The neutrophil in antineutrophil cytoplasmic autoantibody-associated vasculitis

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ABSTRACT
Necrotizing, small-vessel vasculitis develops in patients with circulating ANCAs. Neutrophils and monocytes harbor the two major ANCA antigens, PR3 and MPO. A whole body of in vitro experiments implicated ANCA-activated neutrophil effector functions in the pathogenesis of vasculitis, whereas the role of monocytes is less well-characterized. Mouse models for anti-MPO-induced vasculitis were developed to study ANCA-neutrophil interactions in complex in vivo situations. We not only discuss the significance of ANCA-neutrophil interactions for disease induction but also how a detailed understanding of these interactions helps to identify novel treatment targets for ANCA vasculitis.


ANCAs
ANCAs are found in most patients with systemic, small-vessel vasculitis and pauci-immune NCGN [1]. Four diseases are subsumed under the term AAV: GPA (formerly called Wegener’s granulomatosis), microscopic polyangiitis, eosinophilic GPA (formerly termed Churg-Strauss syndrome), and the renal-limited NCGN [1–3]. ANCAs were first described in 1982 by Davies et al. [4], and their significance as a clinical tool in GPA was established by van der Woude et al. in 1985 [5]. When tested by indirect immunofluorescence on ethanol-fixed and permeabilized neutrophils, two distinct fluorescence patterns are distinguished: a cANCA and a pANCA. The major target antigen for cANCA was discovered to be PR3 and for pANCA, MPO [6, 7]. Recently, a novel ANCA antigen, namely hLAMP-2, was identified [8, 9]. The authors describe ANCA targeting the neutrophil and endothelial cell-expressed hLAMP-2 in a large portion of patients with AAV from Europe. However, a research group in the United States was not able to confirm these data in an independent patient cohort [10]. Whether these discrepancies are explained by technical issues or whether they are a result of geographic and ethnic differences is yet unsettled [11].

NEUTROPHIL ACTIVATION BY ANCA IN VITRO
In addition to being a marker for AAV, ANCAs are believed to be causative in inducing necrotizing vasculitis and consecutive organ damage. Most of the evidence for the pathogenicity of ANCA has arisen from in vitro experiments showing that ANCAs bind to their target antigens PR3 and MPO, which are expressed on cytokine-primed neutrophils [12, 13]. After binding, ANCAs activate neutrophils and monocytes, resulting in a robust respiratory burst with production of intra- and extracellular ROS, degranulation of lytic granule contents, and increased adhesion to endothelial cells, subsequently causing endothelial cell injury [6, 14–19]. All of these responses were described in great detail in in vitro experiments by several groups. However, the true pathogenic in vivo role of these events is not yet clear.

A prerequisite for neutrophil activation by ANCA IgG is a low-dose cytokine pretreatment called priming, which itself does not result in full-blown neutrophil activation. This priming process, however, causes up-regulation of PR3 and MPO on the neutrophil surface, thus enabling interaction between ANCA IgGs and their targets. Low-dose TNF-α is used frequently for in vitro priming, although other mediators, such as C5a or IL-18, prime neutrophils too [6, 13, 20–22]. In addition to ANCA antigen expression up-regulation, TNF-α-induced priming involves activation of the p38 MAPK pathway, and specific p38 MAPK inhibition blocked in vitro ANCA-induced neutrophil activation [13].
Binding of ANCA IgG to PR3 or MPO, expressed on the membrane of neutrophils, leads subsequently to cell activation. This process involves FcR stimulation and antigen cross-linking by F(ab)$_2$ [17, 18, 23]. Recently, Kimberly and colleagues [24] demonstrated an influence of the FcR genotype on in vitro neutrophil activation by ANCA IgG and IgA, as well as by the proinflammatory FcγRIIIA NA$_1$ allele, which was associated with more-severe renal disease compared with the NA$_2$ allele. Ligation of FcRs by ANCA IgG results in downstream activation of a signaling cascade with activation of tyrosine kinases, leading to phosphorylation of Syk, PLC, and PKB [23, 25]. Furthermore, inhibition of upstream tyrosine kinase blocks ANCA IgG-induced activation [25, 26]. In addition, ligation of the membrane-expressed ANCA antigens MPO and PR3 by F(ab)$_2$ leads to activation of the heterotrimeric G protein, finally resulting in downstream stimulation of the PI3K, a process that is necessary but not in and of itself sufficient for ANCA-induced neutrophil activation [27].

In addition to direct activation of neutrophils and monocytes by ANCA IgG, the interaction between endothelial cells and ANCA-activated leukocytes is a prerequisite in the pathogenic sequence, leading finally to vessel wall necrosis. ANCA-activated leukocytes release proinflammatory cytokines that up-regulate endothelial cell adhesion molecules, leading to increased leukocyte adhesion to endothelial cells [28–31]. This multistep process was documented elegantly in an animal model, where anti-MPO IgG enhanced leukocyte adhesion and transmigration through the vessel wall [32]. By intravital microscopy, Kuligowski et al. [33] demonstrated in mice that anti-MPO IgGs induce glomerular leukocyte adhesion via β$_2$- and α$_i$-integrins. Importantly, circulating, damaged endothelial cells were detected in the circulation of patients with active AAV, suggesting that these postulated processes also occur in the human disease [34].

Interestingly, neutrophils express PR3 only on a subset of their population in a genetically determined fashion [35–37]. A higher percentage of mPR3-expressing cells was found to be a risk factor for AAV [37, 38] and was associated with an increased risk for relapse [38]. Recent work identified the NB1 (CD177) as a PR3-presenting membrane receptor [39–41]. As NB1 is a GPI-anchored protein and therefore, lacks an intracellular domain, the mechanism of cell activation by PR3-ANCA was not clear. We demonstrated recently that NB1 and its ligand PR3 colocalize with the β$_2$ integrin, Mac-1 (CD11b), in cholesterol-enriched plasma membrane fractions called lipid rafts. The extracellular domains of NB1 and Mac-1 interact with each other and cooperate in transdermal signal transduction. We provided evidence for the functional importance of this NB1–Mac-1 receptor interaction for PR3-ANCA-mediated neutrophil degranulation and superoxide generation [39].

In addition to NB1-dependent mPR3 expression a NB1-independent, direct mPR3 insertion was described, which depends on a hydrophobic patch within the PR3 molecule [42]. Moreover, neutrophil apoptosis increases mPR3 by processes that are distinct from cell activation and involves phospholipid scramblase 1 [42–44]. It appears that mPR3 on apoptotic neutrophils has proinflammatory effects by stimulating the release of proinflammatory cytokines from phagocytosing macrophages [45].

An interesting phenomenon regarding the transcription status of both ANCA antigens was described by the Chapel Hill group of Falk and Jennette. In contrast to the silenced gene transcription state in mature neutrophils of healthy controls, ANCA patients showed aberrant expression of PR3 and MPO mRNA [46]. The reactivated transcription of the ANCA antigen was found to be caused by lack of epigenetic silencing [47]. Defective silencing was mediated by decreased histone H3K27 methylation, as demonstrated by the absence of the silencing mark H3K27me3. This condition was induced by failure of the transcription factor RUNX3 to recruit EZH2 and methylate H3K27 and by increased demethylation as a result of increased expression of the demethylase JMJD3.

The mechanisms involved in generation of autoreactive B cells producing ANCA IgG are not yet understood. Importantly, it appears that autoreactive B cells escape deletion in AAV [48–50]. Recently, it was described that neutrophils are a primary source of the B cell survival factor B lymphocyte stimulator, which was released from ANCA-stimulated neutrophils in vitro and was increased in patients with AAV [51–53], suggesting that this effect could be possibly involved in perpetuation of ANCA IgG generation in AAV.

**NETs AND ANCA**

Neutrophil activation triggers a variety of responses, including ROS production, phagocytosis, degranulation, and cytokine generation. All of these responses participate in host protection. A novel neutrophil defense mechanism was described by Brinkmann et al. [54], who observed DNA release, together with histones and certain granule proteins into the extracellular space forming NETs. This process, coined “NETosis”, was subsequently found to play a role in microbial defense by trapping and probably killing bacteria in thrombosis formation and transfusion-related acute lung injury [55–58]. Kessenbrock and coworkers [59] showed that ANCA-stimulated neutrophils release NETs that contain the ANCA antigens PR3 and MPO. Furthermore, they demonstrated in vivo local NET formation in kidney biopsies and circulating MPO-DNA complexes in patients’ plasma. However, whether the formation of NETs in AAV merely indicates neutrophil activation or whether NETs also play a functional role is yet unknown. In addition to acting as a scaffold for active proteases, NETs could induce autoimmunity in AAV by presenting ANCA autoantigens PR3 and MPO together with other proteins and DNA. In fact, autoimmunity induction by NETs was demonstrated recently in systemic lupus erythematosus [60, 61]. Several neutrophil proteins are released during NET formation, and most of them show microbicidal effects. For example, LL-37/CAP-18 punches pores into the bacterial cell wall, thereby lysing the cell. In concert with NET-derived ssDNA, LL-37 acts as a trojan horse and enables ssDNA to enter the cell as a LL-37/ssDNA complex. Once within the cell, ssDNA binds to TLR9, causing plasmacytid DC activation and subsequently, IFN-α release. All of these events may participate in breaking immune tolerance, as demonstrated by Lande and coworkers [62] for psori-
asasis. Recently, the potential induction of ANCA autoantibodies by NETs was suggested [63]. The authors demonstrated that NETs present the ANCA antigens PR3 and MPO and transfer these to APCs, such as myeloid DCs. Immunization of mice with NET-treated myeloid DCs induced MPO- and PR3-ANCA, as well as anti-dsDNA autoantibodies and autoimmune vasculitis. In another study, Nakazawa et al. [64] showed ANCA induction by PTU. PTU-induced MPO-ANCA generation is a known phenomenon occurring in patients treated for hyperthyroidism. The authors established that NETs generated in the presence of PTU and the phorbol ester PMA induced MPO-ANCA formation and pulmonary capillaritis. Furthermore, treatment of rats with PTU and i.p. PMA administration led to MPO-ANCA generation and subsequently to NCGN. Both studies support the notion that NETosis may play a role in breaking immune tolerance by presenting autoantigens to APCs. As a limitation, these studies were done with the highly nonphysiologic compound PMA or with isolated and in vitro-preactivated PMN. Several aspects of these experimental conditions do not mimic clinical relevant in vivo conditions in the human AAV disease settings. For example, mice developed antibodies to MPO and PR3 simultaneously, a finding that is highly unusual in patients with AAV. Thus, whether NETosis is indeed applicable to the human disease setting needs further study. It is conceivable that NETs participate in ANCA induction and tissue damage. The latter state of affairs could be mediated by histones, proteases, or other neutrophil proteins. Furthermore, an imbalance between NET formation and degradation by DNase would provide another potential disease aspect that can be investigated in patients and mouse models.

THE ROLE OF MONOCYTES IN ANCA-INDUCED VASCULITIS

Most studies focused on the role of neutrophils in ANCA-induced effector functions. However, monocytes also express both ANCA antigens, PR3 and MPO, and react with ANCA IgG [65–67]. In fact, it was demonstrated that ANCA IgG induced in vitro up-regulation of CD14 and CD18 on monocytes and triggered ROS generation [67]. Recently, anti-PR3 ANCA and mAb to PR3 were found to induce soluble fms-like tyrosine kinase-1 release from monocytes, whereas anti-MPO IgG or mAb to MPO did not [68]. This effect could possibly interfere with endothelial repair during active vasculitis, leading to more severe and prolonged organ damage. Whether monocytes do play an important functional role during the effector phase of ANCA-induced inflammation in vivo still has to be addressed in animal models.

ANIMAL MODELS OF ANCA-INDUCED VASCULITIS

As outlined above, a plethora of in vitro evidence suggests a pathogenic role of ANCA in inducing AAV. However, animal models featuring the human disease were needed to firmly establish pathogenicity in a complex in vivo condition. The first convincing animal model was introduced by Xiao et al. [69] in 2002. Disease was induced by immunization of MPO-deficient mice with native murine MPO, followed by adoptive transfer of splenocytes from these mice into T and B cell-deficient RAG2-deficient mice. Mice developed anti-MPO ANCA and systemic vasculitis, including NCGN. By immunohistology, some background glomerular immune complex deposition was noted that exceeded the pauci-immune character of the human disease. Thus, additional approaches were needed. Xiao and colleagues went on and performed passive antibody transfer experiments into RAG2-deficient and WT mice. These mice developed a reproducible but milder glomerular disease. Importantly, in this setting, underlying immune complex disease was not observed.

We established a modified model of anti-MPO NCGN by immunizing MPO-deficient mice with murine MPO (Fig. 1). After mice had developed anti-MPO antibodies, animals were subjected to lethal irradiation, followed by transplantation of

![Figure 1. Animal model for anti-MPO antibody induced systemic vasculitis, including NCGN. (I) MPO-deficient mice are immunized with murine MPO (muMPO). (II) After generation of anti-MPO IgG, mice are γ-irradiated with 900 rad whole-body dose. (III) Mice are injected i.v. with MPO-positive bone marrow (BM) cells from WT or from different knock-out mice. (IV) Chimeric mice now have both high titers of anti-MPO IgG and MPO-positive, circulating myeloid cells, resulting in systemic vasculitis and NCGN.](image-url)
bone marrow from MPO-positive WT mice. After engraftment with MPO-positive cells for 3–4 weeks, the mice developed a pauci-immune NCGN [70]. This disease model allowed us to demonstrate that circulating MPO-positive cells, neutrophils and monocytes, are the primary target cells for anti-MPO ANCA, are a prerequisite, and are sufficient for inducing NCGN. In addition, this model provides an opportunity to specifically analyze the effector phase and particularly certain aspects of myeloid cells in anti-MPO-induced glomerulonephritis.

A different murine ANCA model was introduced by Holdsworth, Kitching, and coworkers [71]. The investigators immunized WT C57Bl/6 mice with murine or human MPO. Animals received a subsequent injection of a subnephrotogenic dose of nephrotoxic serum. The latter is not a feature of the human disease but served the purpose of attracting neutrophils to the glomerulus. However, this approach allows studying the induction and the effector phase of ANCA disease. Applying this model system, the authors demonstrated a specific role of T cells in the pathophysiology of ANCA-induced NCGN [71–74].

A rat model of anti-MPO-induced NCGN was established by Little et al. [32] in 2005. Here, Wistar Kyoto rats were immunized with human MPO, which resulted in development of antibodies against human MPO but also cross-reactive to rat MPO. Sixty-one percent of the immunized rates demonstrated induction of NCGN.

In contrast to the advances made in anti-MPO antibody-mediated mouse models, a convincing mouse model for anti-PR3-induced glomerulonephritis has yet to be established. Pfister et al. [75] introduced a mouse model, where PR3/elastase double-knockout mice were immunized with murine rPR3, leading to generation of anti-PR3 antibodies. However, passive transfer of these IgGs into WT LPS-primed recipients did not induce NCGN [75]. Why murine anti-PR3 IgG did not induce vasculitis is not entirely clear. Some of problems may pertain to the rather low degree of mPR3 expression in murine neutrophils. Conceivably, important differences between murine and human PR3 within the C-terminal hydrophobic patch contribute to these difficulties. The lack of a hydrophobic patch in murine PR3 and the structural differences between murine and human NB1 could prevent the interaction between murine PR3 and NB1. As a consequence and in contrast to human neutrophils, PR3 presentation by NB1, as well as formation of a PR3-NB1 signaling complex involving Mac-1 could be compromised. Little et al. [76] reported an alternative approach to show an in vivo pathogenic role of PR3-ANCA. The investigators injected human PR3-ANCA IgG into humanized mice. Chimeric mice were generated by transferring human hematopoietic stem cells into irradiated NOD-scid-IL-2Rγ-deficient mice. PR3-ANCA IgG induced a mild pauci-immune NCGN.

Together, these murine model systems strongly support the notion that ANCs are indeed pathogenic in necrotizing vasculitis. In the meantime, further modifications of the above outlined, initial mouse models were introduced and additional animal models developed. Comprehensive discussions of these issues were provided in recent reviews [77–80]. We will retain our focus on mouse models and neutrophils and will discuss below how murine models helped to establish central aspects of neutrophil activation by ANCA (Fig. 2). Moreover, we will discuss data that implicate mouse models as a tool to characterize novel treatment targets and strategies.

I. The role of neutrophil granulocyte in ANCA-induced vasculitis

A whole body of evidence documented manifold in vitro interactions between ANCA and neutrophils. ANCA–monocyte interactions have been less well-studied thus far. Importantly, the aforementioned mouse models established ANCA as a necessary condition for vasculitis induction in vivo. Our bone marrow transplantation model of anti-MPO-induced NCGN proved that circulating myeloid cells are the primary effector cells in ANCA-induced inflammation [70]. These data put the ANCA antigen-expressing neutrophil granulocytes and monocytes into the focus of further research to characterize disease mechanisms and novel treatment targets.

That the neutrophil is indeed the primary effector cell in ANCA vasculitis was demonstrated by Xiao et al. [81] in 2005. The authors used the animal model of NCGN, which was induced by passive anti-MPO IgG transfer into WT mice. Prior to the IgG transfer, neutrophils were depleted by injection of the mAb NIMP-R14, which is directed to Ly-6G and Ly-6C. Neutrophil-depleted mice were protected completely 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. However, one should keep in mind that the NIMP-R14 antibody recognizes Ly-6, which is not entirely specific for neutrophils, as it is also expressed by monocytes. Thus, it is still conceivable that additional cells, particularly monocytes, cooperate with neutrophils in the effector phase of ANCA vasculitis [82].

II. The role of neutrophil FcR engagement by ANCA IgG in ANCA-induced vasculitis

In vitro data suggested that ANCA IgGs activate cytokine-primed neutrophils by antigen binding and cross-linking, as well as by FcR engagement [17, 18, 23, 24, 27]. Until now, there are no published data from animal models that specifically explore the role of FcR ligation by ANCA IgG for induction of vasculitis. However, indirect evidence supports the importance of FcR stimulation in this process. Glycosylation in the CH2 domain is necessary for proper interaction of the ANCA IgG Fc part with FcyRs. Van Timmeren et al. [83] showed that deglycosylation of ANCA IgG by hydrolyzation with endoglycosidase inhibits ANCA IgG-induced neutrophil activation in vitro and reduces the severity of NCGN by anti-MPO IgG in a mouse model.

III. The role of neutrophil priming and signaling pathways in ANCA-induced vasculitis

Several in vitro studies showed that neutrophil priming provides a prerequisite for ANCA stimulation. Priming included not only ANCA-antigen up-regulation but also induction of signaling pathways, such as p38 MAPK and ERK [13]. The
Heeringa group [84] extended these observations by showing that in vivo priming of mice with bacterial LPS increased anti-MPO IgG-induced renal damage, which in turn, was attenuated by anti-TNFα treatment. The investigators confirmed the fact that p38 MAPK is involved in this process. Specific p38 MAPK inhibitors blocked respiratory burst and degranulation of ANCA-activated neutrophils in vitro and reduced anti-MPO-induced NCGN in mice [85]. However, protection was incomplete, suggesting that p38 MAPK inhibition, as a single-treatment approach, might be less promising.

PI3K is a master pathway in cell activation and survival and was implicated in ANCA-induced neutrophil activation in vitro [23, 26, 86]. PI3K consists of a regulatory and one of three distinct p110 catalytic subunits. Two class I subclasses exist, namely, IA and IB. IA enzymes have three types of p110 catalytic subunits: p110α and p110β, which are expressed in many tissues, whereas p110δ is expressed primarily in leukocytes. The single IB enzyme, p110γ, is expressed preferentially in leukocytes. IA enzymes are activated by protein tyrosine kinase receptors, whereas the IB enzyme is activated through GPCRs [87]. To test whether the PI3Kγ isoform is important in ANCA-induced vasculitis, we used PI3Kγ-deficient mice for induction of anti-MPO IgG-induced NCGN. With the use of the bone marrow transplantation model, we transplanted bone marrow from PI3Kγ-deficient or from WT mice into MPO-immunized, MPO-deficient mice. Compared with WT bone marrow, PI3Kγ-deficient bone marrow provided almost complete protection from NCGN [88]. In addition to this genetic approach, the isoform-specific PI3Kγ inhibitor AS605240 inhibited ANCA-induced degranulation, activation of the respiratory burst, and cytokine-stimulated migration in vitro. Oral treatment with AS605240 prevented anti-MPO IgG-induced vasculitic renal disease [88]. These data indicate the importance of different signaling pathways in ANCA-induced NCGN and identified novel treatment targets.

IV. The role of NSPs in ANCA-induced NCGN

Stimulation of neutrophils and monocytes with ANCA IgG results in granule protein release. Degranulated proteins include the NSPs, PR3, NE, and CG. NSPs participate in a broad range of immune functions, such as intracellular killing, degradation of matrix proteins, modification of cytokines, activation of the protease-activated receptor, and cleaving of anti-inflammatory progranulin [89–92]. All of these functions could be impor-

Figure 2. Mechanisms of anti-MPO antibody-induced vasculitis that were established in mouse models. (I) The neutrophil granulocyte is the primary effector cell in anti-MPO-induced NCGN. (II) Priming of neutrophil granulocyte by TNF-α results in aggravated anti-MPO, antibody-induced NCGN. (III) Blockade of p38 MAPK reduces anti-MPO, antibody-induced NCGN. (IV) Interaction between Fc part of anti-MPO IgG with FcRs is necessary for ANCA-induced NCGN. (V) Generation of C5a as an amplification loop in ANCA-induced neutrophil activation. (VI) PI3Kγ activation is an essential part in ANCA-induced neutrophil activation. (VII) NSPs are needed for ANCA-induced monocyte and neutrophil IL-1β generation that participates in ANCA-induced vasculitis and NCGN.
tant in inducing ANCA-induced end-organ damage. We therefore tested the effect of NSPs by using DPPI-deficient mice in the bone marrow transplantation model of anti-MPO-induced vasculitis. NSPs are generated as enzymatically inactive proforms. These proforms are then cleaved and activated by the lysosomal cysteine protease DPPI, which is also known as cathepsin C [93]. Compared with WT bone marrow, bone marrow from DPPI-deficient mice lacking functional, active serine proteases protected from anti-MPO induced NCGN [94]. Interestingly, these mice showed strongly reduced IL-1β levels in kidney tissue. When human monocytes and neutrophils were stimulated with mAb to PR3 or MPO or with human ANCA IgG, IL-1β generation and release in the supernatant were observed. This effect was profoundly higher in monocytes compared with neutrophils. ANCA-induced IL-1β generation was serine protease-dependent, as pretreatment of human monocytes with a specific serine-protease inhibitor reduced this effect. Moreover, compared with WT murine monocytes, DPPI-deficient monocytes released significantly less IL-1β in response to murine anti-MPO antibodies. Further experiments showed that monocytes from PR3/NE-double-deficient mice showed diminished IL-1β production to anti-MPO antibodies. Importantly, compared with WT bone marrow, bone marrow from PR3/NE-double-deficient mice protected from anti-MPO induced NCGN, whereas NE/CG-deficient mice did not. Finally, DPPI-deficient monocytes were rescued from diminished IL-1β generation by addition of exogenous PR3. We therefore suggest that PR3 is the main NSP involved in IL-1β maturation and release in ANCA-stimulated monocytes. With respect to treatment, specific IL-1β blockade by Anakinra reduced ANCA-induced NCGN. Generation of IL-1β is a tightly regulated process, mainly regulated by a multiprotein complex, called the inflammasome. Inflammasome stimulation leads to processing of inactive pro-caspase-1 to active caspase-1, which then cleaves pro-IL-1β into the active IL-1β form [95]. We identified an inflammasome and caspase-1-independent pathway of IL-1β generation in ANCA-induced vasculitis. Whether the classical caspase-1-dependent pathway of IL-1β generation and the NSP-dependent pathway act in parallel has to be elucidated in future experiments.

V. The role of complement in ANCA-induced vasculitis

AAV is characterized by necrotizing inflammation with very weak staining for IgG and complement products, therefore called pauci-immune. In addition to local deposition, complement consumption is not observed in the circulating blood of patients with active ANCA vasculitis. These findings discouraged investigators from pursuing complement as a disease modulator in AAV. However, Xiao et al. [96] demonstrated recently that the alternative complement activation pathway is involved in ANCA-induced NCGN, at least in mice. The investigators observed that complement factor C5- and factor B-deficient mice were protected from anti-MPO-induced NCGN, whereas complement factor C4-deficient mice developed a disease similar to WT mice [96]. Huugen et al. [97] demonstrated that a C5-inhibiting antibody protected mice from anti-MPO-induced NCGN. As no significant complement deposition is observed in murine and human disease, we questioned whether generation of the anaphylatoxin C5a during ANCA-induced complement activation serves as an amplification loop in ANCA-induced neutrophil activation. We found complement activation during ANCA-induced neutrophil activation and documented C5a and C5a generation. rC5a acted as a potent primer for ANCA-induced neutrophil activation, and we could finally show that C5aR deficiency on myeloid cells protected from anti-MPO-induced NCGN [98]. Interestingly, p38 MAPK, ERK, and PI3K signaling pathways were reported to be involved in C5a-primed neutrophil activation by ANCA [99]. These findings are possibly relevant to ANCA vasculitis, given the fact that p38 MAPK and PI3Kβ inhibition was protective in mouse models, as discussed above. Recently, higher plasma and urinary C5a levels were observed in patients with active ANCA vasculitis [22]. Neutrophils could be potentially involved in complement activation by releasing complement-processing proteases. Furthermore, neutrophils release properdin and up-regulate surface properdin expression during activation. Properdin could lead to amplification of the alternative complement pathway and thereby, accelerate neutrophil activation [100, 101]. The exact mechanisms that are involved in complement activation during AAV are incompletely understood and need more experimental and human data. An international multicenter study was launched to explore the effect of C5aR blockade in patients with active ANCA vasculitis and NCGN.

In summary, experimental data strongly suggest that ANCA are pathogenic in necrotizing small-vessel vasculitis. We discussed major findings of ANCA–neutrophil interactions and how mouse models provided an opportunity to confirm key mechanisms in complex in vivo situations. We focused on the main ANCA target antigens PR3 and MPO and on mouse models. Future challenges are to elucidate the mechanisms that break tolerance in ANCA vasculitis, to establish PR3-ANCA animal models, to further elucidate effector mechanisms that mediate tissue damage in vivo, and to characterize novel treatment approaches, to name only a few.

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