

Chapter 2

Animal Models of ANCA-Associated Vasculitides

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Abstract Antibodies against neutrophil proteins myeloperoxidase (MPO) and proteinase-3 (PR3) are responsible for the development of anti-neutrophil cytoplasmic autoantibodies (ANCA)-associated vasculitides (AAV). Although the knowledge of these conditions is remarkably improved in the last few years, their etiology and pathogenetic mechanism(s) are still poorly understood. The establishment of experimental models has been repeatedly attempted with the aim of achieving a deeper understanding of their human counterpart. Here, we discuss the principal animal models currently used to investigate the mechanisms underlying the onset of AAV.

Keywords Animal models • Anti-neutrophil cytoplasmic autoantibodies • Vasculitis

2.1 Introduction

A number of *in vitro* and *in vivo* studies, focusing on different aspects of the neutrophil biology and function, have clearly demonstrated the potential role that neutrophils can exert in the modulation of innate and adaptive immune responses [1].

Anti-neutrophil cytoplasmic autoantibodies (ANCA) were first recognized by van der Woude et al. [2], who described circulating autoantibodies that reacted with cytoplasmic antigens of neutrophils and monocytes in patients with granulomatosis with polyangiitis (GPA). ANCA-associated vasculitides (AAV) are systemic autoimmune disorders characterized by inflammatory necrosis of small blood vessels affecting joints, lungs, kidneys, skin and other tissues [3]. Neutrophils are cardinal

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cells in the pathophysiological process underlying AAV since they are both effector cells responsible for endothelial damage and targets of autoimmunity. It should, however, be emphasized that some patients showing similar disease manifestations as those who are ANCA-positive are nonetheless ANCA-negative [4].

Four diseases are characterized by the presence of ANCA, namely GPA (formerly called Wegener's granulomatosis), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA, formerly termed Churg-Strauss syndrome), and the necrotizing crescenting glomerulonephritis (NCGN). The etiological factors responsible for the production of vessel-damaging ANCA are unknown. Although infectious agents have been repeatedly suspected and *Staphylococcus aureus* has long been known to be associated with GPA, their precise immunologic link with AAV has not been proven.

In the 1980s, autoantibodies to cytoplasmic components of myeloid cells were detected in patients with pauci-immune necrotizing small vessel vasculitis. In AAV, the autoimmune response is directed against neutrophil and monocyte lysosomal enzymes, including myeloperoxidase (MPO) and proteinase 3 (PR3) [5]. MPO is abundantly expressed and exclusively found in azurophilic granules, and is a key component of the phagocyte oxygen-dependent intracellular microbicidal system [6]. On the other hand, PR3, also called myeloblastin, belongs to the neutrophil serine protease family and is classically localized in azurophilic granules. Following phagocytosis of pathogens, PR3 is secreted in the phagolysosome to play its crucial microbicidal function [7].

Clinical and experimental studies have provided extensive evidence for the involvement of autoantibodies to MPO and PR3 in the pathogenesis of AAV [8], thus leading to treatment strategies aimed at ANCA removal. Plasma exchange, for example, has been shown to remove plasma constituents as well as ANCA and to increase the chances of renal recovery in severe renal vasculitis [9].

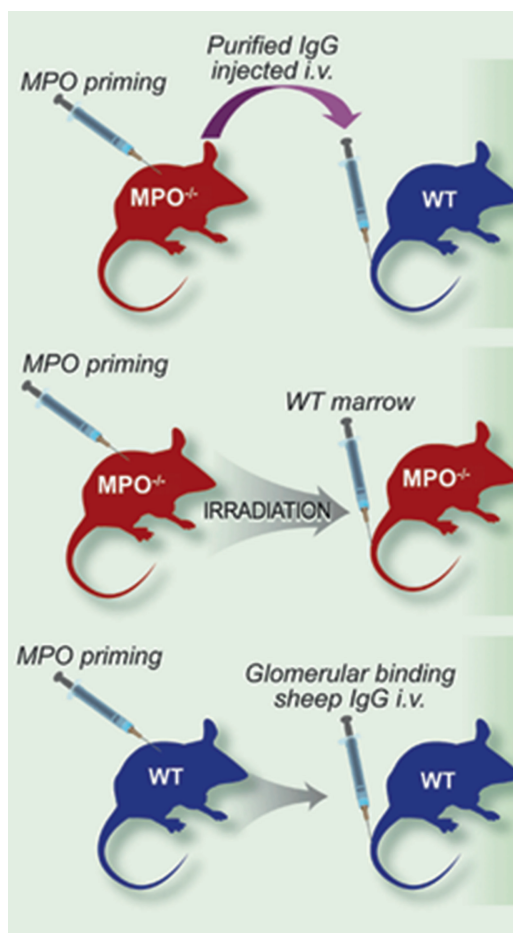
The crucial factors required for animal models of vasculitis are the similarities to the clinical and pathologic phenotypes of human diseases, with the obvious assumption that their study may contribute to the pathogenetic elucidation of human vasculitis. Here, we will briefly discuss the principal animal models currently used to investigate the mechanism(s) of vascular injury in AAV.

2.2 Animal Models Involving Anti-MPO Immune Response

In spite of the large body of *in vitro* studies, unequivocal evidence that ANCA are pathogenic *in vivo* was obtained only recently [10]. The pathogenicity of ANCA has been investigated in mouse models by exploring both passive transfer and active immunization strategies in order to reproduce systemic vasculitis.

The first animal model resembling the human disease was introduced by Xian et al. [11]. They reported that injection of splenocytes, derived from MPO-deficient mice immunized with mouse MPO, into recipient mice lacking mature T and B cells (RAG2-deficient mice) caused severe necrotizing glomerulonephritis. In a second

Fig. 2.1 Summary of three mouse models that have been used to study pathogenetic mechanisms in ANCA-associated vasculitis (Modified from Coughlan et al. Clin Exp Immunol. 2012; 169: 229–37)



approach, IgG were isolated from MPO-deficient mice immunized with MPO and passively transferred into wild type and RAG2 $^{-/-}$ mice, resulting in a pauci-immune glomerulonephritis mimicking the human disease (Fig. 2.1) [11], thus confirming that neutrophil is the primary effector cell in anti-MPO-induced glomerulonephritis [12].

In an additional model, MPO-deficient mice were immunized with murine MPO; after production of anti-MPO IgG, the animals were lethally irradiated and transplanted with bone marrow from MPO-positive wild type mice (Fig. 2.1) [13]. By 8 weeks after bone marrow transplantation, the mice developed a pauci-immune glomerulonephritis with urine abnormalities. The transfer of anti-MPO lymphocytes into immune-deficient mice has also resulted in necrotizing glomerulonephritis with glomerular immune deposits [14].

A third mouse model is based on the induction of both humoral and cellular autoimmune responses to MPO (Fig. 2.1) [15]. Wild type mice were in fact

immunized with MPO and subsequently injected with a sub-nephritogenic dose of nephrotoxic serum (anti-GBM), this procedure resulting in the development of glomerulonephritis. The advantage of this model was the generation of an autoimmune response to MPO in wild type mice.

The models of anti-MPO-mediated glomerulonephritis shortly described above have proven to be useful tools for testing experimental therapies. For example, therapeutic interventions aimed at blocking the pro-inflammatory effects of tumor necrosis factor-alpha (TNF α) have been evaluated in both the MPO-ANCA mouse model [16] and the experimental autoimmune vasculitis rat model [17].

2.3 Animal Models Involving Anti-PR3 Immune Response

Following immunization with recombinant human mouse PR3, non-obese diabetic (NOD) mice develop specific anti-PR3 autoantibodies. The transfer of splenocytes from these mice into immunodeficient NOD/severe combined immunodeficiency disease (SCID) mice has been shown to result in vasculitis and severe segmental and necrotizing glomerulonephritis, leading to acute kidney failure and death [18].

Little et al. [19] have described an interesting model consisting of humanized immunodeficient NOD/SCID interleukin-2 (IL-2)- receptor knockout mice, which received human hematopoietic stem cells and developed a human-mouse chimeric immune system. These mice developed glomerulonephritis following passive transfer of PR3-ANCA IgG derived from patients with severe systemic vasculitis [19].

2.4 Concluding Remarks

Interesting animal models of anti-MPO-related vasculitis, closely resembling clinical and pathological features in humans, have been established. By inducing an abnormal immune response to MPO, these models mimic the clinical aspects of the human MPO-AAV and are contributing to elucidate how ANCA cause vasculitis.

Animal models of anti-PR3 associated disease are much less advanced, and generation of an experimental model that implies both anti-PR3-associated vasculitis and granuloma formation is a major challenge in this field. Further investigations are needed to identify the molecular mechanisms that control the complex neutrophil/endothelium interactions and to establish whether they are dysregulated in AAV [20].

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