Lectures


L10. Animal models of ANCA-associated vasculitis: Effector mechanisms and experimental therapies

ANCA and their interaction with neutrophils and monocytes

Four distinct diseases are characterized by the appearance of anti-neutrophil cytoplasmic autoantibodies (ANCA), namely granulomatosis with polyangiitis (GPA, formerly called Wegener’s granulomatosis), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA, formerly...
term Churg-Strauss syndrome), and the renal-limited necrotizing crescentic glomerulonephritis (NCGN) [1–3]. Beside from being a disease marker, ANCA are viewed as causative agent in ANCA-associated vasculitis (AAV). Initial evidence came from in vitro studies, demonstrating that ANCA IgG can bind to human neutrophil granulocytes and monocytes. The ANCA antigens proteinase 3 (PR3) and myeloperoxidase (MPO) are expressed on the surface of cytokine-primed neutrophils, which allows a direct interaction between the autoantibodies and their target antigen. This process results in full activation with a robust respiratory burst and generation of reactive oxygen species (ROS), increased adhesion to endothelial cells, release of pro-inflammatory cytokines and degranulation of lytic granule contents [4–11]. However, more firm evidence of the ANCA IgG pathogenicity comes from animal models, which have been introduced in recent years and have substantially broadened our understanding of this autoimmune entity.

**ANCA animal models**

The first appropriate animal model resembling the human disease was introduced by Xiao et al. in 2002 [12]. The authors were the first to demonstrate that anti-MPO IgG in itself can induce NCGN: MPO-deficient mice were immunized with native murine MPO followed by adoptive splenocytes transfer from these mice into T- and B-cell deficient Rag2−/− mice, which developed anti-MPO ANCA, systemic vasculitis and NCGN. In a second approach, IgG were isolated from MPO-immunized MPO-deficient mice and passively transferred into wildtype and Rag2−/− mice. Pauci-immune NCGN developed in both model systems. We have modified this model by immunization MPO-deficient mice with murine MPO, followed by lethal irradiation (after development of anti-MPO IgG) and transplantation of bone marrow from MPO-positive wildtype mice. These mice developed a pauci-immune NCGN [13]. Another murine model of anti-MPO induced NCGN was established by the group from Kitching and Holdsworth [14]. Here wildtype mice were immunized with MPO followed by subsequent injection of a subnephritogenic dose of nephrotoxic serum (anti-GBM). The combination of anti-MPO immune-response and anti-GBM antibodies resulted in development of a NCGN. Using this model, the authors subsequently dissected the specific role of T-cells in developing anti-MPO-induced NCGN, which however is beyond the focus of this review [14–18]. A rat model of anti-MPO-induced NCGN was established by Little et al. in 2005 [19]. WKY rats were immunized with human MPO which resulted in development of antibodies against human MPO but also cross-reactive to rat MPO. 61% of the immunized rats demonstrated induction of NCGN. All of these studies were done on anti-MPO immune-response. For the pathogenicity of anti-PR3-ANCA, two different animal models have been reported: Primo et al. showed that adoptive transfer from splenocytes from rMP3 immunized NOD-mice into NOD-SCID immune-incompetent mice resulted in development of NCGN [20]. A different approach was introduced by Little and coworkers: the investigators generated chimeric mice by transferring human hematopoietic stem cells into irradiated NOD-scid-IL2Rγ-deficient mice. Passive transfer of PR3-ANCA IgG induced a mild pauci-immune NCGN [21].

**Effector mechanisms established in animal models**

Different effector mechanisms involved in the pathogenesis of ANCA-induced vasculitis and NCGN have been ultimately elucidated by the use of the aforementioned animal models. Most of the intervention studies focused on the neutrophil granulocyte. Xiao et al. demonstrated in 2005 that the neutrophil is indeed the primary effector cell in anti-MPO-induced NCGN [22]. Antibody-depletion of circulating neutrophils completely protected from anti-MPO IgG-induced NCGN. This landmark study was the first to strongly suggest that the neutrophil granulocyte is the main effector cell in ANCA-induced NCGN.

From in vitro studies it has been known that cytokine-priming of neutrophils is a requisite for ANCA-induced stimulation [6,9,10,23]. Different animal studies have focused on the process of priming and have broadened our understanding of this process. Heeringa et al. were the first to established in vivo that lipopolysaccharide (LPS) increases anti-MPO-induced NCGN by generation of the pro-inflammatory cytokine TNFα [24]. The involvement of TNFα in ANCA-induced NCGN was confirmed by Little et al. in a rat model by showing that blockade with an anti-rat TNFα mAb reduces both the renal and lung phenotype of anti-MPO induced vasculitis [25]. However, a randomized controlled trial of the use of etanercept in GPA did not show a treatment benefit of TNFα-blockade [26].

The explanation for this contradiction between mouse and human data is not clear. The signaling cascade involved in cytokine-priming of neutrophils for subsequent ANCA-stimulation has been elucidated in vitro. Here a role of the p38 mitogen-activated protein kinase (MAPK) in ANCA-induced neutrophil activation was suggested [27]. Heeringa and coworkers expanded these studies recently by demonstrating in vivo that specific inhibition of this signaling pathway reduces ANCA-induced NCGN only partially [28]. Therefore, treatment of the human disease with p38 MAPK inhibitors seems not to be the most promising approach.

Binding of ANCA IgG to their antigens on the membrane of neutrophils leads subsequently to cell activation, a process that involves both classical Fc receptor stimulation and antigen crosslinking by F(ab)2. Both processes activate a signaling cascade with stimulation of Syk, phospholipase C, protein kinase B and phosphoinositol-3 kinase [4,29–32]. We have recently demonstrated in vivo that the PI3k isomor γ is essentially involved in the activation process by ANCA IgG and that treatment of mice with a highly specific inhibitory compound reduces anti-MPO induced NCGN [33]. Treatment of human
ANCA disease with a specific PI3k isoform γ inhibitor could therefore be a potential new approach. However, this strategy has not yet been tested in patients. The interaction between ANCA IgG and Fc receptors was studied by van Timmeren et al.; the authors demonstrated that deglycosylation of ANCA IgG by hydrolyzation with the enzyme endoglycosidase S inhibited ANCA IgG-induced neutrophil activation in vitro and reduces the severity of NCGN by anti-MPO IgG in a mouse model [34]. We could recently show that pharmacologic MPO-specific plasma cells depletion by the proteasome inhibitor Bortezomib reduced anti-MPO titers and prevented anti-MPO-induced NCGN [35].

A formerly not known pathogenic role of the complement system in ANCA-induced NCGN was recently demonstrated in different animal studies. Xiao et al. implicated involvement of the alternative pathway of complement activation in the pathogenesis of anti-MPO IgG-induced NCGN by showing that both complement factor C5 and factor B deficient mice were protected from disease, whereas C4 deficient mice were not [36]. This study was extended by Heeringa’s group, who showed that specific inhibition of complement factor C5 protected mice form anti-MPO-induced NCGN [37]. Our group could recently demonstrate that ANCA-induced neutrophil activation induces C5a generation, which then in turn acts as a potent aggravation loop for further ANCA-induced neutrophil activation [38]. C5aR-deficiency on myeloid cells protected from anti-MPO-induced NCGN. A recently started international multicenter study currently tests the feasibility of a treatment with a specific C5a receptor inhibitor in ANCA patients.

ANCA-stimulation of neutrophils and monocytes results in generation of ROS and degranulation of lytic granule contents, among them the neutrophil serine proteases (NSPs) proteinase 3, neutrophil elastase (NE), and cathepsin G (CG). NSPs are among them the neutrophil serine proteases (NSPs) proteinase 3, neutrophil elastase (NE), and cathepsin G (CG). NSPs are generated as enzymatically inactive pro-forms, which are generated as enzymatically inactive pro-forms, which are cleaved and activated by the lysosomal cysteine protease dipeptidyl peptidase I (DPPI). We recently demonstrated that DPPI-deficient mice lacking functional active serine proteases were protected from anti-MPO induced NCGN. In addition, we found that PR3/NE-double-deficient mice were protected from anti-MPO-induced NCGN. In different in vitro and in vivo assays, we finally established that NSP are essentially involved in the process of activating the pro-inflammatory cytokine IL-1β in monocytes stimulated with ANCA IgG [39]. Interestingly, specific IL-1β blockade by Anakinra reduced ANCA-induced NCGN. Thus treatment of the human disease with this drug could serve as a novel treatment option.

Summary

Animal models established the pathogenicity of ANCA IgG in the induction of vasculitis and NCGN. Furthermore, different activation pathway and aggravation loops have been identified and could finally lead to novel treatment approaches in the future.

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

References

L11. Hepatitis C virus mixed cryoglobulinemia vasculitis: Therapeutic options

With the discovery of hepatitis C virus (HCV) as the etiologic agent for most cases of mixed cryoglobulinemia (Cryovas), new opportunities and problems for crafting therapy of HCV-Cryovas have emerged [1,2]. A new and major concern was the potential adverse effects that immunosuppressive therapy with glucocorticoids and cytotoxic drugs could have on an underlying chronic viral infection. Alternatively, the discovery of HCV provided the opportunity to control HCV-Cryovas with antiviral therapy as the underlying infection drives immune complex formation and resultant vasculitis [3]. The cornerstone of HCV therapy has been and continues to be interferon alpha (IFN) which has the potential to exacerbate autoimmune disease states [4]. Aggressive antiviral therapy with Peg-IFNα and ribavirin should be considered as induction therapy for HCV-Cryovas with mild to moderate disease severity and activity. Very recent advances using a triple combination with Peg-IFNα,

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