# The role of the endothelium in systemic small vessel vasculitis

Mark A. Little, Caroline O.S. Savage

Division of Immunity and Infection, University of Birmingham, Edgbaston, Birmingham B15 2TH

Please address correspondence to: Caroline Savage, Professor of Nephrology, University of Birmingham, Edgbaston, Birmingham B15 2TH, UK. E-mail: c.o.s.savage@bham.ac.uk

Received on March 6, 2008; accepted in revised form on April 1, 2008. Clin Exp Rheumatol 2008; 26 (Suppl. 49): S136-S141.

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**Key words:** Vasculitis, ANCA, endothelium, protease, adenosine, Wegener's granulomatosis.

# ABSTRACT

The endothelium is the back-drop against which the effects set in train by interactions between Anti-Neutrophil Cytoplasm Antibodies (ANCA) and neutrophils are played out. This review considers the mechanisms of the endothelial cell injury that may result but also questions the impact of endothelial heterogeneity and endothelial cell activation in facilitation of vasculitic lesions, as well as the potential roles for endothelial-dependent anti-inflammatory mechanisms in controlling inflammation.

# Introduction

"The disseminated necrotising arteritis seen in Wegener's granulomatosis may be due to an immunological reaction against an antigen circulating in the blood stream" (1)

This prescient statement by Kahn in 1954 predicted the discovery of Anti-neutrophil Cytoplasm Antibodies (ANCA), autoantibodies directed against components of circulating neutrophils that are often found in patients with systemic small vessel vasculitis. The pathogenic potential of these antibodies now appears likely to be important in the initiation of vasculitis (2), but it is not the target of these antibodies, neutrophils, that one finds in the tissues of patients with this devastating condition. Rather, one sees the aftermath of their destructive capability on the single-cell lining of the cardiovascular tree that forms the crucial interface between the blood and its components on one side, and the tissues and organs on the other: the vascular endothelium. Lying as they do at this interface, the endothelial cells are well placed to perform many synthetic and metabolic functions including secretion of prostacyclin, nitric oxide, von Willebrand factor, plateletderived growth factor, endothelin-1 and chemokines. The endothelium is a non-thrombogenic gatekeeper that controls, by means of selective adhesion molecule and chemokine expression, which cells to usher into sites of inflammation and repair. This review places these functions of endothelium in the context of the physiological dysregulation witnessed in ANCA-associated vasculitis.

# The endothelium as site of initial

injury in ANCA-associated vasculitis Although the histopathological appearance of established, clinically evident, systemic vasculitis is usually one of frank trans-mural necrosis of the blood vessel wall, the early pathological lesion is detachment of endothelial cells from the basement membrane progressing through endothelialitis (3). Therefore, it was logical for initial studies to focus on the effect of ANCA-stimulated neutrophils on endothelial cells. The potential for endothelial injury in this setting was first demonstrated in static assays using radio-labelling of endothelial cells as a readout for cytotoxicity (4, 5). This was complemented by studies demonstrating enhanced static ANCA-stimulated neutrophil adhesion to TNFα-stimulated HUVEC (6-8). In addition, if ANCA-stimulated neutrophils are incubated with endothelium, it is possible to measure a marked increase in Von-Willebrand factor release (reflecting acute endothelial cell activation / stress) (9), and Von Willebrand factor levels have been found to be elevated in the serum of patients with Wegener's granulomatosis (10). The MPO released by degranulating neutrophils is highly cationic and has been shown to bind non-covalently to endothelial cells, thus potentially allowing ANCA binding and possible complement fixation with resultant endothelial injury (11, 12). However, leukocyte adhesion occurs in vivo as part of a co-ordinated multi-step process ultimately resulting in extravasation

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into the extravascular space, a process ultimately controlled by local chemokine expression and activation characteristics of the endothelial cells. Thus, the logical extension of these studies was to use assays that mimic this process under conditions of flow.

Using a flow chamber to mimic the physiological shear stresses observed in vivo, Radford et al. found that treatment of neutrophils rolling over a platelet or endothelial monolayer with ANCA IgG resulted in CD11b dependent conversion to firm adhesion and acceleration of neutrophil transmigration (13, 14). These static and flow-based experiments began to paint a picture of ANCA-induced dysregulation at the point where the neutrophil interacts with endothelium and were subsequently confirmed in vivo using a rat and a mouse model of systemic vasculitis (15, 16), with evidence of acute ANCA-induced endothelial injury in mesenteric blood vessels and lung capillaries, as characterised by localised haemorrhage (15) (Fig. 1). Nolan et al. went on to demonstrate that this ANCA-induced enhancement of leukocyte-endothelial interaction was dependent upon the presence of the adhesion molecule CD18 and Fcy receptor expression on leukocytes (16).

One can imagine that, if such endothelial injury is occurring in patients with active ANCA-associated vasculitis, as suggested by the earliest electron microscopy studies of Davis (3), some blood vessels would lose their endothelial cell lining, leaving areas of exposed basement membrane. Indeed, it is possible to detect a marked increase in circulating endothelial cells, which are necrotic or strongly pro-inflammatory (17), in patients developing new active vasculitis and in those relapsing with vasculitis (Fig. 2) (18, 19). Levels of these circulating endothelial cells fall back towards those seen in people without vasculitis when the vasculitic process is brought under control with appropriate therapy. De Groot and colleagues went on to demonstrate that these putative endothelial defects may be replenished by CD34+ haematopoetic and endothelial progenitor cells, levels of which were found to approximately double as



**Fig. 1.** Microvascular injury resulting in localised haemorrhage from mesenteric vessels following infusion of anti-MPO antibodies into a WKY rat. This image was captured during the course of an intravital microscopy experiment (H&E, x10).



**Fig. 2.** Schema of events following microvascular injury in the context of systemic vasculitis. **A.** Local endothelial cell injury results in the appearance of circulating (pro-inflammatory or necrotic) endothelial cells, which return to near normal levels with the induction of remission (graph reproduced with permission from *The Lancet*; **B.** The amount of circulating CD34<sup>+</sup> endothelial progenitor cells (EPC) increases dramatically as remission is induced. (Image reproduced with kind permission from Dr Kirsten De Groot, Klinikum Offenbach, Germany).

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# Fenestrated with diaphragm Pancreas Arenal cortex Pancreas Arenal cortex International cortex Brenerular capillaries Communicational contex Brenerular capillaries Fenestrated without diaphragm Skeletal muscle Lung alveolar Continuous

**Fig. 3.** Heterogeneity of the vascular endothelium. Some endothelia are continuous and some are fenestrated (with and without diaphragm). This heterogeneity reflects the function of the underlying tissue and may underlie some of the variation in vasculitic injury in systemic vasculitis. (Image adapted with permission from anatomium.com and from *The Journal of Cell Biology* 1985; 100: 418-28. <sup>©</sup>1985 the Rockefeller University Press).

the patients entered remission (Fig. 2) (20). Intriguing data from Holmen *et al.* also raise the possibility that, as well as being a potential biomarker for active vasculitis, circulating highly activated endothelial cells may actually inhibit endothelial repair by these progenitor cells, thus potentially pushing the vascular injury towards scar formation (17).

What we have little understanding of at present is whether age has any impact on the development of endothelial injury, given that ANCA-associated vasculitis is a disease primarily of the elderly, with a peak incidence in the 60-70 year old age group. It is conceivable that micro-endothelial damage from the effects of pre-existing hypertension, smoking or ischaemia may hasten or amplify vasculitic injury.

# Heterogeneity of the vascular endothelium

Why does systemic vasculitis particularly affect the lungs, upper respiratory tract and glomeruli? This is an important question, the answer to which will be very illuminating for the overall issue of the molecular pathogenesis of vasculitis. Various reasons have been proposed, including exposure of the particular tissue to exogenous antigens/infectious agents, variations in blood flow and endothelial shear stress, cytokine/ chemokine patterns in the perivascular

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tissue and factors pertaining to characteristics of the endothelial cells themselves. The latter concept applies to both expression of particular endothelial cell adhesion molecules as well as ultrastructural variation in endothelial cell anatomy. For example, endothelial cells from glomeruli are very different in structure from those in skeletal muscle, the former displaying large fenestrations (without covering diaphragm) consistent with the primary glomerular function of plasma filtration (Fig. 3) (21). In addition, endothelial beds from certain organs have differential expression of adhesion molecules in response to injury, such as Vascular Adhesion Protein-1 (VAP-1, strongly expressed in the inflamed kidney) (17) or Vascular Cell Adhesion Molecule-1 (VCAM-1, strongly expressed in the glomerular endothelium of patients with vasculitis) (22). Thus, the endothelium is well placed to account for some of the variation in organ injury in vasculitis; the specific question as to whether glomerular endothelial cells are particularly effective at recruiting ANCA-stimulated leukocytes is the subject of ongoing intense research.

# Anti-endothelial cell antibodies in vasculitis

Interest in the possible role of autoantibodies directed against individual components of the endothelium in systemic vasculitis (AECA) has waxed and waned over the past 20 years. Depending on methods used and the stage of disease, the fraction of patients with small vessel vasculitis harbouring these antibodies ranges from 20 (23, 24) through 59% (25) and up to 80% (26). This large degree of variability and technical difficulty with the assay, coupled with difficulties in differentiating the auto from the allo-immune response (27), has meant that the clinical utility of AECA has largely receded in the wake of the rise of ANCA. However, there has been some renewed interest in recent years, a study by Holmen et al. reporting a high incidence (71%) of antibodies directed against human kidney microvascular endothelial cells in patients with Wegener's granulomatosis (17). Interestingly, when the

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same samples were used on HUVEC (the substrate used in many of the early studies), this group could detect antibodies in only 7% of cases. This study extended previous work by demonstrating clear activation of SAPK/JNK intracellular signalling pathways and intracellular calcium flux following binding of AECA.

One of the principal barriers when considering how AECA may effect endothelial cell injury via complement-mediated lysis or antibody dependent cellular cytotoxicity in ANCA-associated vasculitis is the absence of significant amounts of deposited complement or immunoglobulin in this condition. It has been suggested that immune deposits may be present early in the disease course (before tissue biopsy) (28), but the prevailing opinion is that this is a truly "pauci-immune" condition, implying that alternative mechanisms of AECA-induced endothelial injury would need to be entertained. Such potential mechanisms could include induction of endothelial cell apoptosis by AECA (29), a concept that requires further study.

# Role of serine proteases and other neutrophil degranulation products in modulating the local immune response

The most extensively studied neutrophil response to ANCA is the exaggerated respiratory burst and release of superoxide. However, as indicated below, this effect is strongly inhibited at the endothelial surface. Indeed, when co-cultures ANCA-stimulated one neutrophils with endothelial cells in the presence of the NADPH oxidase inhibitor diphenylene iodonium (DPI) superoxide production is almost completely inhibited, but it is still possible to detect release of Von Willebrand factor, indicating non-oxidative endothelial injury (9). This has led researchers to investigate other neutrophil degranulation products as possible mediators of ANCA-induced endothelial injury. Foremost in this regard are the serine proteases Proteinase-3 (PR3) and Human Neutrophil Elastase (HNE). Serine protease activity is detectable in the supernatants of ANCA-stimulated

neutrophil/endothelial cell co-cultures and addition of the protease inactivator diisopropyl fluorophosphate (DFP) almost completely inhibits Von Willebrand factor release in these co-culture experiments (9). Furthermore, it is clear that PR3 itself is capable of inducing endothelial cell cytolysis (30) and Von Willebrand factor release from endothelium (9), that secreted PR3 has angio-regulatory properties and that it is capable of inducing endothelial cell apoptosis directly (31, 32).

It is thus interesting that there is an association between partially inactivating polymorphisms of the primary endogenous PR3 inhibitor, α1-anti-trypsin (A1AT), and development of ANCAassociated vasculitis (33-35). This protease inhibitor ensures that the potentially deleterious effect of PR3 and HNE released from neutrophils at the endothelial surface is limited to the immediate peri-neutrophil environment; relative lack of efficacy of this system in patients with, for example, the PiZZ polymorphism of A1AT is unlikely to be a primary cause of vasculitis, but may allow the initial ANCA-induced injury to be more severe.

Recent studies also point to other factors released from human neutrophils that may be capable of activating the alternate complement pathway. In vivo studies with the murine model of MPO-ANCA vasculitis have shown that animals deficient in the final common pathway component C5 or the alternate pathway component Factor B are protected against development of vasculitic lesions, while animals deficient in the classical and lectin pathway component C4 are not (36). How the alternate complement pathway provides such an apparently strong permissive effect will need to be determined but, given the pauci-immune nature of the lesions, it is likely that these effects are operating through non-classical mechanisms.

# The role of the endothelium in switching off local immune responses

As well as being a potential target and mediator of uncontrolled local inflammation, the endothelium itself plays an important role in acting as a brake to inflammation (37). Endothelial cells:

- are an important source of adenosine (which can down-regulate neutrophil responses via interaction with the A<sub>2AI</sub> adenosine receptor),
- are capable of preferentially inducing expansion of CD4<sup>+</sup>CD25<sup>+</sup>*FoxP3*<sup>+</sup> regulatory T-lymphocytes with the capacity for inhibiting proliferation of alloreactive T-cells (38)
- are a potential source of transcellular production of the anti-inflammatory aioscapoids the Lipoving (38)

tory eicosanoids, the Lipoxins (38). When one co-incubates neutrophils with endothelial cells in the presence of ANCA, the normal ANCA-induced superoxide response seen in the absence of endothelial cells is strongly inhibited (9). Lu et al. went on to show that this inhibitory effect is largely reversed when adenosine deaminase (which breaks adenosine down) is introduced into the system, suggesting that endothelial adenosine production may be important in damping down potentially injurious neutrophil activation at the endothelial surface. Adenosine is produced in vivo by ecto 5'-nucleotidases, the most important of which is membrane bound CD73, which is expressed on endothelial cells. Mice lacking this molecule display exaggerated VCAM-1 expression, enhanced monocyte capture and more severe plaque formation in a carotid wire injury model (39). Thus, it is possible that defects in local adenosine production may contribute to focal endothelial injury in systemic vasculitis.

Other natural neutrophil braking systems include the lipoxin and annexin pathways. Lipoxins are eicosanoids generated in neutrophils and platelets from arachidonic acid by preferential activation of 5- and 15-Lipoxygenase, thereby pushing prostaglandin and Leukotriene synthesis towards production of the anti-inflammatory compounds Lipoxin A4 and B4. Aspirin is particularly effective at directing these pathways towards Lipoxin synthesis. These agents inhibit neutrophil and eosinophil recruitment to sites of inflammation, block many of the actions of TNF- $\alpha$ and enhance macrophage phagocytosis of apoptotic leukocytes (40). They are important in promoting resolution of the inflammatory process and, although there is no direct evidence of an impaired Lipoxin response in vasculitis, it is intriguing to contemplate whether simple use of aspirin would be of benefit in accelerating resolution of endothelialitis.

# Conclusion

The endothelium may be both a facilitator for development of vasculitis as well as a target for injury, with additional roles for switching off an ongoing inflammatory response. Timing and strength of responses during these three phases may dictate the severity of vascular injury. Expanding knowledge of these processed will assist in the development of novel therapeutic targets that will either inhibit the initiation of vascular injury at the level of the endothelial cell, or accelerate resolution of uncontrolled inflammation.

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