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 results in pauci-immune glomerulonephritis mimicking the human disease [5]. These results provided the first direct in vivo evidence that MPO-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.
These results, however, highlighted the potential benefits of interventions targeting complement activation as novel therapies in AAV and have led to the initiation of a clinical trial that aims to test the safety and efficacy of a CsA receptor inhibitor in AAV patients. Overall, the observations in the passive anti-MPO IgG transfer model provide convincing evidence that MPO-ANCA are pathogenic and can induce disease manifestations typical of human anti-MPO vasculitis.

**Effector mechanisms of autoreactive T cells in AAV: insights from animal models**

In the original study by Xiao and colleagues adoptive transfer of splenocytes from mMPO-immunized MPO-deficient mice into mice that lack mature B and T cells caused severe glomerulonephritis [5]. In follow-up studies it was found that transfer of pure B cells alone also induced disease manifestations whereas transfer of pure CD4⁺ T cells did not, indicating that in this model MPO-specific CD4⁺ T cells are not essential in the effector phase of the disease [11]. However, these observations do not rule out a role for (autoreactive) T cells in the maintenance and propagation of the immune response.

To study the role of autoreactive T cells in AAV in more depth, an alternative strategy for modeling MPO autoimmunity has been employed. Immunization of C57BL/6 mice with either murine or human MPO in adjuvant was found to generate a cellular and humoral anti-MPO response but alone did not result in vasculitic manifestations [12]. However, when MPO immunized mice are injected with a subnephritogenic dose of heterologous anti-glomerular basement membrane antibodies the development of necrotizing crescentic glomerulonephritis is triggered. Interestingly, this model was found to depend on T cell mediated immunity because mice lacking the ability to produce circulating antibody still develop disease while CD4⁺ T cell depletion inhibits glomerulonephritis development [12]. Subsequent experiments in this model have demonstrated that Th17 cells are essential in directing the pathogenic anti-MPO autoimmune response [13]. In addition, it was shown that Toll-like receptor ligands can differentially orchestrate the anti-MPO autoimmune response [14]. Immunization with MPO and a TLR2 ligand promoted Th17-mediated immunity characterized by the production of IL-17 whereas immunization with MPO and TLR9 ligand induced a Th1 immune response characterized by enhanced IFNγ production [14]. Importantly, recent studies in this model have identified an immunodominant MPO T cell epitope that initiates cell mediated glomerular injury [15]. From these studies it was proposed that vascular injury in MPO-ANCA vasculitis is a two-step process that requires:

- local release of MPO by activated neutrophils, conceivably induced by MPO-specific autoantibodies followed by;
- recognition of extracellular MPO by MPO-specific autoreactive CD4⁺ T causing delayed type hypersensitivity like vascular injury [15].

Similar to the studies in mice, immunization of Wistar Kyoto rats with human MPO (hMPO) induces hMPO-ANCA that cross-react with rat MPO and a MPO-specific T cell response [16]. After 6 to 8 weeks, these rats develop pauci-immune crescentic glomerulonephritis and, in some animals, pulmonary capillaritis. In this model, both arms of the adaptive immune response are activated although their individual contribution to vasculitis development has not been studied yet.

In summary, using various approaches, animal models of MPO-ANCA vasculitis have substantiated the pathogenic potential of MPO-ANCA and indicate that MPO reactive T cell mediated effector mechanisms contribute to disease development as well. The most likely scenario is that autoantibody and autoreactive T cell driven effector mechanisms act in concert to cause the severe vasculitic manifestations that are typically observed in AAV.

**Disclosure of interest** the author declares that he has no conflicts of interest concerning this article.

**References**


L9. The role of genetic background in an animal model of ANCA-associated vasculitis

Introduction

There is increasing evidence for genetic influences affecting vasculitis and glomerulonephritis caused by antineutrophil cytoplasmic autoantibodies (ANCA) [1–21]. A genome-wide association study demonstrated a genetic basis for differences between diseases associated with ANCA specific for myeloperoxidase (MPO-ANCA) versus proteinase 3 (PR3-ANCA) [1]. Additional evidence for genetic influences on ANCA-associated vasculitis (AAV) includes familial occurrences [2–6], prevalence in first-degree relatives of AAV patients [7], racial influences on incidence [7–11], and correlations between polymorphisms in genes that influence immune responses and the clinical and pathologic manifestations of AAV [12–21].

A genetic influence also has been demonstrated in a rat model of MPO-ANCA glomerulonephritis. Immunization of Wistar Kyoto (WKY) rats with human MPO results in anti-MPO antibodies that cross-react with rat MPO and cause necrotizing and crescentic glomerulonephritis (NCGN) that resembles human ANCA NCGN [22]. Identical immunization of Lewis, Wistar Furth, and Brown Norway rats does not induce NCGN even though the rats have similar levels of circulating anti-MPO [22]. The authors propose that this difference in strain susceptibility is more likely caused by genetic influences on the innate immune response rather than the adaptive immune response.

A mouse model of AAV that closely mimics human disease, including the characteristic pauci-immune NCGN, is induced by injection of anti-MPO IgG derived from MPO knockout mice that have been immunized with murine MPO [23]. Investigations using this model demonstrate that neutrophils are the primary effector cells of acute injury, and that activation of the alternative complement pathway and engagement of leukocyte Fc receptors are important inflammatory mediators [24–28]. Intravenous injection of anti-MPO IgG into C57Bl/6 (B6) mice consistently induces NCGN in all recipient mice with crescent formation in approximately 5% to 10% of glomeruli [23–25]. Although the individual murine glomerular lesions have a remarkable resemblance to human ANCA NCGN, the NCGN is less severe and less variable than NCGN in AAV patients who often have severe disease although there is substantial variability among patients, ranging from 100% to less than 5% crescents and averaging 50% [29]. A minority of patients who have systemic AAV have no NCGN. The homogeneous genetic background among B6 mice could explain the lack of variability in severity of anti-MPO GN among B6 mice, and, mice with different genetic background might have different susceptibility to and severity of anti-MPO NCGN. We confirmed this possibility by comparing NCGN induction by anti-MPO IgG in 13 mouse strains and performed genotyping to try to identify candidate loci that influenced disease severity [30]. We also used bone marrow chimeric mice and in vitro neutrophil activation assays to demonstrate that genetic differences were mediated primarily by effects on neutrophil function [30].

Severity of anti-MPO IgG induced NCGN is influenced by genetic background

C57Bl/6j (B6), 129S6/SvEv, 129S1/SvImJ, LP/J (LP), WSB/Eij (WSB), NZO/H1l1J (NZO), PWK/PhJ (PWK), NOD/LtJ (NOD), DBA1, DBA2, AJ, C3H and CAST/Ei (CAST) mice were injected with the same dose of anti-MPO IgG. As shown in table 1 and figure 1, severity of NCGN as measured by percentage of glomeruli with crescents ranged from greater than 60% in 129S6 and CAST mice to no disease induction in NOD, DBA1 and DBA2 mice. The observed differences in pathogenicity could be determined either by protective alleles in mice with less severe NCGN or disease promoting alleles in mice with more severe NCGN.

The nephritogenicity of anti-MPO IgG was tested in F1 mice generated by B6 backcross with 129S6 mice, and F2 mice generated by (B6x129S6) F1 intercross [30]. The severity of NCGN in 129S6 x B6 F2 spanned the extremes between B6 and...