When ANCA were first described in the early 1980s [1, 2], it rapidly became apparent that the presence of these antibodies was very useful diagnostically. A debate ensued that logically asked the question as to whether these antibodies were merely useful biomarkers, or whether they were in fact part of the pathogenic process and capable of inducing the pathological features seen in this disease. Various attempts at modelling the autoimmune reaction to the autoantigens myeloperoxidase (MPO) [4–9] and proteinase-3 (PR3) [10] followed, with the seminal studies of Xiao, Heeringa, Falk and Jennette [11] proving that, under certain circumstances, antibodies directed against MPO were sufficient to recapitulate the glomerular pathology seen in human MPO-ANCA vasculitis. Once the acute vasculitic pathology had been successfully modelled in this and related animal models, it opened the door to dissecting the biological components that were either necessary or not required for development of pathology, including neutrophils (essential) [12, 13], T cells (variably dispensable depending on model) [12, 14], B cells (variably essential depending on model) [12, 14], Toll-like receptors (TLR2, 4, 9 exacerbate) [15–23], mast cells (attenuate) [18], tumour necrosis factor α [19, 20] and the alternative complement pathway (essential) [21–23]. The latter was surprising, given that complement was not conventionally thought to be important in ANCA vasculitis, and has led directly to a clinical trial investigating the efficacy of an oral C5a receptor antagonist in affected patients, to date the only new therapeutic agent ever developed and tested for use in this disease (clinical trial ID NCT01363388). Thus, the last decade has seen an explosion of our knowledge in MPO-ANCA vasculitis flowing from the ability to recapitulate the disease in a pliable animal model.

**L7. Animal models of PR3-ANCA vasculitis: Approaches and controversies**

**In vivo modelling of ANCA-associated systemic small vessel vasculitis**

When ANCA were first described in the early 1980s [1–3], it rapidly became apparent that the presence of these antibodies was very useful diagnostically. A debate ensued that logically asked the question as to whether these antibodies were merely useful biomarkers, or whether they were in fact part of the pathogenic process and capable of inducing the pathological features seen in this disease. Various attempts at modelling the autoimmune reaction to the autoantigens myeloperoxidase (MPO) [4–9] and proteinase-3 (PR3) [10] followed, with the seminal studies of Xiao, Heeringa, Falk and Jennette [11] proving that, under certain circumstances, antibodies directed against MPO were sufficient to recapitulate the glomerular pathology seen in human MPO-ANCA vasculitis. Once the acute vasculitic pathology had been successfully modelled in this and related animal models, it opened the door to dissecting the biological components that were either necessary or not required for development of pathology, including neutrophils (essential) [12, 13], T cells (variably dispensable depending on model) [12, 14], B cells (variably essential depending on model) [12, 14], Toll-like receptors (TLR2, 4, 9 exacerbate) [15–23], mast cells (attenuate) [18], tumour necrosis factor α [19, 20] and the alternative complement pathway (essential) [21–23]. The latter was surprising, given that complement was not conventionally thought to be important in ANCA vasculitis, and has led directly to a clinical trial investigating the efficacy of an oral C5a receptor antagonist in affected patients, to date the only new therapeutic agent ever developed and tested for use in this disease (clinical trial ID NCT01363388). Thus, the last decade has seen an explosion of our knowledge in MPO-ANCA vasculitis flowing from the ability to recapitulate the disease in a pliable animal model.

**In vivo modelling of proteinase-3-ANCA vasculitis**

A natural extension of the antibody transfer techniques of Xiao et al. was to attempt the same in PR3 associated disease. Pfister et al. reported experiments describing the induction of PR3 antibodies in PR3/elasatse−/− mice, with passive transfer of these antibodies to PR3 replete 129 Sv/Ev mice in a manner directly analogous to the MPO−/− approach [10]. The recipients did not develop the same pathological features of vasculitis, although a subtle increase in inflammation was observed in TNFα exposed skin. Why did this strategy fail when it worked so dramatically for MPO immunity? Although PR3 is expressed on the surface of human neutrophils in association with CD177 [24, 25], it is not detectable on the surface of unstimulated murine neutrophils, so there is no available antigen for the antibody to bind to. In addition, the PR3 molecule is only 68% homologous with its human homologue, whereas it is 86% identical in the case of myeloperoxidase, and PR3 has very different physico-chemical properties to MPO (isoelectric point 6.7 and 10 respectively). Finally, it is conceivable that PR3 autoimmunity is not actually a central component of the clinical syndrome Granulomatosis with polyangiitis (GPA, Wegener), that it is in fact an epi-phenomenon. However, the results of a recent genome wide association study investigating the
This genetic contribution to ANCA vasculitis argue strongly against this [26]: three SNP’s were identified as having a significant association with vasculitis, which were located within the genes encoding HLA-DP (in the MHC region), PRTN3 (the putative autoantigen, PR3) and SERPINA1 (alpha1 anti-trypsin, the natural physiological inhibitor of PR3). Furthermore, these genetic associations were only observed in the cohort with PR3-ANCA vasculitis. Therefore, this places PR3 autoimmunity at the heart of PR3-ANCA vasculitis and provides a strong rationale for continuing the search for a means of modelling this. Several other groups have switched their experimental approaches in an attempt to achieve this:

- Van der Geld et al. exploited this difference in human and murine PR3 by generating a series of chimeric PR3 molecules and immunising both mice and WKY/Brown Norway rats [27]. A robust antibody response was observed but no pathological features of vasculitis were observed;

- Primo et al. mirrored the splenocyte transfer approach used by Xiao et al. in the MPO antibody model, but shifted to the autoimmune-prone NOD strain [28]. Again, after immunising with recombinant murine PR3 (rmpR3), high antibody titres were observed in the immunised mice, which displayed a typical c-ANCA pattern when serum was incubated with fixed neutrophils. Interestingly, 20% of circulating Mac-1 positive cells from immunised mice actually had antibody (presumed directed against PR3) coating their surface, which is surprising as one would expect these cells to be removed rapidly from the circulation. Although, none of these mice developed pathological features of vasculitis, immunodeficient NOD-SCID recipients of splenocytes from these rmpR3 immunised mice developed severe focal necrotising glomerulonephritis (as was observed in the analogous experiment using MPO). Conversely, immunodeficient C57BL/6-RAG1-/- recipients of splenocytes from rmpR3 immunised C57BL/6 mice developed no pathology despite having high titres of anti-PR3 antibodies, illustrating the critical importance of the strain in the disease phenotype. Of note, in the successful anti-MPO splenocyte transfer model of Xiao et al., the recipient strain was C57BL/6-RAG2-/-, which further underlines the difference between MPO and PR3 immunity. The Primo model illustrates the potential for PR3 immunity to induce vasculitic injury but is let down by one major flaw: the propensity (as demonstrated by Xiao et al.) of the splenocyte transfer technique to induce marked immune complex deposition in the kidney, thereby rendering it unrepresentative of the pauci-immune human condition. The immunostaining of the kidney is not reported in this paper, but one must assume that the same issue with immune deposits pertains;

- in collaboration with Jeremy Duffield (University of Washington, Seattle), we have attempted to circumvent the disparity between murine and human PR3 structure and expression by generating mice with a human immune system [29]. This technology, which makes use of engraftment of human haemopoetic stem cells into immunodeficient mice, is advancing rapidly. We used NOD-SCID-IL2R-/- (NSG) mice to generate mice with on average 22% chimerism (notably including a small number of circulating human neutrophils) and infused human PR3-ANCA purified from patients with acute pulmonary-renal syndrome. We chose the source of ANCA carefully, screening a bank of 65 plasma samples for IgG preparations that were strong activators of neutrophils in vitro. When combined with low dose LPS these ANCA IgG preparations induced pulmonary haemorrhage and mild proliferative glomerulonephritis. Interestingly, when stained with anti-human and mouse CD45 to identify infiltrating cells, extracapillary proliferation of leukocytes comprising cells from both species could be identified in affected glomeruli.

Although a potential way forward for investigation of both ANCA vasculitis and other autoimmune diseases, there are a number of major barriers to overcome when adopting this humanised mouse strategy. Firstly, the chimeric nature of the inflammatory response, occurring in the context of murine endothelium, complement and pericytes, makes results difficult to interpret. Secondly, the experiments are technically challenging and expensive, require availability of haemopoetic stem cells (ideally of foetal origin) and mandate very large experimental group sizes because of the inherent variability of chimera generation. Thirdly, the immune system generated in these mice is deficient in many ways. For example, in the NSG mouse the absence of thymus means that there are very few T cells and those that are present have not been appropriately educated, making induction of adaptive immune responses virtually impossible. Additionally, myeloid chimerism, presumably critical for this neutrophil driven disease, tends to be poor. These issues are being circumvented through the use of transgenic (e.g. NSG-SGM3 mice transgenic for human Stem cell factor, GMCSF and IL3 [30]) and so-called “BLT” mice (engrafted with foetal bone marrow, liver and thymus) [31]. In addition, Coughan et al. recently reported a marked increase in functional circulating neutrophils in NSG mice following treatment with exogenous human gCSF [32]. Therefore, this is a rapidly evolving field with significant challenges but great potential;

- Relle et al. successfully created transgenic FVB mice that expressed human PR3 into which they infused monoclonal anti-PR3 antibodies [33]. Bizarrely, however, they elected to express the PR3 under the control of the podocin promoter, which meant that the protein was expressed in glomeruli only. Not surprisingly, they did not detect any pathological features of vasculitis, given that the pathogenesis paradigm mandates interaction of the antibody with PR3 on neutrophils and monocytes (and not glomerular cells) with resultant activation of these cells and bystander microvascular injury. Indeed, it is a hallmark of ANCA vasculitis that the glomerular...
lesions are pauci-immune, i.e.: there is little or no local glomerular immune complex formation. One wonders whether different results may have been obtained if the human PR3 had been expressed on the surface of myeloid cells, although it is likely that one would need to link it to other membrane bound proteins such as CD177 to see the intracellular effect of ANCA binding.

**In vivo modelling of granulomatosis with polyangiitis**

I believe that the next 5 years will see robust techniques to model the acute vasculitic effects of PR3 (auto)immunity. However, the major challenge will be additionally modelling the necrotising granulomatous inflammation that causes such destructive lesions in our patients. This is an arena where there has been little research in recent years. Kesel et al. recently published elegant experiments involving transplantation of inflamed GPA mucosa into immunodeficient pfp/RAG2−/− mice [34], showing that fibroblasts in the granulomatous tissue are associated with destruction of bone fragments. However, there was no immune system in these animals so the pathogenesis of the granuloma itself could not be investigated. It is conceivable that adopting similar approaches in humanised mouse models of PR3-ANCA vasculitis as described above may be a rewarding avenue, although issues of alloimmune effects will always arise if granuloma tissue is derived from a different source to the stem cells used to reconstitute the immune system. Thus, the holy grail of humanised mouse investigation of GPA will be identification of patients who are undergoing bone marrow examination from whom it may be possible to derive sufficient stem cells to generate a cohort of humanised mice. This would allow full re-capitulation in the mouse of the immune system of that patient with GPA, and would permit analysis of patient-derived cellular material, such as leukocyte sub-populations or granuloma, in the humanised murine “test-tube”. Therefore, this is a plea to clinical researchers around the world to please get in contact if such a patient undergoing bone marrow aspiration crosses your path!

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**References**


L8. Animal models of ANCA associated vasculitis: The contribution of autoantibodies and autoreactive T cells

Introduction

Anti-neutrophil cytoplasmic autoantibody-associated vasculitides (AAV) are severe systemic autoimmune disorders that attack primarily small blood vessels culminating in tissue injury and organ failure [1]. AAV can involve any organ but the upper airways, lungs and kidneys are most frequently affected. In AAV, the autoimmune response targets neutrophil and monocyte lysosomal enzymes, in particular myeloperoxidase (MPO) and proteinase 3 (PR3), and is considered to be the cause of the disease [2]. Here, I will discuss the evidence derived from studies in animal models that indicate that both cellular and humoral (auto)immune effector mechanisms contribute to the pathogenesis of AAV.

Autoantibody mediated effector mechanisms in AAV: insights from animal models

Numerous in vitro studies have demonstrated that ANCA are able to activate cytokine-primed neutrophils leading to the extracellular release of proteolytic enzymes and the production of toxic oxygen radicals [3]. The ability of ANCA to induce neutrophil activation is considered a central pathogenic mechanism in AAV and based on this contention various animal models of ANCA mediated vasculitis have been developed in particular for MPO-ANCA vasculitis [4]. In 2002, Xiao and colleagues reported that injection of splenocytes derived from mouse MPO (mMPO) immunized MPO-deficient mice into recipient mice lacking mature T and B cells (RAG2-deficient mice) causes severe necrotizing glomerulonephritis [5]. Also, injection of purified antibodies isolated from mMPO-immunized MPO-deficient mice into C57Bl6 mice results in pauci-immune glomerulonephritis mimicking the human disease [5]. These results provided the first direct in vivo evidence that MPO-ANCA are pathogenic and set the stage for further investigations that aimed to elucidate the effector mechanisms involved in ANCA mediated vascular injury. Studies in the passive MPO-ANCA transfer model demonstrated that neutrophils are the main effector cells early on in the disease process and that disease severity can be augmented by systemic injection of lipopolysaccharide or proinflammatory cytokines [6,7]. Subsequent studies also revealed that disease induction and progression in this model involves interactions of antibodies with leukocyte Fcγ receptors and is critically dependent on activation of the alternative pathway of complement activation [8–10]. The latter observations were quite unexpected given the lack of clinical evidence of complement activation in AAV patients.


