Vascular Cytoprotection, Autoimmune Disease, and Premature Atherosclerosis

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Abstract

A healthy vascular endothelium is critical to health, and interference with endothelial homeostasis disrupts hemostasis, regulation of vascular tone and blood pressure, leukocyte trafficking, angiogenesis and tissue repair. Endothelial injury and apoptosis leads to endothelial dysfunction, which is closely associated with increased generation of reactive oxygen species, reduced endothelial nitric oxide (NO) synthase, increased consumption, and impaired synthesis of NO. Systemic inflammatory diseases including rheumatoid arthritis and systemic lupus erythematosus are associated with endothelial dysfunction, increased aortic stiffness, and accelerated atherogenesis. Premature cardiovascular disease is well-recognized feature of many rheumatic diseases. The cell and molecular mechanisms related to this remain poorly understood. Specific diseases display individual and common attributes that likely influence cardiovascular risk. A key challenge is the development of the means by which those at highest risk can be identified. Likewise, the ability of current therapies to mitigate risk and the identification of novel vasculoprotective therapies represent important areas of research focus. Similarly, close liaison between rheumatologists and cardiologists is essential to minimize the cardiovascular impact on patients and to ensure that patients with rheumatic disease and coexistent coronary heart disease receive appropriate therapy. Identification of safe therapeutic approaches that combine the targeted immunosuppression required, along with comprehensive vascular protection to control the primary disease and prevent secondary complications over the longer term, remains the ultimate challenge.

Key Words: Atherosclerosis, endothelium, inflammation, vascular injury

Introduction

The vascular endothelium lies at a critical interface between the blood constituents and the tissues. It is a highly diverse, multifunctional organ, central for the maintenance of an anticoagulant state, for leukocyte trafficking, blood pressure regulation, angiogenesis, and control of vascular permeability. Its importance to health is critical, and the negative effects of impaired endothelial homeostasis should not be underestimated. Abnormalities in endothelial function contribute to clinical features associated with sepsis, diabetes mellitus, atherosclerosis, thrombembolism, hypertension, and renal disease. The association between rheumatoid arthritis (RA) and premature cardiovascular (CV) death was reported in the 1950s.[1] More recently, systemic lupus erythematosus (SLE), gout, psoriatic arthritis, vasculitides, and ankylosing spondylitis has all been linked to an enhanced risk of premature CV events.[2]

The phenotype of the vascular endothelium is dynamic and can change rapidly in response to exogenous stimuli including rapid-acting mediators such as thrombin and histamine. Longer term changes are dependent upon gene transcription driven by pro-inflammatory cytokines including tumor necrosis factor (TNF)-α and interleukin (IL)-1β and by proangiogenic growth factors. In response, the endothelium increases expression of cell surface adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) to facilitate leukocyte trafficking or initiates development of endothelial sprouts and neovessels. Interference with the regulation of...
endothelial function is a characteristic feature of chronic inflammation.[3] Indeed, in RA, TNF-α drives enhanced adhesion molecule expression in the rheumatoid synovium, which is reversed by anti-TNF-α therapy, while increased vascular endothelial growth factor drives neoangiogenesis in the synovium.

Endothelial dysfunction is apparent in many diseases and is reversible in the face of effective treatment, at least transiently. Endothelial dysfunction increases the risk of apoptosis and atherogenesis. Its precise definition remains somewhat controversial. Classically, a local excess of reactive oxygen species (ROS) consumes nitric oxide (NO)-generating peroxynitrite and in turn favors uncoupling of tetrahydrobiopterin from endothelial NO synthase (eNOS) and thus impaired NO biosynthesis. More broadly, endothelial dysfunction can be considered as a maladaptive response resulting in impaired function which disrupts homeostatic functions including maintenance of vascular tone, thrombosis-hemostasis, control of angiogenesis, redox signaling, and predisposes to chronic inflammation[4] [Figure 1].

Vascular Injury, Systemic Inflammation, and Atherothrombosis

A multitude of factors may prove harmful to the vascular endothelium during prolonged systemic inflammatory responses. These include pro-inflammatory cytokines (TNF-α, IL-1, IL-6, and interferon (IFN)-α), autoantibodies, activation and deposition of complement components, and pro-inflammatory microvesicles.[2] Oxidized low-density lipoproteins and pro-inflammatory high-density lipoproteins, which are often raised in patients with inflammatory connective tissue diseases, may activate and injure the endothelium predisposing to atherogenesis. Downregulation of thrombomodulin and reduced prostacyclin synthesis, along with increased release of tissue factor, is prothrombotic. Local damage may be wrought by activated neutrophils and neutrophil extracellular traps (netosis) in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Increased circulating CD4+CD28− T-cells and a Th1/T regulator cell imbalance may be harmful in diseases including RA.[5]

Increasingly, there is recognition of the role played by noncoding RNAs in vascular disease pathogenesis. Micro-RNAs (miRs) act to inhibit mRNA translation and miR126, miR236, miR27a, miR143, and miR145 act to reduce atherogenesis.[6] In contrast, miR92a may inhibit the function of protective transcription factors, Kruppel-like factors 2 and 4.[7] miR34a favors upregulation of cellular adhesion molecules VCAM-1 and ICAM-1 and consequently monocyte adhesion.[8]

The central role of inflammatory pathways in atherogenesis is now established. This recognition formed the catalyst for collaborative basic science and clinical research programs and brought together immunologists, cell biologists, rheumatologists, and cardiologists. Migration of monocytes into the subintimal space is facilitated by endothelial injury and increased permeability. The local inflammatory response is fueled in part by IL-1β and TNF-α which induce expression of cellular adhesion molecules including VCAM-1. Monocyte adhesion to endothelial surface-expressed VCAM-1 precedes migration into the subintimal space along a chemotactic gradient involving chemokine CCL-2. Monocytes mature into macrophages and use their scavenger receptors to ingest lipid and create foam cells. A variety of other cells play critical roles including T- and B-cells, dendritic cells, vascular smooth muscle cells, and mast cells.[9] The importance of T- and B-cells in this process has been reinforced by the improved definition of cellular subsets, understanding of the proatherogenic actions of IFN-γ, and the role of B1-cells, which appear protective, and B2 cells proatherogenic.[9] The balance between the latter subsets is critical and may be of relevance given the wider use of B-cell depleting and modulating therapies in rheumatic diseases.

Rheumatic Diseases, Inflammation, and Premature Cardiovascular Events

Size of the problem

Although patients with rheumatic diseases typically exhibit more traditional CV risk factors than age- and sex-matched controls, this fact alone does not account for the increased prevalence of premature CV events.[2] Poorly controlled inflammation may enhance the impact of traditional risk factors. The association between systemic rheumatic diseases and CV disease (CVD) is most widely studied
in RA and SLE. It is important to note that accelerated atherogenesis is not the only concern, with myocarditis, dysrhythmias, nonischemic heart failure, and pulmonary arterial hypertension all contributing to the increased CV death rate.\[10\]

In RA, patients are prone to endothelial dysfunction and increased aortic stiffness which predispose to atherogenesis. Intimal-medial thickness (IMT) and plaque development proceeds rapidly in the first 6 years following diagnosis, followed by the appearance of increased CV mortality between years 7 and 10. This is reflected in the doubling of the risk of sudden cardiac death.\[11\]

Although the mechanisms involved may differ, the CVD risk is also significantly increased in those with SLE. This is particularly striking given the age range and female: male preponderance of the disease. Again abnormal endothelial function is apparent when assessed by flow-mediated dilatation and carotid artery IMT is increased early in the disease course. Taking CV events as a whole a 2.6-fold increase is revealed when compared to the general population, evident most frequently in those <40 years.\[12\]

Disease-related risk factors

The range of rheumatic diseases associated with premature CV events suggests that both shared and unique disease-related risk factors are important [Figure 2]. Poorly controlled disease activity has been directly linked to an increased incidence of CV events in both SLE\[12\] and RA.\[13\] Thus, in RA, raised acute phase reactants, seropositivity, active synovitis, erosive disease, and extra-articular features have all been linked to CVD.\[14\] In SLE, clinical activity indices, disease duration, anti-dsDNA titers, renal involvement, and steroid therapy have all been implicated in CVD.\[12\]

Emerging evidence from biologic registries suggest that drugs targeting TNF-\(\alpha\) and IL-6 may reduce the risk of premature CV events. This may reflect the effect of the cytokines on endothelial apoptosis, endothelial activation, and adhesion molecule expression. This may contribute to inflammatory vascular injury and exert a direct effect on atherosclerotic plaque propagation. Immunological disturbance in the form of increased CD4+CD28-cytotoxic T-cells, persistent complement activation and an imbalance of Th\(_1\) and Th\(_{10}\) cells have all been implicated in accelerated atherogenesis.\[2\] In the ANCA-associated vasculitides, activated neutrophils may directly injure the vascular endothelium and the generation of ROS may exacerbate endothelial injury.

The contribution of genetic predisposition to CVD and its relationship with environmental influences remains to be established. Perhaps, the best example is the combined role of the HLA-DRB1-shared epitope in disease susceptibility to RA and in premature CVD, an impact that is further exacerbated by cigarette smoking.\[15\]

Predisposition to premature cardiovascular events

When considering why individuals suffering rheumatic diseases are prone to premature CV events, it is often assumed that they have more extensive atherosclerosis than matched controls. However, in the main, the pattern and extent of coronary artery atherosclerosis does not appear to differ. Study focus has, therefore, shifted to analysis of plaque biology. Comparing atherosclerotic plaques in RA and matched controls, 48% were graded unstable in RA versus 22% in controls.\[16\] The inflammatory environment may also predispose to endothelial erosion, plaque instability, and rupture.\[16\]

How Can We Identify Patients at Risk?

Identification of those with rheumatic diseases most at risk of premature CV complications remains to be optimized. Not only would this allow targeting of diagnostic and therapeutic resources to those most at risk, it would also likely reduce morbidity and mortality. However, discovery and validation of novel CV risk biomarkers in rheumatic diseases is required. Cardiac positron emission tomography in patients with RA and SLE with angiographically normal epicardial arteries demonstrated abnormalities in microvascular blood flow and coronary flow reserve in a significant number when compared with controls and these may represent those most at risk.\[17\] Carotid artery ultrasound in experienced hands may act as an early indicator of atherosclerotic disease, demonstrating increased IMT and/or early plaque development, and help predict risk of CV events.\[18,19\]

Attempts are being made to refine CV risk prediction scores for use in those with rheumatic diseases and ideally to develop disease-specific tools. EULAR have proposed multiplying a standard CV risk calculator (mSCORE)
by 1.5× while others have proposed the addition of carotid US data to the score.[20] Likewise, consideration has been given to adding endothelial dysfunction and/or aortic stiffness data. The recently published QRISK3 prediction algorithm allows incorporation of additional clinical variables including a diagnosis of SLE and/or the presence of renal impairment.[21] All of these approaches now require extensive prospective validation in specific disease groups.

Management Approaches to Those with Cardiovascular Disease

While the prevention of CVD is the principle aim (see below), closer collaboration between rheumatologists and cardiologists is required to optimize the management of CVD in those with rheumatic diseases. Although published evidence in this area is somewhat disturbing, the situation is rapidly changing and multidisciplinary management is far more common. Historically, perhaps, due to concerns regarding risks associated with the underlying autoimmune disease, patients with RA or SLE often received less aggressive primary and secondary prevention therapy.[20] Moreover, they exhibit worse outcomes following a myocardial infarction than matched controls.[22]

In addition to concerns regarding coronary intervention in the face of a systemic inflammatory disease and immunosuppressive therapy, there is a biological reason why prognosis may be worse. In vivo experiments in murine models have demonstrated that systemic or distant inflammation in the vasculature predisposes to increased inflammation in atherosclerotic plaques.[23] Although, with wider use of acute percutaneous coronary intervention, rheumatic disease patients are more likely to receive equivalent therapy, the effect of angioplasty on the culprit lesion might inadvertently and deleteriously affect other sites of atherosclerotic disease in those with systemic inflammation. This hypothesis needs to be considered and appropriate monitoring strategies devised.

Impact of Antirheumatic Therapies on Cardiovascular Events

Data concerning the impact of disease-modifying antirheumatic therapies on CVD and CV events remain somewhat sparse. This reflects the relative rarity of CV events and hence the need for large prospective clinical trials. CV events have typically been included as secondary end points, and the majority of trials have proved underpowered.

Disease-modifying antirheumatic drugs

Methotrexate remains the most widely used disease-modifying antirheumatic drug (DMARD) for the treatment of RA. Available data also suggest that its use in patients with RA or psoriatic arthropathy (PsA) can reduce the number of CV events and associated mortality.[24] Recent data extend and reinforce these findings. In patients with RA or PsA, methotrexate therapy reduced the incidence of all CV events by 28%,[25] Control of disease activity is key, a 10-point reduction in the time-averaged Clinical Disease Activity Index resulted in a 21% reduction in CV risk.[26]

Sulfasalazine and hydroxychloroquine therapy are reported to have a lesser but still positive effect on CV events in RA and SLE.[27,28] Mycophenolate mofetil has been shown to exert direct effects on the biology and progress of atherosclerotic plaques.[29]

Biologic therapies

The introduction of biologic therapies has markedly impacted on disease activity in RA and reduced the incidence of erosive disease. Meanwhile, biologics are proving similarly effective in seronegative spondyloarthropathies, PsA, and ANCA vasculitis while experience and understanding of their use and efficacy is growing in large vessel vasculitides and SLE. In those with RA who respond to TNF-α inhibitors within 6 months, there is a reduced rate of myocardial infarction.[30] Meta-analyses reveal a 30% CV event risk reduction.[31] This benefit for both TNF-α antagonists and methotrexate is most obvious in those presenting with active RA and four traditional risk factors, where a rapid reduction in carotid IMT can be observed in treatment responders.[14]

Data concerning CV risk and other biologics is relatively sparse. Rituximab or tocilizumab therapy has been associated with reduced aortic stiffness as measured by pulse wave velocity.[32] Although tocilizumab may increase plasma low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), no correlation with changes in lipid levels and future CV events was observed, while in contrast, improved control of disease activity by tocilizumab was protective.[32]

Nonsteroidal anti-inflammatory drugs

The controversy concerning the CV risk posed by traditional nonsteroidal anti-inflammatory drugs (NSAIDs) and particularly cyclo-oxygenase-2-specific antagonists (COXIBs) has been widely reported. Concern regarding an increased risk of atherothrombosis was largely but not exclusively predicated upon clinical trials involving rofecoxib and the “prostanoid hypothesis,” whereby COXIBs shifted the balance between thromboxane A2 and prostacyclin synthesis to favor a prothrombotic environment. Current data suggest that prolonged use of either NSAIDs or COXIBs confers a small and manageable CV risk that these drugs should be avoided wherever possible in those with preexisting CVD. Of note, increased CV events were not seen in RA patients treated with these drugs,[33] presumably reflecting a beneficial effect on the pro-inflammatory environment.
On account of significant heterogeneity, current data suggest that the safety profiles of each drug should be considered individually, rather than as part of a class. For example, the antiplatelet effects of naproxen confer a CV benefit when compared to other NSAIDs. Likewise, the risk of hospital admission with cardiac failure was significantly increased by etoricoxib, rofecoxib, and a group of NSAIDs but not by celecoxib.\textsuperscript{[34]} The recently reported Prospective Randomized Evaluation of Celecoxib Integrated Safety versus Ibuprofen or Naproxen (PRECISION) trial of 24,081 RA and OA patients has also revealed important differences. Although the dropout rate in the trial was high, celecoxib was shown to be noninferior or better than ibuprofen or naproxen in terms of CV, gastrointestinal, and renal safety.\textsuperscript{[35]} Further analysis has demonstrated that celecoxib is significantly less likely to increase blood pressure or induce new hypertension than ibuprofen or naproxen.\textsuperscript{[36]} Indeed, it appears that the concern about COXIBs as a class was largely due to the effect of rofecoxib alone.

These data have raised interest in COX-2-independent actions of COXIBs. Our group reported that celecoxib but not rofecoxib induced the anti-inflammatory, antioxidant, antiapoptotic protein heme oxygenase-1 (HO-1) in human endothelium. The mechanism underlying this response involved activation of Akt and modulation of mitochondrial redox signaling.\textsuperscript{[37]} The ability of celecoxib to alter TNF-\(\alpha\) signaling in the endothelium involves inhibition of JNK mitogen-activated protein kinase. This action prevented the induction of tissue factor, a response that was not reproduced by rofecoxib.\textsuperscript{[38]}

**Corticosteroid therapy**

Glucocorticoids remain an essential component of drug therapy for many rheumatic diseases. Strides toward minimizing their use have been made and are ongoing. Steroid-sparing therapies are more widely used to allow more rapid glucocorticoid tapering toward a dose of prednisolone of \(\leq 7.5\) mg daily. However, a significant corticosteroid side effect burden remains in many conditions. These include a deleterious effect on CV risk profiles with a propensity toward hypertension, hyperlipidemia, and steroid-induced diabetes mellitus. While these are well recognized, it is important to note that insufficient use of glucocorticoids leading to persistent disease activity may enhance the risk of atherogenesis in SLE.\textsuperscript{[19]} However, in RA, a 47% increase in CV events in those receiving corticosteroids was revealed.\textsuperscript{[22]}

**Statins**

Hydroxy-methyl CoA reductase antagonists (statins) reduce CV risk by lowering LDL-C and through additional beneficial lipid-independent actions including modulation of redox signaling, thrombotic, inflammatory, and immune signaling cascades.\textsuperscript{[39]} Although widely prescribed in patients with systemic inflammatory rheumatic diseases, specific clinical trial data in support of the routine use of statins are sparse.\textsuperscript{[2,40]} Although guidelines are lacking, recommended indications for statins in RA and SLE include use in those with LDL-C \(\geq 190\) mg/dL and 100 mg/dL, respectively.\textsuperscript{[41,42]} Notwithstanding, demonstration of a clear benefit of statins in CV event prevention in those with SLE has proved elusive.\textsuperscript{[43,44]}

**The Vascular Endothelium and Systemic Inflammatory Diseases**

Endothelial dysfunction has been linked to all stages of atherogenesis and hence represents an important therapeutic target.\textsuperscript{[4]} Endothelial dysfunction is evident early in the course of diseases including SLE, RA, and the vasculitides. Similarly, pulse wave velocity analysis reveals evidence of aortic stiffness while cardiac microvascular blood flow and coronary flow reserve are diminished. Although the precise mechanistic relationship between systemic inflammation and disruption of endothelial function is multifactorial and context dependent and remains to be fully understood, it is likely to be directly related to the increased risk of premature CV events.\textsuperscript{[42]}

This association between inflammation and endothelial dysfunction can also be observed in arterial endothelium exposed to disturbed blood flow, generated by arterial curves and branch points.\textsuperscript{[45]} Although direct evidence is lacking, the systemic inflammatory milieu seen in chronic rheumatic diseases is likely to exacerbate the abnormal endothelial function at these atherosclerosis-prone sites. Likewise, developing atherosclerotic plaques are covered by arterial endothelium. Increased endothelial cell apoptosis in the face of systemic inflammatory disease may predispose to loss of integrity of the endothelium overlying atherosclerotic plaques. This endothelial erosion leads to enhanced risk of coronary thrombosis. Intriguingly, ligation of endothelial toll-like receptor 2 and the local actions of neutrophils contribute to increased endothelial apoptosis, detachment, and superficial erosion.\textsuperscript{[46]}

In established plaques, the pro-inflammatory actions of TNF-\(\alpha\) induce release of matrix metalloproteinases so increasing the risk of plaque rupture. Although direct evidence is required, the pro-inflammatory environment of active systemic rheumatic diseases might enhance this process, and hence, effective anti-TNF-\(\alpha\) therapy reduces the risk of CV events.

**Therapeutic vascular protection**

Given its reversibility and links to atherosclerosis, endothelial dysfunction offers a potential therapeutic target which has yet to be fully realized [Figure 3]. This fact is emphasized by the premature CV events described in the systemic inflammatory diseases, where preventative therapy remains an important goal. As suggested above,
Mason: Vascular protection in autoimmune disease

In vivo

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vasculoprotective in a murine model of SLE-associated reproduced this response and MTX therapy proved pathway to induce the expression of antioxidant proteins (a member of the cAMP response element -binding (CREB) kinase (AMPK) may be important for vascular protection.

Our recent work supports this hypothesis. In addition to CREB1, the vasculoprotective transcription factors, Kruppel-like factors 2 and 4 may offer important therapeutic targets for endothelial dysfunction. Endothelial KLF-2 and KLF-4 activity is increased in arterial endothelium exposed to atheroprotective laminar shear stress. Moreover, statin therapy enhances KLF-2 activity and increases endothelial expression of protective proteins including eNOS, HO-1, and the complement inhibitory protein CD59. These actions also involve the protective protein kinases AMPK and Erk5.

In the search for effective vasculoprotective therapeutic combinations, we noted reports from the transplant field indicating that rapamycin had specific benefits in terms of preventing postcardiac transplant arteriopathy. Given the common co-prescription of rapamycin and statins in such patients, we investigated potential drug synergy. In therapeutically relevant concentrations, atorvastatin and rapamycin exhibited a synergistic response, protecting endothelium against complement-mediated injury through maximal induction of the inhibitory protein decay-accelerating factor, CD55. This response was dependent upon protein kinase C-α, AMPK, and CREB signaling and was reproduced in a murine model. Further consideration should now be given to optimizing drug combinations in those with systemic inflammatory disease.

Conclusions and Future Challenges

In an era, when immunosuppressive targeting of systemic inflammatory diseases has improved very significantly, an important outstanding therapeutic challenge for rheumatologists remains, namely, vascular protection and prevention of premature CVD. To achieve this, research efforts are directed at the identification of novel biomarkers and molecular targets, utilizing high throughput technologies including proteomics, metabolomics, and genomics. Alongside this, further clinical trials incorporating primary CV end-points are needed.

The recently published CANTOS trial tested the link between inflammation and atherothrombosis. The randomized double-blind placebo-controlled trial investigated the effect of IL-1β antagonist canakinumab and in >10,000 participants with a history of previous myocardial infarction and a CRP level ≥2 mg/L. After median follow-up of 3.7 years, 150 mg s/c canakinumab every 3 months significantly reduced further CV events compared to placebo and independently of lipid lowering.

intramyocardial arteriopathy. The ongoing CV inflammation reduction trial (NCT01594333) will test the positive CV effects of MTX seen in RA in a separate group of patients, namely, those with stable coronary disease and diabetes, but without a rheumatic disease, to look for protection against future CV events. The results are awaited with interest.
analysis showed that responders who achieved a CRP < 2 mg/L demonstrated a 25% reduction in future CV events, a response not seen in those whose CRP remained ≥2 mg/L.

These data are likely to be especially relevant for those with systemic inflammatory rheumatic disease and offer further encouragement to rheumatologists that effective targeting of inflammation with a treatment-to-target strategy combining immunosuppression with vascular protection can improve long-term patient outcomes.

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Conflicts of interest

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