent with this, there was a different IGHV gene usage distribution at this time point, and the B-cell repertoire was enriched for class-switched BCRs.<sup>115</sup> Whilst this demonstrates significant remodelling of the B-cell repertoire after B-cell depletion therapy, the level of clonal persistence and association with disease outcome has not yet been assessed.

#### **Key outstanding questions in RA**

Most studies to date have focused on synovium B-cell population BCR repertoires, highlighting differences from peripheral blood. It is unsurprising that there are significant differences in BCR repertoire between these compartments. However, the most exciting results arise from the presence of dominant clonal expansions in the peripheral blood that precede clinical presentation and therefore might be predictive of disease development. This is especially pertinent as such clones migrate to the synovium in active disease. The next steps will be to determine the sites of autoreactive B-cell generation in

RA: whether these clonal expansions arise within synovial germinal centres, or within other anatomic sites and undergo migration to the synovium. Indeed, sampling of different anatomic sites and B-cell clonal tracking through the BCR in high-risk or early clinical RA patients may shed more light on the early dynamics of autoreactive B-cells.

#### **Concluding remarks**

B-cell receptor repertoire sequencing has, so far, been used most effectively to characterize and track B-cell clones between different tissues and blood. Such studies suggest migration of B-cells between peripheral blood and the CSF in MS, and between blood and synovium in RA.

Often, these autoreactive B-cells have also undergone SHM and/or CSR, suggesting a role for the germinal centre in their generation. However, the exact location where affinity maturation occurs is not always clear and is an important question. Indeed, the presence of ectopic germinal centres in synovial tissue or CSF in RA and MS, respectively, still allows for the possibility that B-cell clones matured in one site cannot then migrate to another. In agreement with this, the circulation of members of B-cell clones present in the inflamed tissues in MS and RA demonstrates that these autoreactive B-cells are able to undergo systematic immunosurveillance.

A second key finding across these diseases is the distinct patterns of variable gene usages and CDR3 lengths between healthy individuals and patients with SLE, MS or RA. It has been hypothesized that these differences may signify differences in B-cell tolerance. However, different B-cell subpopulations exhibit significantly different variable gene usages and CDR3 lengths. Some of these disease-specific differences may therefore reflect changes in the relative size of B-cell subpopulations.

The relative contributions of central and peripheral tolerance in developing autoreactive B-cell clones in these diseases are currently unknown. Autoreactive B-cell clones clearly arise from a breakdown in tolerance, though whether this arises at the level for central or peripheral tolerance is unknown. BCR repertoire sequencing of sorted naïve and antigen-experienced B-cell populations may provide some insight into central tolerance mechanisms in different diseases. It has been argued that defective central tolerance would generate a naïve B-cell population with a higher proportion of B-cells with higher affinities to autoantigen. A difference in naïve B-cell populations could be assessed by skewed V gene usages and CDR3 lengths as well as assessment of affinity for auto-antigen affinity ELISA. Dissecting the role of peripheral tolerance may be more complex, particularly when central tolerance may also be defective. Differences in the levels and patterns of SHM and CSR have been demonstrated most clearly between SLE and healthy



individuals. However, whether this is a result of abnormal naïve B-cell repertoire generation is not clear.

Much can be learnt about these processes from investigations into Mendelian disorders of B-cell development. Indeed, the study of the effect of monogenic drivers or GWAS associations of autoimmune disease may help to unravel the role of different stages of B-cell selection. This was demonstrated by the defective counter-selection of autoreactive B-cells in individuals with the *PTPN22* gene polymorphism. The advent of exome and whole-genome sequencing of individuals with inherited disorders of B-cell development and function is set to pave the way to identify hitherto unknown coding and non-coding variation that influences the B-cell development (and thus antibody) repertoire (Fig. 1).<sup>61</sup> Indeed, with distinct genetic signals from each autoimmune disease, we are likely to observe distinct differences between different autoimmune diseases.

As presented here, most of the studies to date on BCR sequencing in autoimmune diseases are limited by small numbers of patients. A meta-analysis of published data is not possible due to the different BCR sequencing methods or sample sources used. The combined approaches of isotype-specific BCR sequencing coupled with cell sorting of specific B-cell subsets and other complementary technologies are likely to make great advances in the field. Furthermore, (i) understanding the changes in B-cell populations over disease course, (ii) associations with disease severity, and (iii) how this responds to different immune-modulatory therapies will be critical to understand how to improve patient outcomes. While the advances that have been made in treating autoimmune disease have been substantial, many patients experience treatment-resistant disease despite the armoury of immunosuppressants now available. Similarly, many currently used immunomodulatory drugs have pleiotropic effects and an extensive side-effect profile. BCR sequencing in the context of autoimmune diseases is therefore both timely and essential.

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## Disclosures

The authors declare no competing interests.

## References

- Cooper GS, Bynum MLK, Somers EC. Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases. *J Autoimmun* 2009; **33**:197–207.
- Lydyard PM, Whelan A, Fanger MW. Instant notes series; instant notes in immunology. 2000:i–x, 1–318.

- Schatz DG, Swanson PC. V(D)J Recombination: mechanisms of initiation. *Annu Rev Genet* 2010; **45**:167–202.
- Latchman D. Gene Regulation (Advanced Texts), Garland Science, 2005
- Schatz DG, Baltimore D. Stable expression of immunoglobulin gene V(D)J recombination activity by gene transfer into 3T3 fibroblasts. *Cell* 1988; **53**:107–15.
- Oettinger MA, Schatz DG, Gorka C, Baltimore D. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* 1990; **248**:1517–23.
- Tonegawa S. Somatic generation of antibody diversity. *Nature* 1983; **302**:575–81.
- Kalinina O, Doyle-Cooper CM, Miksanek J, Meng W, Prak EL, Weigert MG. Alternative mechanisms of receptor editing in autoreactive B cells. *Proc Natl Acad Sci USA* 2011; **108**:7125–30.
- Vettermann C, Schlissel MS. Allelic exclusion of immunoglobulin genes: models and mechanisms. *Immunol Rev* 2010; **237**:22–42.
- ten Boekel E, Melchers F, Rolink AG. Precursor B cells showing H chain allelic inclusion display allelic exclusion at the level of pre-B cell receptor surface expression. *Immunity* 1998; **8**:199–207.
- Woof JM, Burton DR. Human antibody-Fc receptor interactions illuminated by crystal structures. *Nat Rev Immunol* 2004; **4**:89–99.
- Geisberger R, Lamers M, Achatz G. The riddle of the dual expression of IgM and IgD. *Immunology* 2006; **118**:429–37.
- Grandien A, Fucs R, Nobrega A, Andersson J, Coutinho A. Negative selection of multi-reactive B cell clones in normal adult mice. *Eur J Immunol* 1994; **24**:1345–52.
- Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science* 2003; **301**:1374–7.
- Theofilopoulos AN, Kono DH, Bacala R. The multiple pathways to autoimmunity. *Nat Immunol* 2017; **18**:716–24.
- McHeyzer-Williams LJ, McHeyzer-Williams MG. Antigen-specific memory B cell development. *Annu Rev Immunol* 2005; **23**:487–513.
- Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA *et al.* Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003; **349**:1526–33.
- Ehrenstein MR, Katz DR, Griffiths MH, Papadaki L, Winkler TH, Kalden JR *et al.* Human IgG anti-DNA antibodies deposit in kidneys and induce proteinuria in SCID mice. *Kidney Int* 1995; **48**:705–11.
- Gaynor B, Putterman C, Valadon P, Spatz L, Scharff MD, Diamond B. Peptide inhibition of glomerular deposition of an anti-DNA antibody. *Proc Natl Acad Sci USA* 1997; **94**:1955–60.
- Chen L, Morris DL, Vyse TJ. Genetic advances in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 2017; **29**:423–33.
- Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. *Nat Rev Immunol* 2001; **1**:147–53.
- Sanz I, Lee FE. B cells as therapeutic targets in SLE. *Nat Rev Rheumatol* 2010; **6**:326–37.
- Odendahl M, Jacobi A, Hansen A, Feist E, Hiepe F, Burmester GR *et al.* Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J Immunol* 2000; **165**:5970–9.
- Yurasov S, Wardemann H, Hammersen J, Tsuiji M, Meffre E, Pascual V *et al.* Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J Exp Med* 2005; **201**:703–11.
- Yurasov S, Tiller T, Tsuiji M, Velinzon K, Pascual V, Wardemann H *et al.* Persistent expression of autoantibodies in SLE patients in remission. *J Exp Med* 2006; **203**:2255–61.
- Wei C, Anolik J, Cappione A, Zheng B, Pugh-Bernard A, Brooks J *et al.* A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J Immunol* 2007; **178**:6624–33.
- Huang W, Sinha J, Newman J, Reddy B, Budhai L, Furie R *et al.* The effect of anti-CD40 ligand antibody on B cells in human systemic lupus erythematosus. *Arthritis Rheum* 2002; **46**:1554–62.
- Anolik JH, Barnard J, Cappione A, Pugh-Bernard AE, Felgar RE, Looney RJ *et al.* Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum* 2004; **50**:3580–90.
- Kaminski DA, Wei C, Rosenberg AF, Lee FE, Sanz I. Multiparameter flow cytometry and bioanalytics for B cell profiling in systemic lupus erythematosus. *Methods Mol Biol* 2012; **900**:109–34.
- Cho ML, Chen PP, Seo YI, Hwang SY, Kim WU, Min DJ *et al.* Association of homozygous deletion of the Humh3005 and the VH3-30.3 genes with renal involvement in systemic lupus erythematosus. *Lupus* 2003; **12**:400–5.
- Huang DF, Siminovich KA, Liu XY, Olee T, Olsen NJ, Berry C *et al.* Population and family studies of three disease-related polymorphic genes in systemic lupus erythematosus. *J Clin Invest* 1995; **95**:1766–72.
- Mo L, Leu SJ, Berry C, Liu F, Olee T, Yang YY *et al.* The frequency of homozygous deletion of a developmentally regulated Vh gene (Humh3005) is increased in patients with chronic idiopathic thrombocytopenic purpura. *Autoimmunity* 1996; **24**:257–63.



- 33 Tsai FJ, Lee YC, Chang JS, Huang LM, Huang FY, Chiu NC *et al.* Identification of novel susceptibility Loci for Kawasaki disease in a Han Chinese population by a genome-wide association study. *PLoS ONE* 2011; **6**:e16853.
- 34 Watson CT, Breden F. The immunoglobulin heavy chain locus: genetic variation, missing data, and implications for human disease. *Genes Immun* 2012; **13**:363–73.
- 35 Demaison C, David D, Fautrel B, Theze J. V(H) gene-family representation in peripheral activated B cells from systemic lupus erythematosus (SLE) patients. *Clin Exp Immunol* 1996; **104**:439–45.
- 36 Tipton CM, Fucile CF, Darce J, Chida A, Ichikawa T, Gregoretto I *et al.* Diversity, cellular origin and autoreactivity of antibody-secreting cell population expansions in acute systemic lupus erythematosus. *Nat Immunol* 2015; **16**:755–65.
- 37 Silberstein LE, Jefferies LC, Goldman J, Friedman D, Moore JS, Nowell PC *et al.* Variable region gene analysis of pathologic human autoantibodies to the related i and I red blood cell antigens. *Blood* 1991; **78**:2372–86.
- 38 Pascual V, Victor K, Lelsz D, Spellerberg MB, Hamblin TJ, Thompson KM *et al.* Nucleotide sequence analysis of the V regions of two IgM cold agglutinins. Evidence that the VH4-21 gene segment is responsible for the major cross-reactive idiotype. *J Immunol* 1991; **146**:4385–91.
- 39 Stevenson FK, Longhurst C, Chapman CJ, Ehrenstein M, Spellerberg MB, Hamblin TJ *et al.* Utilization of the VH4-21 gene segment by anti-DNA antibodies from patients with systemic lupus erythematosus. *J Autoimmun* 1993; **6**:809–25.
- 40 Mockridge CI, Chapman CJ, Spellerberg MB, Sheth B, Fleming TP, Isenberg DA *et al.* Sequence analysis of V(4-34)-encoded antibodies from single B cells of two patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1998; **114**:129–36.
- 41 van Vollenhoven RF, Bieber MM, Powell MJ, Gupta PK, Bhat NM, Richards KL *et al.* VH4-34 encoded antibodies in systemic lupus erythematosus: a specific diagnostic marker that correlates with clinical disease characteristics. *J Rheumatol* 1999; **26**:1727–33.
- 42 Isenberg DA, McClure C, Farewell V, Spellerberg M, Williams W, Cambridge G *et al.* Correlation of 9G4 idiotope with disease activity in patients with systemic lupus erythematosus. *Ann Rheum Dis* 1998; **57**:566–70.
- 43 Kraj P, Friedman DF, Stevenson F, Silberstein LE. Evidence for the overexpression of the VH4-34 (VH4.21) Ig gene segment in the normal adult human peripheral blood B cell repertoire. *J Immunol* 1995; **154**:6406–20.
- 44 Schickel JN, Glauzy S, Ng YS, Chamberlain N, Massad C, Isnardi I *et al.* Self-reactive VH4-34-expressing IgG B cells recognize commensal bacteria. *J Exp Med* 2017; **214**:1991–2003.
- 45 Fraser NL, Rowley G, Field M, Stott DI. The VH gene repertoire of splenic B cells and somatic hypermutation in systemic lupus erythematosus. *Arthritis Res Ther* 2003; **5**:R114–21.
- 46 Liu S, Hou XL, Sui WG, Lu QJ, Hu YL, Dai Y. Direct measurement of B-cell receptor repertoire's composition and variation in systemic lupus erythematosus. *Genes Immun* 2017; **18**:22–7.
- 47 Yin L, Hou W, Liu L, Cai Y, Wallet MA, Gardner BP *et al.* IgM repertoire biodiversity is reduced in HIV-1 infection and systemic lupus erythematosus. *Front Immunol* 2013; **4**:373.
- 48 Meffre E, Milili M, Blanco-Betancourt C, Antunes H, Nussenzweig MC, Schiff C. Immunoglobulin heavy chain expression shapes the B cell receptor repertoire in human B cell development. *J Clin Invest* 2001; **108**:879–86.
- 49 Galson JD, Truck J, Fowler A, Clutterbuck EA, Munz M, Cerundolo V *et al.* Analysis of B cell repertoire dynamics following hepatitis B vaccination in humans, and enrichment of vaccine-specific antibody sequences. *EBioMedicine* 2015; **2**:2070–9.
- 50 Dorner T, Heimbacher C, Farner NL, Lipsky PE. Enhanced mutational activity of V<sub>kappa</sub> gene rearrangements in systemic lupus erythematosus. *Clin Immunol* 1999; **92**:188–96.
- 51 Jacobi AM, Hansen A, Burmester GR, Dorner T, Lipsky PE. Enhanced mutational activity and disturbed selection of mutations in V(H) gene rearrangements in a patient with systemic lupus erythematosus. *Autoimmunity* 2000; **33**:61–76.
- 52 Dorner T, Foster SJ, Farner NL, Lipsky PE. Immunoglobulin kappa chain receptor editing in systemic lupus erythematosus. *J Clin Invest* 1998; **102**:688–94.
- 53 Sfikakis PP, Karali V, Lilakos K, Georgiou G, Panayiotidis P. Clonal expansion of B-cells in human systemic lupus erythematosus: evidence from studies before and after therapeutic B-cell depletion. *Clin Immunol* 2009; **132**:19–31.
- 54 Isenberg DA, Ravirajan CT, Rahman A, Kalsi J. The role of antibodies to DNA in systemic lupus erythematosus—a review and introduction to an international workshop on DNA antibodies held in London, May 1996. *Lupus* 1997; **6**:290–304.
- 55 Witte T. IgM antibodies against dsDNA in SLE. *Clin Rev Allergy Immunol* 2008; **34**:345–7.
- 56 Dema B, Pellefigues C, Hasni S, Gault N, Jiang C, Ricks TK *et al.* Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis. *PLoS ONE* 2014; **9**:e90424.
- 57 Villalta D, Bizzaro N, Bassi N, Zen M, Gatto M, Ghirardello A *et al.* Anti-dsDNA antibody isotypes in systemic lupus erythematosus: IgA in addition to IgG anti-dsDNA help to identify glomerulonephritis and active disease. *PLoS ONE* 2013; **8**:e71458.
- 58 Jost SA, Tseng LC, Matthews LA, Vasquez R, Zhang S, Yancey KB *et al.* IgG, IgM, and IgA antinuclear antibodies in discoid and systemic lupus erythematosus patients. *Sci World J* 2014; **2014**:171 028.
- 59 Shi B, Yu J, Ma L, Ma Q, Liu C, Sun S *et al.* Short-term assessment of BCR repertoires of SLE patients after high dose glucocorticoid therapy with high-throughput sequencing. *Springerplus* 2016; **5**:75.
- 60 Mei HE, Frolich D, Giesecke C, Lodenkemper C, Reiter K, Schmidt S *et al.* Steady-state generation of mucosal IgA+ plasmablasts is not abrogated by B-cell depletion therapy with rituximab. *Blood* 2010; **116**:5181–90.
- 61 Menard L, Saadoun D, Isnardi I, Ng YS, Meyers G, Massad C *et al.* The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J Clin Invest* 2011; **121**:3635–44.
- 62 Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P *et al.* Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 2005; **37**:1317–9.
- 63 Arechiga AF, Habib T, He Y, Zhang X, Zhang ZY, Funk A *et al.* Cutting edge: the PTPN22 allelic variant associated with autoimmunity impairs B cell signaling. *J Immunol* 2009; **182**:3343–7.
- 64 Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC *et al.* A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004; **75**:330–7.
- 65 Michou L, Lasbleiz S, Rat AC, Migliorini P, Balsa A, Westhovens R *et al.* Linkage proof for PTPN22, a rheumatoid arthritis susceptibility gene and a human autoimmunity gene. *Proc Natl Acad Sci USA* 2007; **104**:1649–54.
- 66 Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M *et al.* A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet* 2004; **36**:337–8.
- 67 Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VE *et al.* Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 2004; **75**:504–7.
- 68 Compston A, Coles A. Multiple sclerosis. *Lancet* 2008; **372**:1502–17.
- 69 Qin Y, Duquette P, Zhang Y, Olek M, Da RR, Richardson J *et al.* Intrathecal B-cell clonal expansion, an early sign of humoral immunity, in the cerebrospinal fluid of patients with clinically isolated syndrome suggestive of multiple sclerosis. *Lab Invest* 2003; **83**:1081–8.
- 70 Ebers GC, Sadovnick AD, Risch NJ. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. *Nature* 1995; **377**:150–1.
- 71 Wekerle H. The viral triggering of autoimmune disease. *Nat Med* 1998; **4**:770–1.
- 72 International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium 2, Sawcer S, Hellenthal G, Pirinen M, Spencer CC *et al.* Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011; **476**:214–9.
- 73 Prineas JW. Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science* 1979; **203**:1123–5.
- 74 Kabat EA, Glusman M, Knaub V. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. *Am J Med* 1948; **4**:653–62.
- 75 Walter MA, Gibson WT, Ebers GC, Cox DW. Susceptibility to multiple sclerosis is associated with the proximal immunoglobulin heavy chain variable region. *J Clin Invest* 1991; **87**:1266–73.
- 76 Sawcer S, Jones HB, Feakes R, Gray J, Smaldon N, Chataway J *et al.* A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat Genet* 1996; **13**:464–8.
- 77 Warren KG, Catz I. Autoantibodies to myelin basic protein within multiple sclerosis central nervous system tissue. *J Neurol Sci* 1993; **115**:169–76.
- 78 Wucherpfennig KW, Catz I, Hausmann S, Strominger JL, Steinman L, Warren KG. Recognition of the immunodominant myelin basic protein peptide by autoantibodies and HLA-DR2-restricted T cell clones from multiple sclerosis patients. Identity of key contact residues in the B-cell and T-cell epitopes. *J Clin Invest* 1997; **100**:1114–22.
- 79 Stern JN, Yaari G, Vander Heiden JA, Church G, Donahue WF, Hintzen RQ *et al.* B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes. *Sci Transl Med* 2014; **6**:248, ra107.
- 80 Eggers EL, Michel BA, Wu H, Wang SZ, Bevan CJ, Abounasr A *et al.* Clonal relationships of CSF B cells in treatment-naive multiple sclerosis patients. *JCI Insight* 2017; **2**:92724.
- 81 Walsh MJ, Tourtellotte WW, Roman J, Dreyer W. Immunoglobulin G, A, and M—clonal restriction in multiple sclerosis cerebrospinal fluid and serum—analysis by two-dimensional electrophoresis. *Clin Immunol Immunopathol* 1985; **35**:313–27.
- 82 Baranzini SE, Jeong MC, Butunoi C, Murray RS, Bernard CC, Oksenberg JR. B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. *J Immunol* 1999; **163**:5133–44.

- 83 Palanichamy A, Apeltsin L, Kuo TC, Sirota M, Wang S, Pitts SJ *et al.* Immunoglobulin class-switched B cells form an active immune axis between CNS and periphery in multiple sclerosis. *Sci Transl Med* 2014; **6**:248, ra106.
- 84 Beltran E, Obermeier B, Moser M, Coret F, Simo-Castello M, Bosca I *et al.* Intrathecal somatic hypermutation of IgM in multiple sclerosis and neuroinflammation. *Brain* 2014; **137**:2703–14.
- 85 Colombo M, Dono M, Gazzola P, Roncella S, Valetto A, Chiorazzi N *et al.* Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. *J Immunol* 2000; **164**:2782–9.
- 86 Cepok S, Rosche B, Grummel V, Vogel F, Zhou D, Sayn J *et al.* Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. *Brain* 2005; **128**:1667–76.
- 87 Wu YC, Kipling D, Leong HS, Martin V, Ademokun AA, Dunn-Walters DK. High-throughput immunoglobulin repertoire analysis distinguishes between human IgM memory and switched memory B-cell populations. *Blood* 2010; **116**:1070–8.
- 88 Lehmann-Horn K, Wang SZ, Sagan SA, Zamvil SS, von Budingen HC. B cell repertoire expansion occurs in meningeal ectopic lymphoid tissue. *JCI Insight* 2016; **1**: e87234.
- 89 Maurer MA, Tuller F, Gredler V, Berger T, Lutterotti A, Lunemann JD *et al.* Rituximab induces clonal expansion of IgG memory B-cells in patients with inflammatory central nervous system demyelination. *J Neuroimmunol* 2016; **290**:49–53.
- 90 Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 2003; **423**:356–61.
- 91 Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR *et al.* Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med* 2004; **350**:2572–81.
- 92 Samuels J, Ng YS, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. *J Exp Med* 2005; **201**:1659–67.
- 93 Samuels J, Ng YS, Coupillaud C, Paget D, Meffre E. Human B cell tolerance and its failure in rheumatoid arthritis. *Ann N Y Acad Sci* 2005; **1062**:116–26.
- 94 Tan YC, Kongpachith S, Blum LK, Ju CH, Lahey LJ, Lu DR *et al.* Barcode-enabled sequencing of plasmablast antibody repertoires in rheumatoid arthritis. *Arthritis Rheumatol* 2014; **66**:2706–15.
- 95 Kerkman PF, Rombouts Y, van der Voort EI, Trouw LA, Huizinga TW, Toes RE *et al.* Circulating plasmablasts/plasmacells as a source of anticitrullinated protein antibodies in patients with rheumatoid arthritis. *Ann Rheum Dis* 2013; **72**:1259–63.
- 96 Duke O, Panayi GS, Janossy G, Pouler LW. An immunohistological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies. *Clin Exp Immunol* 1982; **49**:22–30.
- 97 Young CL, Adamson TC III, Vaughan JH, Fox RI. Immunohistologic characterization of synovial membrane lymphocytes in rheumatoid arthritis. *Arthritis Rheum* 1984; **27**:32–9.
- 98 Schroder AE, Greiner A, Seyfert C, Berek C. Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. *Proc Natl Acad Sci USA* 1996; **93**:221–5.
- 99 Kim HJ, Krenn V, Steinhäuser G, Berek C. Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. *J Immunol* 1999; **162**:3053–62.
- 100 Owczarczyk K, Lal P, Abbas AR, Wolslegel K, Holweg CT, Dummer W *et al.* A plasmablast biomarker for nonresponse to antibody therapy to CD20 in rheumatoid arthritis. *Sci Transl Med* 2011; **3**:101, ra92.
- 101 Olee T, Yang PM, Siminovich KA, Olsen NJ, Hillson J, Wu J *et al.* Molecular basis of an autoantibody-associated restriction fragment length polymorphism that confers susceptibility to autoimmune diseases. *J Clin Invest* 1991; **88**:193–203.
- 102 Vencovsky J, Zd'arsky E, Moyes SP, Hajeer A, Ruzickova S, Cimburek Z *et al.* Polymorphism in the immunoglobulin VH gene V1-69 affects susceptibility to rheumatoid arthritis in subjects lacking the HLA-DRB1 shared epitope. *Rheumatology (Oxford)* 2002; **41**:401–10.
- 103 Voswinkel J, Pfreundschuh M, Gause A. Evidence for a selected humoral immune response encoded by VH4 family genes in the synovial membrane of a patient with RA. *Ann N Y Acad Sci* 1997; **815**:312–5.
- 104 Pascual V, Capra JD. VH4-21, a human VH gene segment overrepresented in the autoimmune repertoire. *Arthritis Rheum* 1992; **35**:11–8.
- 105 Doorenspleet ME, Klarenbeek PL, de Hair MJ, van Schaik BD, Esveldt RE, van Kampen AH *et al.* Rheumatoid arthritis synovial tissue harbours dominant B-cell and plasma-cell clones associated with autoreactivity. *Ann Rheum Dis* 2014; **73**:756–62.
- 106 Lee SK, Bridges SL Jr, Koopman WJ, Schroeder HW Jr. The immunoglobulin kappa light chain repertoire expressed in the synovium of a patient with rheumatoid arthritis. *Arthritis Rheum* 1992; **35**:905–13.
- 107 Fong S, Chen PP, Crowley JJ, Silverman GJ, Carson DA. Idiotypic characteristics of rheumatoid factors. *Scand J Rheumatol Suppl* 1988; **75**:58–65.
- 108 Tak PP, Doorenspleet ME, de Hair MJH, Klarenbeek PL, van Beers-Tas MH, van Kampen AHC *et al.* Dominant B cell receptor clones in peripheral blood predict onset of arthritis in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis* 2017; **76**:1924–30.
- 109 Amara K, Steen J, Murray F, Morbach H, Fernandez-Rodriguez BM, Joshua V *et al.* Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. *J Exp Med* 2013; **210**:445–55.
- 110 Morbach H, Wiegeling V, Richl P, Schwarz T, Suffa N, Eichhorn EM *et al.* Activated memory B cells may function as antigen-presenting cells in the joints of children with juvenile idiopathic arthritis. *Arthritis Rheum* 2011; **63**:3458–66.
- 111 Moller B, Aeberli D, Egli S, Fuhrer M, Vajtai I, Vogelin E *et al.* Class-switched B cells display response to therapeutic B-cell depletion in rheumatoid arthritis. *Arthritis Res Ther* 2009; **11**:R62.
- 112 Michelutti A, Gremese E, Morassi F, Petricca L, Arena V, Toluoso B *et al.* B-cell subsets in the joint compartments of seropositive and seronegative rheumatoid arthritis (RA) and No-RA arthritides express memory markers and ZAP70 and characterize the aggregate pattern irrespectively of the autoantibody status. *Mol Med* 2011; **17**:901–9.
- 113 Souto-Carneiro MM, Mahadevan V, Takada K, Fritsch-Stork R, Nanki T, Brown M *et al.* Alterations in peripheral blood memory B cells in patients with active rheumatoid arthritis are dependent on the action of tumour necrosis factor. *Arthritis Res Ther* 2009; **11**:R84.
- 114 Rudolph U, Rzepka R, Batsford S, Kaufmann SH, von der Mark K, Peter HH *et al.* The B cell repertoire of patients with rheumatoid arthritis. II. Increased frequencies of IgG+ and IgA+ B cells specific for mycobacterial heat-shock protein 60 or human type II collagen in synovial fluid and tissue. *Arthritis Rheum* 1997; **40**:1409–19.
- 115 Rouziere AS, Kneitz C, Palanichamy A, Dorner T, Tony HP. Regeneration of the immunoglobulin heavy-chain repertoire after transient B-cell depletion with an anti-CD20 antibody. *Arthritis Res Ther* 2005; **7**:R714–24.
- 116 Melamed D, Nemazee D. Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest. *Proc Natl Acad Sci USA* 1997; **94**:9267–72.
- 117 Verkoczy L, Duong B, Skog P, Ait-Azzouzene D, Puri K, Vela JL *et al.* Basal B cell receptor-directed phosphatidylinositol 3-kinase signaling turns off RAGs and promotes B cell-positive selection. *J Immunol* 2007; **178**:6332–41.
- 118 Benschop RJ, Cambier JC. B cell development: signal transduction by antigen receptors and their surrogates. *Curr Opin Immunol* 1999; **11**:143–51.
- 119 Derudder E, Cadera EJ, Vahl JC, Wang J, Fox CJ, Zha S *et al.* Development of immunoglobulin lambda-chain-positive B cells, but not editing of immunoglobulin kappa-chain, depends on NF-kappaB signals. *Nat Immunol* 2009; **10**:647–54.
- 120 Lange MD, Huang L, Yu Y, Li S, Liao H, Zemlin M *et al.* Accumulation of VH replacement products in IgH genes derived from autoimmune diseases and anti-viral responses in human. *Front Immunol* 2014; **5**:345.
- 121 van der Velden VH, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER *et al.* Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia* 2007; **21**:604–11.
- 122 Koralov SB, Novobrantseva TI, Konigsmann J, Ehlich A, Rajewsky K. Antibody repertoires generated by VH replacement and direct VH to JH joining. *Immunity* 2006; **25**:43–53.
- 123 Gojbori T, Nei M. Relative contributions of germline gene variation and somatic mutation to immunoglobulin diversity in the mouse. *Mol Biol Evol* 1986; **3**:156–67.
- 124 Griffiths GM, Berek C, Kaartinen M, Milstein C. Somatic mutation and the maturation of immune response to 2-phenyl oxazolone. *Nature* 1984; **312**:271–5.
- 125 Eisen HN, Siskind GW. Variations in affinities of antibodies during the immune response. *Biochemistry* 1964; **3**:996–1008.
- 126 Zhang W, Wang IM, Wang C, Lin L, Chai X, Wu J *et al.* IMPre: an accurate and efficient software for prediction of T- and B-cell receptor germline genes and alleles from rearranged repertoire data. *Front Immunol* 2016; **7**:457.
- 127 Janda A, Eryilmaz E, Nakouzi A, Cowburn D, Casadevall A. Variable region identical immunoglobulins differing in isotype express different paratopes. *J Biol Chem* 2012; **287**:35 409–17.
- 128 Dodev TS, Bowen H, Shamji MH, Bax HJ, Beavil AJ, McDonnell JM *et al.* Inhibition of allergen-dependent IgE activity by antibodies of the same specificity but different class. *Allergy* 2015; **70**:720–4.
- 129 Visciano ML, Tagliamonte M, Tornesello ML, Buonaguro FM, Buonaguro L. Effects of adjuvants on IgG subclasses elicited by virus-like particles. *J Transl Med* 2012; **10**:4.
- 130 Chaudhuri J, Alt FW. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. *Nat Rev Immunol* 2004; **4**:541–52.
- 131 Lomakin YA, Zakharova MY, Stepanov AV, Dronina MA, Smirnov IV, Bobik TV *et al.* Heavy-light chain interrelations of MS-associated immunoglobulins probed by deep sequencing and rational variation. *Mol Immunol* 2014; **62**:305–14.