Editorial

Perspectives: modelling the vasculitis and granulomatous tissue destruction of granulomatosis with polyangiitis (GPA)

S.D. Gadola

Department of Rheumatology, University Hospital of Southampton NHS Foundation Trust, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, United Kingdom.

Please address correspondence to:
Prof. Stephan D. Gadola, DM, PhD, FRCP,
University Hospital of Southampton NHS Foundation Trust,
Tremona Road,
Southampton SO16 6YD,
United Kingdom.
E-mail: s.gadola@southampton.ac.uk

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A wealth of new publications in 2011, as illustrated in this issue of *Clinical Experimental Rheumatology,* has provided new insights into the mechanisms of ANCA-associated vasculitis (AAV) and granuloma formation in granulomatosis with polyangiitis (GPA; Wegener's granulomatosis). However, the aetiopathogenesis of this fascinating syndrome remains enigmatic. While a large body of epidemiological and *in vitro* evidence strongly indicates critical involvement of proteinase 3 (PR3) specific autoantibodies in the appearance of small-vessel vasculitis in GPA, direct *in vivo* evidence for the pathogenicity of these anti-PR3 Antibodies, *e.g.* in rodent models of GPA, has long been lacking. An obvious reason for this is the known poor structural homology between rodent and human PR3 and, accordingly, the failure of patient-derived anti-PR3 antibodies to bind to rodent PR3 on neutrophils (1). In contrast, murine models of several different human autoimmune diseases have been successfully established in autoimmune prone mouse strains by vaccinating animals with autoantigens in the presence of a strong inflammatory stimulus, *e.g.* complete Freund's adjuvant (CFA). Using such an approach, in 2009, Primo *et al.* actually achieved high anti-PR3 antibody titers in the autoimmune prone NOD mouse strain, yet these mice did not develop any signs of vasculitis (2). Of course, it has been long known that high titers of anti-PR3 antibodies can persist in patients with GPA who are in complete clinical remission. Potential factors that might explain this are, on the one hand, differences in epitope specificity and IgG subclass of the anti-PR3 antibodies, and on the other hand, Fc-receptor polymorphism and PR3-expression levels on myeloid cells. PR3-expression on PMN and monocytes is induced via inflammatory stimuli, *e.g.* TNF-α or TLR4 activation, which is why anti-PR3 antibodies are not likely to exert significant pathogenic effects in the absence of inflammation. Moreover, human neutrophils, the presumed key target cells of anti-PR3 autoantibodies, are not uniform in their ability to express PR3 on the cell surface. In fact, a higher percentage of PMN is able to express PR3 on their surface in GPA, even during times of complete remission, compared to healthy controls. When Primo *et al.* injected splenocytes from PR3-immunised mice into immune-deficient NOD-SCID mice, the recipient mice developed a rapidly progressive glomerulonephritis (RPGN). However, RPGN in these mice seemed to be immune-complex mediated and was therefore not representative of the characteristic pauci-immune RPGN in human GPA. The above facts underpin the notion that the pathogenesis of small-vessel vasculitis in GPA is fundamentally different from other, immune complex and complement mediated forms of vasculitis, and they illustrate some of the hurdles that need to be overcome in order to generate realistic *in vivo* models of GPA in rodents.

Now, in a tour de force, Little *et al.* have succeeded, for the first time, to induce pauci-immune glomerulonephritis and haemorrhagic alveolitis in mice by injecting the animals with human anti-PR3 antibody+ immunoglobulins that were purified from patients with active GPA (3). To make this possible, the authors generated chimeric NOD-SCID mice which contained both human and mouse neutrophils and monocytes, and they injected the mice with lipopolysaccharide (LPS) to induce expression of the target antigen, PR3 on their surface. Another important precaution of the authors was their careful selection of GPA

patient IgG preparations that induced strong degranulation and superoxide release from human neutrophils *in vitro*. While only 17% of the animals developed severe pauci-immune glomerular injury, 72% of the mice developed capillaritis and focal haemorrhagic lesions in the lungs and 83% showed evidence of at least mild glomerulonephritis. This demonstration that IgG from anti-PR3 antibody+ GPA patients can transfer characteristic pauci-immune AAV to LPS-primed rodents is an important breakthrough, and further refinement of the model may soon allow novel insights into the earliest stages and dynamics of anti-PR3 antibody induced AAV. Already, some new insight into the pathogenesis of AAV can be gained from the data generated by Little *et al*. For example, as T cells are almost completely deficient in the above mice, T cells are unlikely to play an important role for the induction of AAV once anti-PR3 antibodies have arisen. Second, based on the fact that most tissue infiltrating neutrophils and monocytes were of mouse and not human origin, monocytes and neutrophils participate in the inflammatory process even if they are not activated by anti-PR3 antibodies. However, the authors have not, with lasting certainty, directly demonstrated that anti-PR3 antibodies were mediating disease in the chimeric mice since they have not used PR3-absorbed IgG preparations in their experiments. Therefore, the possibility remains that other neutrophil specific antibodies in the patient IgG fraction were responsible for the transfer of disease.

While generating a representative mouse model of anti-PR3 antibody induced pauci-immune AAV has finally become feasible, modelling the characteristic granulomatous inflammation of human GPA in rodents still seems utopic. Nevertheless, a mouse model of granulomatous tissue destruction in GPA has now been developed by Ulrich *et al*., and novel insights can be derived from the model (4). These researchers transplanted human granulomatous GPA tissue together with allogeneic human cartilage subcutaneously into immune-deficient pfp/rag2-/- SCID mice, which simply served as a living environment providing the human tissue samples with oxygen and nutrients. A similar approach has been previously used by others to study the tissue destructive behaviour of rheumatoid pannus. The most interesting observation of the study by Ulrich *et al*. was the dominant role of inflammatory fibroblasts in cartilage destruction. These fibroblasts exhibited an increased resistance to apoptosis and upregulated production of different metalloproteinases (MMP 1/3/13) and pro-inflammatory cytokines (IL-6/IL-8). Thus, in analogy to erosive rheumatoid arthritis, granulomatous tissue destruction in GPA seems to be mediated by an aggressive fibroblast rich pannus. The possibility to model this process *in vivo* in mice now allows to study the kinetics of the destructive process and its response to therapies in greater detail. In fact, Ulrich *et al*. already showed that Dexamethasone treatment of the mice inhibits the fibroblast mediated tissue destruction in the transplanted tissue, thereby providing a proof of concept for future therapeutic studies in their model.

Our understanding of the aetiopathogenesis of GPA is certainly rapidly improving, and the arrival of the above discussed new animal models of anti-PR3 antibody induced AAV and granulomatous tissue destruction may soon provide new valuable insights and also support drug development. On the other hand, we are still at the beginning when it comes to the question of how granulomatous inflammation and AAV are interconnected, and it may take a very long time indeed before full-blown GPA can – if that is ever possible – be modelled in rodents.

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