Pathogenesis and therapeutic interventions for ANCA-associated vasculitis

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Abstract

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a vasculitis that affects systemic small vessels, accompanied by the presence of ANCAs in the serum. This disease entity includes microscopic polyangiitis, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis and drug-induced AAV. Similar to other autoimmune diseases, AAV develops in patients with a predisposing genetic background who have been exposed to causative environmental factors. The mechanism by which ANCAs cause vasculitis involves ANCA-mediated excessive activation of neutrophils that subsequently release inflammatory cytokines, reactive oxygen species and lytic enzymes. In addition, this excessive activation of neutrophils by ANCAs induces formation of neutrophil extracellular traps (NETs). Although NETs are essential elements in the innate immunity, excessive NET formation is harmful to small vessels. Moreover, NETs are involved not only in ANCA-mediated vascular injury but also in the production of ANCAs themselves. Therefore, a vicious cycle of NET formation and ANCA production is considered to be involved in the pathogenesis of AAV.

In addition to this role of NETs in AAV, some other important discoveries have been made in the last few years. Incorporating these new insights into our understanding of the pathogenesis of AAV is needed to fully understand and ultimately overcome this disease.

[H1] Introduction

Following the initial discovery of anti-neutrophil cytoplasmic antibodies (ANCAs) in 1982,[1] ANCA-associated vasculitis (AAV) was established as a disease entity distinct from other vasculitides. AAV is now recognized as a systemic vasculitis of small vessels that is accompanied by the presence of ANCAs in the serum. The major antigens targeted by these ANCAs are myeloperoxidase (MPO) and proteinase 3 (PR3, also known as myeloblastin).

AAV includes microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA) and drug-induced AAV.[2] MPA affects systemic small vessels — preferentially those of the renal glomeruli — and the majority of patients with MPA are positive for MPO-ANCA. The characteristic histopathological feature of MPA is necrotizing vasculitis; granulomatous inflammation is generally absent. By contrast, GPA is characterized by granulomatous inflammation with necrosis that usually involves the respiratory tract and the simultaneous development of necrotizing small-vessel vasculitis. Typically, patients with GPA are positive for PR3-ANCA. Pauci-immune necrotizing and crescentic glomerulonephritis (NCGN) is associated with both MPA and GPA. EGPA is an eosinophil-rich necrotizing vasculitis with a granulomatous reaction that predominantly affects small-to-medium-sized vessels. This disease is associated with adult onset asthma and allergic sinusitis; high levels of eosinophils in peripheral blood and affected tissues are its essential feature. About 50% of patients with EGPA are positive for MPO-ANCA. Finally, drugs such as propylthiouracil (an anti-thyroid drug), hydralazine (an anti-hypertension drug) and cocaine can induce production of ANCAs and lead to the development of drug-induced AAV. Epidemiological studies have revealed ethnic differences in both ANCA specificity and manifestations of AAV.[3-6] For example, PR3-ANCA and GPA are most common in

Western countries, whereas MPO-ANCA and MPA predominate in East Asian countries (including Japan).[3-6]

International collaborative studies have published guidelines for diagnosis[7,8] and treatments aimed at remission induction[9,10] and maintenance of remission of AAV.[11] Although these efforts have resulted in a remarkable improvement in the prognosis of affected patients, more work is needed to fully understand and ultimately overcome AAV. In this Review, therefore, we present an updated understanding of the aetiology and pathogenesis of AAV.

[H1] Aetiology

[H2] Genetic factors

Genome-wide association studies (GWASs)[12-14] have identified several genes involved in either susceptibility or resistance to AAV (Table 1). Among these, the strongest associations with AAV are displayed by class II major histocompatibility complex (MHC) genes. A GWAS in an European population demonstrated that GPA with PR3-ANCA is most strongly associated with the *HLA-DP* region, and that MPA with MPO-ANCA is highly associated with the *HLA-DQ* region.[12] Interestingly, these data also indicated that the HLA genomic signature was associated with ANCA specificity (that is, PR3-ANCA or MPO-ANCA) rather than with the clinical manifestation (GPA or MPA). A GWAS conducted in a North American population also found that the *HLA-DP* locus showed the strongest association with GPA.[13] That study further identified *HLA-DPB1*04* as a risk allele for GPA. By contrast, *HLA-DRB1*09:01*, which is common in East Asian but rare in European populations, is strongly associated with MPA (MPO-ANCA) in the Japanese population.[15] These ethnic differences in class II MHC allele frequencies reflect the epidemiological differences observed in AAV; that is, the predominance of GPA with PR3-ANCA in white European populations and the predominance of MPA with MPO-ANCA in Asian populations.[3]

Non-MHC genes identified as associated with AAV include PTPN22 (encoding tyrosine-protein phosphatase non-receptor type 22),[16] *SERPINA1* (encoding α1-antitrypsin), *PRTN3*

(encoding PR3), and *SEMA6A* (encoding semaphorin 6a).[12-14] The frequency of a gain-of-function single nucleotide polymorphism (SNP) in *PTPN22* is significantly higher in white patients with AAV than in the general American white population.[16] The PTPN22 gain-of-function variant also negatively regulates the production of IL-10, an immunosuppressive cytokine, resulting in hyper-responsiveness of the immune system in patients with AAV.[16] A SNP near *SERPINA1* is associated with resistance to GPA (PR3-ANCA).[17] Because α1-antitrypsin is an inhibitor of PR3, this SNP might affect the function of PR3.[17] A SNP in *PRTN3* is associated with resistance to PR3-ANCA AAV,[12] whereas a SNP in *SEMA6A* is associated with resistance to GPA.[13,14] Future studies will reveal how these molecules are involved in the pathogenesis of AAV.

[H2] Epigenetic factors

The epigenetic modifications, trimethylation of histone 3 lysine 27 (H3K27me3) and DNA methylation, are implicated in the regulation of *MPO* and *PRTN3* gene expression.[18,19] Reduced H3K27me3 is associated with aberrant expression of *MPO* and *PRTN3* genes in patients with active AAV.[18] The levels of *MPO* and *PRTN3* promoter methylation are negatively correlated with the levels of mRNA transcripts of these genes.[19] Moreover, *MPO* and *PRTN3* promoter methylation is reduced in patients with active AAV and increased during remission of AAV.[19] Elevated expression of MPO and PR3, mediated by these epigenetic modifications, is also linked with AAV.[19]

[H2] Environmental factors

Environmental factors that can trigger the development of AAV include infectious agents, drugs such as propylthiouracil, hydralazine and cocaine, and airborne particulates such as silica dust. For example, toxic shock syndrome toxin-1, which is secreted by *Staphylococcus aureus*, is a risk factor for disease relapse in GPA.[20] Although the association of silica with AAV has been intensely debated, a systematic review and meta-analysis confirmed the association between silica dust exposure and development of AAV.[21] Moreover, morbidity and disease severity increased substantially in patients

with AAV after the great earthquakes in Japan (Hanshin-Awaji in 1995[22] and East Japan in 2011[23]). These observations suggest that silica dust in the air, caused by the massive destruction and subsequent reconstruction of the cities[22] and by the marine sludge and sediments deposited in the subsequent tsunami disaster,[23] might have affected the presentation of AAV, especially its respiratory manifestations such as pulmonary haemorrhage and interstitial pneumonitis. However, in a contrasting study, no difference in the incidence of AAV was observed before versus after the February 2011 earthquake in Christchurch, New Zealand.[24] Therefore, whether environmental pollution from earthquakes can affect the incidence or presentation of AAV remains unresolved.

[H2] ANCAs

The two major types of ANCAs show different staining patterns, which can be detected by indirect immunofluorescence of ethanol-fixed neutrophils. One type of ANCA is associated with staining around the nucleus, and is known as perinuclear ANCA (p-ANCA), whereas the other type is associated with diffuse staining of the cytoplasm, and is known as cytoplasmic ANCA (c-ANCA). The major antigen targeted by p-ANCA is MPO, and that targeted by c-ANCA is PR3. At present, ANCAs are usually detected quantitatively by enzyme-linked immunosorbent assay (ELISA) using plates coated with these antigens. ANCAs detected by ELISA are called MPO-ANCA or PR3-ANCA according to the target antigen used.

In addition to MPO and PR3, several other neutrophil-derived molecules can be targeted by ANCAs, including α-enolase, azurocidin, bactericidal permeability-increasing protein (BPI), cathepsin G, elastase, defensin, lactoferrin, lysosome-associated membrane glycoprotein 2 (LAMP2), and moesin.[25-32] The pathogenicity of these ‘minor’ ANCAs is generally low, and p-ANCAs other than MPO-ANCA are not usually associated with vasculitis; however, discussion is ongoing as to whether some minor ANCAs might show pathogenic involvement in AAV.[25-32]

[H3] Mechanisms of ANCA production. Disordered regulation of neutrophil extracellular traps (NETs) is now known to contribute to ANCA production. Although NETs are an element of the innate immune system that is essential for host defence,[33] the formation and breakdown of NETs is strictly regulated because excessive exposure to NETs can cause angiopathy.[34,35] NETs are degraded mainly by DNase I in serum;[36] however, the administration of propylthiouracil produces abnormal NETs that are resistant to degradation by DNase I and thus can persist *in vivo*.[38] Tolerance to MPO present in these NETs is thereafter broken, resulting in MPO-ANCA production. Of interest, MPO-ANCAs are produced in ~30% of patients treated with propylthiouracil.[37] The molecular modification of MPO induced by propylthiouracil — a change in the protein structure surrounding the haem iron from a rhombic to an axial form[39] — could contribute to the breakdown of tolerance to MPO.[38]

In mice, the injection of a mixture of myeloid dendritic cells and neutrophils that have formed NETs induces ANCA production, whereas the injection of a mixture of myeloid dendritic cells and DNase I-treated NET-forming neutrophils does not induce ANCA production.[40] This finding suggests that the DNA in NETs is required for MPO-ANCA and PR3-ANCA formation. The DNA in NETs can also activate B cells via Toll-like receptor (TLR) 9 to accelerate antibody production.[41]

In summary, several steps are involved in the process of ANCA production. During NET formation, the contents of neutrophilic granules, including MPO and PR3, are mixed with chromatin fibres and bind to DNA, which might modify the antigenicity of these autoantigens.[40] This physiological first step is not specific to patients with AAV. The second step is the incomplete degradation of NETs, which seems to be specific to some autoimmune diseases. Although NETs are appropriately degraded by serum DNase I under physiological conditions,[36] NET degradation activity is reduced in patients with AAV[42] as well as in patients with systemic lupus erythematosus (SLE).[36] The third step is antigen presentation. Susceptibility to AAV is strongly associated with specific class II MHC genotypes.[12,13,15] Therefore, patients who express class II MHC molecules that are apt to present MPO or PR3 are those most likely to produce ANCAs. This phenomenon could be specific to patients with AAV. Misfolded MPO presented by MPA-susceptible class II MHC (HLA-DR) can also be

a target of ANCAs.[43] These misfolded MPO proteins are transported to the cell surface (via a chaperone-like function of HLA-DR) where cryptic epitopes of MPO are recognized by the immune system[43].

[H3] Pathogenicity of ANCAs. Several animal models of AAV have been established so far. They are classified into five categories, including passive immune models,[44,46-49] active immune models[45,50-53], drug-induced models,[38,54] molecular mimicry models[27] and spontaneous models.[55] The characteristics of these models are summarized in Supplementary Tables 1–3. Studies in these models have shown that ANCAs not only serve as a biomarker of AAV but also have pathogenic potential themselves. For example, injection of either MPO-ANCA (obtained by the immunization of MPO-deficient mice with mouse MPO) or splenocytes from such immunized mice into immunocompromised or wild-type mice can induce NCGN.[44] In other animal models, such as Wistar-Kyoto rats immunized with human MPO, the anti-human MPO antibodies generated cross-react with rat MPO, resulting in the development of NCGN and pulmonary haemorrhage.[45] These animal models clearly indicate the pathogenicity of ANCAs.

The mechanism by which ANCAs cause vasculitis has been explained as follows.[56] Proinflammatory cytokines such as tumour necrosis factor (TNF) and interleukin 1β (IL-1β) are generated during infection and activate neutrophils, which then express target antigens (namely MPO and PR3) on their cell surface. ANCAs bind to these antigens. At the same time, the Fc portion of these ANCAs bind to Fcγ receptors on neutrophils, inducing excessive activation of the neutrophils. This hyperactivation results in abnormal cytokine production accompanied by the release of reactive oxygen species (ROS) and lytic enzymes, which injure vascular endothelial cells.[57, 58] The excessive activation of neutrophils elicited by ANCAs also induces the formation of NETs.[42,59] Moreover, humoral factors other than ANCAs, such as damage-associated molecular patterns (DAMPs), are involved in the formation of NETs in patients with AAV.[60] This excessive NET formation — specifically, exposure to NET components such as histones and matrix metalloproteinases (MMPs) — is harmful

to vascular endothelial cells.[34,35,56]

Despite the critical role of ANCAs in the pathogenesis of AAV, the histological hallmark of AAV is necrotizing glomerulonephritis without apparent deposition of immune complexes (pauci-immune glomerulonephritis). However, when TNF-primed neutrophils were exposed to ANCAs in vitro, the ANCAs disappeared after induction of NETs owing to their digestion by neutrophil elastase derived from NET-forming neutrophils.[64] These findings suggest that NETs contribute to the disappearance of immunoglobulins from AAV lesions. Immunofluorescence studies can detect NETs in the necrotizing lesions of AAV by the presence of citrullinated histones accompanied by extracellular DNA.[59,61,62,63] Also, in our own unpublished research, we have found scattering of citrullinated histones, in a manner that cannot be explained by an intracellular distribution, in vessels affected by AAV (Figure 1). Interestingly, almost no NET deposition can be detected in the lesions of ANCA-unrelated necrotizing vasculitis, such as polyarteritis nodosa (PAN).

[H3] Association of ANCAs with disease activity. Although AAV is an ANCA-associated disease, ANCA titres do not necessarily reflect disease activity except for renal involvement.[65] The lack of correlation between ANCA titres and vasculitic disease activity can be explained by differences in the epitopes and affinity of ANCAs. MPO is a 158 kDa heterotetrameric glycoprotein consisting of two light chains (14 kDa each, two heavy chains (59 kDa each) and two haem molecules.[66] ANCAs in patients with MPO-AAV typically recognize an epitope at the *N*-terminus of the MPO heavy chain;[68] this specificity is most likely in patients with the most severe disease activity. MPO-ANCAs are subdivided into high-affinity and low-affinity antibodies.[69] Vasculitic disease activity is higher in patients with high-affinity MPO-ANCAs than in those with low-affinity MPO-ANCAs.[42,69] In patients with low vasculitic disease activity despite high MPO-ANCA titres, the MPO-ANCAs might target epitopes located in a low-risk region, instead of at the N-terminus of the MPO heavy chain, or the MPO-ANCAs might have low antigen affinity.

ANCAs cannot be detected in some patients with clinical manifestations of MPO-AAV. In

some of these patients, laboratory tests for ANCAs can give false-negative results because 50 kDa fragments of ceruloplasmin bind to the ANCA in the serum.[70] However, the clinical relevance of the production of ceruloplasmin degradation products *in vivo* remains unclear.

[H1] Pathogenesis

[H2] The common pathways involved in the pathogenesis of AAV

The pathogenesis of AAV partly overlaps among MPA, GPA, EGPA, and drug-induced AAV. The common pathways in the pathogenesis of AAV based on collective evidence is illustrated in Figure 2. This is almost applicable to MPA because MPA can be considered as a prototype of AAV.

[H3] Priming of neutrophils. Upon the capture of infectious agents, dendritic cells produce transforming growth factor-β (TGFβ) and IL-6, which induce the differentiation of naive T cells into T_H17 cells.[71] IL-17 (which is released from T_H17 cells stimulated by dendritic-cell-derived IL-23) can induce the release of proinflammatory cytokines, such as TNF and IL-1β, from macrophages.[72] These proinflammatory cytokines prime neutrophils.

Disturbed T-cell immune homeostasis is critically involved in both this priming and subsequent processes. For example, functional and numerical abnormalities of regulatory T cells (T_{reg} cells, which suppress the proliferation of autoreactive T-cells) are associated with the development of AAV.[73] In patients with AAV, a proportion of T_{reg} cells differentiate into T_H17 cells, resulting in elevated levels of IL-17.[72] In addition, low expression of CD122 protein (IL-2 receptor β-chain, an important signal transducer of T cells) on $CD4^+CD25^+$ T cells and T_{reg} cells is associated with systemic vasculitis with renal involvement, and with relapse of AAV.[74]

The complement system is also involved in priming of neutrophils, and accordingly the role of complement proteins in the pathogenesis of MPO-AAV has been studied in mouse models. C4-deficient mice injected with MPO-ANCA also develop vasculitis, as do wild-type mice injected with MPO-ANCA; however, mice deficient in both C5 and factor B do not develop vasculitis after the

injection of MPO-ANCA.[75] This finding suggests that the alternative complement pathway is involved in the pathogenesis of MPO-AAV. Interestingly, injection of MPO-ANCA into C6-deficient mice also results in vasculitis; therefore, an alternative pathway mechanism that is not mediated by the membrane attack complex (MAC) is likely to contribute to the development of MPO-AAV.[76] Neutrophil priming by C5a — an activated C5 fragment — is one such mechanism that deserves further consideration.[56] Binding of C5a to its receptor on the neutrophil cell surface induces neutrophil activation and ANCA-mediated glomerulonephritis in mice.[77] In addition, the C5a-induced release of tissue factor from neutrophils can promote hypercoagulability in patients with AAV.[78] Furthermore, elevated serum levels of C3a and C5a, which suggest activation of the alternative complement pathway and neutrophil priming via the C5a receptor, have been demonstrated in patients with active AAV.[79,80]

[H3] MPO-ANCA production. Neutrophils stimulated by bacteria form NETs, which are then degraded by various enzymes, the most important of which in serum is DNase I.[36] Serum DNase I activity is markedly lower in patients with MPA than in healthy individuals.[42] In patients with MPA who have low NET degradation activity, NETs persist *in vivo* and tolerance to MPO is disrupted, resulting in MPO-ANCA production.[81] The structural modification of MPO proteins[39] could contribute to this mechanism by being recognized as neoantigens.

Dendritic cells are involved in the presentation of MPO contained in NETs to $CD4^+$ T cells.[40] These $CD4^+$ T cells induce the differentiation of B cells into plasma cells that produce MPO-ANCA, via the production of IL-21.[82] TNF ligand superfamily member 13B (also known as B-cell-activating factor (BAFF) or B-lymphocyte stimulator (BLyS)) released from activated neutrophils is also involved in the activation of B cells.[82]

[H3] Excessive activation of neutrophils. Primed neutrophils express ANCA antigens on their plasma membrane. ANCAs bind to the antigens and, at the same time, the Fc region of ANCA binds to the

Fcγ receptor on neutrophils. This binding induces excessive activation of these neutrophils, leading to abnormal cytokine production accompanied by the release of ROS and lytic enzymes, and eventually to NET formation.[57-59,83] The most important angiopathic molecules in NETs are histones dissociated from DNA, and MMPs such as MMP2 and MMP9.[34,35,56] Accumulating evidence indicates that a vicious cycle of NET and ANCA formation is involved in the pathogenesis of AAV (Figure 3).

Semaphorin 4D regulates neutrophil activation in small vessels, and impairment of this regulation has been demonstrated to lead to NET-mediated vascular injury.[84,85] Serum levels of soluble semaphorin 4D are elevated in patients with AAV, and correlate with disease activity.[84,85] The same researchers further determined that the cell-surface expression of semaphorin 4D is decreased in neutrophils from patients with AAV, as a consequence of the proteolytic cleavage of membrane semaphorin 4D.[84,85] Interestingly, membranous semaphorin 4D on neutrophils binds to plexin B2 on vascular endothelial cells, and this interaction decreases NET formation.[84,85] Collectively, these findings suggest that a physiological mechanism that inhibits NET formation in small vessels is impaired in patients with AAV, and that this impairment is associated with increased NET formation.

Some researchers have found that serum levels of NET do not correlate with either AAV disease activity[86] or ANCA titres.[60,87] However, a gold-standard method for quantifying NETs *in vivo* has not yet been established;[88] therefore, future studies are needed to define the association between NETs and AAV.

[H2] Granulomatosis with polyangiitis

Neutrophil priming and ANCA-mediated excessive activation of neutrophils also occur in GPA, similarly to MPA. However, necrotizing granulomas in the respiratory tract and PR3-ANCA production are distinct features of GPA.

[H3] Necrotizing granuloma formation. The mechanism of necrotizing granuloma formation is thought to involve infection, possibly by *Staphylococcus aureus* (*S. aureus*), which activates

tissue-resident macrophages in the bronchial epithelium via TLRs.[89] These macrophages release proinflammatory cytokines, including TNF and IL-1β, which promote recruitment of neutrophils and monocytes from the blood into the developing lesion. Recruited neutrophils that encounter microbes release ROS and lytic enzymes and undergo lysis, resulting in the formation of the necrotic core of the lesion.[89] The recruited monocytes differentiate into macrophages that secrete IL-23, resulting in the differentiation of T cells toward a T_H17 phenotype. IL-17 released from T_H17 cells is critically implicated in formation of the granuloma that surrounds the necrotic region.[89]

PR3 on the surface of apoptotic neutrophils interferes with the induction of anti-inflammatory mechanisms following phagocytosis of these cells by macrophages.[90] In mouse models, concomitant injection of PR3-ANCAs and PR3-expressing apoptotic neutrophils induced a T_H17 response, revealing a GPA-specific mechanism of immune polarization.[91] Furthermore, levels of IL-17 and IL-23 are increased during active disease in patients with GPA.[92] Effector memory T cells, proliferation of which is dependent on IL-15, also contribute to granuloma formation in patients with GPA.[93] PR3-stimulated dendritic cells derived from patients with GPA induced a higher IFNγ response in PR3-specific $CD4^+$ T cells than did PR3-stimulated dendritic cells from healthy individuals.[94] This T_H1-type response might favour granuloma formation in patients with GPA.

[H3] PR3-ANCA production. MPO-ANCA and PR3-ANCA seem to share a fundamental underlying mechanism of production. Indeed, both types of ANCA are produced when mice are injected with NETs and myeloid dendritic cells.[40] The major factor that affects ANCA specificity seems to be class II MHC genotype, although other factors are specific to the production of each type of ANCA. For example, a complementary-PR3-mediated mechanism has been specifically implicated in the production of PR3-ANCA in GPA.[95] The initial immune response in GPA has been identified as directed against complementary PR3 transcripts, after which PR3-ANCA develop during a secondary anti-idiotypic immune response.[95] Interestingly, pathogens such as *S. aureus* bear genetic sequences that are complementary to the human *PRTN3* gene, which encodes PR3; thus, this observation

suggests that the complementary PR3 transcripts have an exogenous origin. In support of this notion, chronic carriage of *S. aureus* in the nasal cavity increases the risk of relapse of GPA.[96]

[H2] EGPA

EGPA is characterized by allergic manifestations such as adult-onset asthma and sinusitis. Eosinophilia and a prominent infiltration of eosinophils into vasculitic lesions are distinct features of EGPA. About 50% of patients with EGPA are positive for MPO-ANCA, and the presence of these autoantibodies shows a positive correlation with renal involvement but an inverse correlation with cardiac involvement.[97] Interestingly, the prevalence of ANCAs in patients with EGPA decreases over time.[98]

[H3] Eosinophilia and infiltration of eosinophils. EGPA is regarded as a T_H2-cytokine-mediated disease, because elevated levels of T_H2 cytokines (such as IL-4, IL-5, and IL-13) are associated with eosinophilia in patients with EGPA.[99] C-C motif chemokine 26 (CCL26, also known as eotaxin 3) released from vascular endothelial cells is implicated in the tissue infiltration of eosinophils.[100] The tissue-infiltrating eosinophils secrete eosinophilic granules, including eosinophilic neurotoxin, major basic proteins and eosinophilic cationic proteins, resulting in tissue destruction.[101] Although eosinophil peroxidase shares 68% amino acid identity with neutrophil MPO,[102] the mechanism of MPO-ANCA production in patients with EGPA remains to be revealed.

[H3] Anti-lactoferrin antibody in EGPA. Anti-lactoferrin antibodies (one of the 'minor' ANCAs) are found in a subgroup of patients with EGPA but not in patients with MPA or GPA.[32] The frequency of renal involvement, serum C-reactive protein levels, and disease activity are all considerably higher in anti-lactoferrin antibody-positive than in anti-lactoferrin antibody-negative patients with EGPA. Lactoferrin is present in neutrophil-specific granules and is immediately secreted (via degranulation) upon their activation. Lactoferrin is an endogenous suppressor of NET formation;[103] thus,

anti-lactoferrin antibodies inhibit the NET-suppressing activity of lactoferrin, and consequently promote NET formation after activation of neutrophils.[32] Therefore, the presence of anti-lactoferrin antibodies is thought to be associated with NET-related disease activity in patients with EGPA.

[H2] Drug-induced AAV

Many drugs, such as propylthiouracil, benzylthiouracil, methimazole, carbiomazole, minocycline, cefotaxime, nitrofurantoin, D-penicillamine, hydralazine, allopurinol, levamisole, phenytoin, sulfasalazine, rifampicin and anti-TNF agents, can induce AAV.[104] Cocaine can also cause AAV.[105] Although several different pathways might lead to the development of drug-induced AAV, the innate and adaptive immune responses share some common mechanisms.

Propylthiouracil treatment induces the formation of DNase I-resistant NETs.[38] Because propylthiouracil does not inhibit DNase I activity directly, metabolites of propylthiouracil are hypothesized to mask the DNase I recognition sites in DNA extruded in NETs.[38] Cocaine and levamisole induce the formation of NETs enriched in neutrophil elastase and, potentially, in mitochondrial DNA, which is highly inflammatory.[106] Hydralazine also enhances NET formation,[107] and NETs induced by these drugs or reagents could be a source of antigens leading to ANCA formation. Some drugs, such as hydralazine, induce the promoter demethylation of DNA in T cells, resulting in their activation.[107] Activated T cells contribute to autoantibody production by B cells and plasma cells.

[H1] Novel therapeutic targets in AAV

International collaborative studies led to the publication of treatment guidelines for both remission induction[9,10] and maintenance of remission.[11] Although these efforts have resulted in a remarkable improvement in the prognosis of patients with AAV, development of novel therapies that target molecules specifically involved in the pathogenesis of AAV is needed to ultimately overcome this disease. In the following sections, we focus on the therapeutic possibilities inspired by recent

discoveries in the pathogenesis of AAV in the last five years (Table 2).

[H2] Targeting B cells

BAFF is produced by neutrophils in large amounts and has an important role in B-cell survival. Serum levels of this molecule increase with progression of AAV.[108,109] A clinical trial of combination therapy with methotrexate plus the BAFF antagonist blisibimod has demonstrated promising results.[110]

Bortezomib inhibits proteasomes, which have an essential role in the intracellular clearance of unwanted peptides. Treatment with bortezomib results in depletion of plasma cells, which produce antibodies, including pathogenic ANCAs. In a mouse model, bortezomib treatment led to reduction in MPO-AAV disease activity as a result of the deletion of MPO-ANCA-producing plasma cells.[111] In humans, bortezomib treatment ameliorates disease activity in patients with refractory AAV.[112]

[H2] Targeting T cells

Cytotoxic lymphocyte antigen 4 (CTLA4), which is expressed specifically and constitutively on T_{reg} cells, also has a key role in the regulation of self-tolerance. Abatacept, a selective co-stimulation inhibitor, is a soluble fusion protein composed of the Fc region of the human IgG1 linked to the extracellular domain of CTLA4. This agent binds to CD80 or CD86 on the surface of antigen-presenting cells, thereby inhibiting T-cell activation. A clinical study has demonstrated that abatacept treatment is well tolerated and associated with a high frequency of disease remission in patients with relapsing GPA.[113] The results of a double-blind randomized controlled trial of abatacept as first-line therapy for AAV (the ABAVAS study, NCT00482066, https://clinicaltrials.gov/ct2/show/NCT00482066) are expected to be released soon.

[H2] Targeting cytokines

Patients with AAV can develop high levels of circulating cytokines. Serum levels of proinflammatory

cytokines, particularly TNF and IL-6, are much higher in patients with AAV than in healthy individuals.[114,115] Serum levels of IL-6 are also substantially increased in patients with active MPA and GPA.[114,115] Thus, treatment with anti-cytokine biologic agents has been studied in patients with these diseases.

Although conflicting reports have been published on the efficacy of TNF blockade in patients with AAV,[116] IL-6 blockade seems to be a promising approach. IL-6 not only promotes B-cell differentiation but also has a central role in macrophage activation, T-cell differentiation, plasma-cell survival and induction of other cytokines. Tocilizumab, a humanized anti-IL-6 receptor antibody, achieved a complete and sustained remission in one patient with MPA who did not respond to standard immunotherapy.[117] However, large-scale clinical studies are required to define the adverse events associated with anti-IL-6 therapy and to determine the long-term prognosis of treated patients.

IL-5 binds to the IL-5 receptor, which leads to the growth of B cells and activation of eosinophils. A clinical study has demonstrated that about 50% of patients with EGPA treated with the anti-IL-5 antibody mepolizumab will enter remission.[118]

[H2] Targeting the complement system

The complement system is presumed to be crucial for the development of AAV. C5a, a product derived from activation of the alternative complement pathway, is thought to be a key neutrophil-activating molecule.[119] In mice, treatment with C5 and C5a receptor-inhibiting antibodies suppresses the development of MPO-AAV.[76,120] The C5a receptor antagonist avacopan blocks C5a-mediated neutrophil activation and infiltration into the vascular endothelium. A clinical study of avacopan in patients with AAV demonstrated the therapeutic potency of this treatment, which was capable of replacing glucocorticoids.[121] A larger clinical trial (ADVOCATE, NCT02994927, https://clinicaltrials.gov/ct2/show/NCT02994927) is underway that will determine the efficacy of this drug in patients with AAV.

[H2] Targeting humoral factors

Not only pathogenic ANCAs but also other humoral factors are involved in the pathogenesis of AAV. Some clinical studies have demonstrated that plasma exchange, which removes these factors, improves renal dysfunction in patients with AAV and shortens the duration of hospitalization.[122,123] A clinical trial of plasma exchange and glucocorticoid treatment in patients with AAV (the PEXIVAS study, NCT00987389, https://clinicaltrials.gov/ct2/show/NCT00987389) has been completed and analysis of the results is now ongoing.

[H1] Conclusions

In this Review, we present an update on the aetiology and pathogenesis of AAV. Strenuous investigations into the genetic and environmental factors associated with AAV, ANCA pathogenicity, and the roles of neutrophils, other immune cells and humoral factors in the pathogenesis of AAV have led to several breakthroughs in the understanding and treatment of this disease. Consequently, promising new therapeutic agents that target B cells, T cells, and cytokines have been developed. However, no therapies have yet been developed that target neutrophils, the pivotal players in this disease. Moreover, glucocorticoids remain at the forefront of AAV treatment, and these agents are known to prolong neutrophil survival.[124] Future strategies should specifically address the role of these cells in AAV. Finally, C5 and C5a receptor inhibitors could become promising treatments for AAV, and NET inhibitors (if they can be developed) might also be potently effective. In the meantime, continued collaborative studies and interactions between basic and clinical research are needed to fully understand the aetiology and pathogenesis of AAV and ultimately to develop safe and effective treatments.

References

1. Davies, D.J., Moran, J.E., Niall, J.F. & Ryan, G.B. Segmental necrotising glomerulonephritis with

antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed)* **285**, 606 (1982).

2. Jennette, J.C*., et al.* 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* **65**, 1-11 (2013).
3. Fujimoto, S*., et al.* Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the U.K. *Rheumatology (Oxford)* **50**, 1916-1920 (2011).
4. Furuta, S*., et al.* Comparison of phenotype and outcome in microscopic polyangiitis between Europe and Japan. *J Rheumatol* **41**, 325-333 (2014).
5. Furuta, S*., et al.* Comparison of the Phenotype and Outcome of Granulomatosis with Polyangiitis Between UK and Japanese Cohorts. *J Rheumatol* **44**, 216-222 (2017).
6. Pearce, F.A., Craven, A., Merkel, P.A., Luqmani, R.A. & Watts, R.A. Global ethnic and geographic differences in the clinical presentations of anti-neutrophil cytoplasm antibody-associated vasculitis. *Rheumatology (Oxford)* **56**, 1962-1969 (2017).
7. Seeliger, B*., et al.* Are the 1990 American College of Rheumatology vasculitis classification criteria still valid? *Rheumatology (Oxford)* **56**, 1154-1161 (2017).
8. Bossuyt, X*., et al.* Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. *Nat Rev Rheumatol* **13**, 683-692 (2017).
9. Ntatsaki, E*., et al.* BSR and BHPR guideline for the management of adults with ANCA-associated vasculitis. *Rheumatology (Oxford)* **53**, 2306-2309 (2014).
10. Yates, M*., et al.* EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis* **75**, 1583-1594 (2016).
11. Puechal, X*., et al.* Long-Term Outcomes Among Participants in the WEGENT Trial of Remission-Maintenance Therapy for Granulomatosis With Polyangiitis (Wegener's) or Microscopic Polyangiitis. *Arthritis Rheumatol* **68**, 690-701 (2016).
12. Lyons, P.A*., et al.* Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med* **367**,

214-223 (2012).

13. Xie, G.*, et al.* Association of granulomatosis with polyangiitis (Wegener's) with HLA-DPB1*04 and SEMA6A gene variants: evidence from genome-wide analysis. *Arthritis Rheum* **65**, 2457-2468 (2013).

14. Rahmattulla, C.*, et al.* Genetic variants in ANCA-associated vasculitis: a meta-analysis. *Ann Rheum Dis* **75**, 1687-1692 (2016).

15. Kawasaki, A.*, et al.* Protective Role of HLA-DRB1*13:02 against Microscopic Polyangiitis and MPO-ANCA-Positive Vasculitides in a Japanese Population: A Case-Control Study. *PLoS One* **11**, e0154393 (2016).

16. Cao, Y.*, et al.* High basal activity of the PTPN22 gain-of-function variant blunts leukocyte responsiveness negatively affecting IL-10 production in ANCA vasculitis. *PLoS One* **7**, e42783 (2012).

17. Relle, M., Fohr, B., Fasola, F. & Schwarting, A. Genetics and pathophysiology of granulomatosis with polyangiitis (GPA) and its main autoantigen proteinase 3. *Mol Cell Probes* **30**, 366-373 (2016).

18. Ciavatta, D.J.*, et al.* Epigenetic basis for aberrant upregulation of autoantigen genes in humans with ANCA vasculitis. *J Clin Invest* **120**, 3209-3219 (2010).

19. Jones, B.E.*, et al.* Gene-Specific DNA Methylation Changes Predict Remission in Patients with ANCA-Associated Vasculitis. *J Am Soc Nephrol* **28**, 1175-1187 (2017).

20. Popa, E.R.*, et al.* Staphylococcal toxic-shock-syndrome-toxin-1 as a risk factor for disease relapse in Wegener's granulomatosis. *Rheumatology (Oxford)* **46**, 1029-1033 (2007).

21. Gomez-Puerta, J.A., Gedmintas, L. & Costenbader, K.H. The association between silica exposure and development of ANCA-associated vasculitis: systematic review and meta-analysis. *Autoimmun Rev* **12**, 1129-1135 (2013).

22. Yashiro, M.*, et al.* Significantly high regional morbidity of MPO-ANCA-related angitis and/or nephritis with respiratory tract involvement after the 1995 great earthquake in Kobe (Japan). *Am J Kidney Dis* **35**, 889-895 (2000).

23. Takeuchi, Y.*, et al.* The influence of the Great East Japan earthquake on microscopic polyangiitis: A retrospective observational study. *PLoS One* **12**, e0177482 (2017).

24. Farquhar, H.J.*, et al.* Incidence of anti-neutrophil cytoplasmic antibody-associated vasculitis before and after the February 2011 Christchurch Earthquake. *Intern Med J* **47**, 57-61 (2017).

25. Moodie, F.D., Leaker, B., Cambridge, G., Totty, N.F. & Segal, A.W. α-enolase: a novel cytosolic autoantigen in ANCA positive vasculitis. *Kidney Int* **43**, 675-681 (1993).

26. Yu, F.*, et al.* Clinical and pathological features of renal involvement in propylthiouracil-associated ANCA-positive vasculitis. *Am J Kidney Dis* **49**, 607-614 (2007).

27. Kain, R.*, et al.* Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med* **14**, 1088-1096 (2008).

28. Nagao, T.*, et al.* Direct activation of glomerular endothelial cells by anti-moesin activity of anti-myeloperoxidase antibody. *Nephrol Dial Transplant* **26**, 2752-2760 (2011).

29. Kontic, M., Radovanovic, S., Nikolic, M. & Bonaci-Nikolic, B. Concomitant drug- and infection-induced antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis with multispecific ANCA. *Med Princ Pract* **21**, 488-491 (2012).

30. Fukuhara, A.*, et al.* Systemic vasculitis associated with anti-neutrophil cytoplasmic antibodies against bactericidal/permeability increasing protein. *Intern Med* **52**, 1095-1099 (2013).

31. Suzuki, K.*, et al.* A novel autoantibody against moesin in the serum of patients with MPO-ANCA-associated vasculitis. *Nephrol Dial Transplant* **29**, 1168-1177 (2014).

32. Shida, H.*, et al.* The Presence of Anti-Lactoferrin Antibodies in a Subgroup of Eosinophilic Granulomatosis with Polyangiitis Patients and Their Possible Contribution to Enhancement of Neutrophil Extracellular Trap Formation. *Front Immunol* **7**, 636 (2016).

33. Brinkmann, V.*, et al.* Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532-1535 (2004).

34. Grayson, P.C. & Kaplan, M.J. At the Bench: Neutrophil extracellular traps (NETs) highlight novel aspects of innate immune system involvement in autoimmune diseases. *J Leukoc Biol* **99**, 253-264 (2016).

35. Schreiber, A.*, et al.* Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis. *Proc Natl Acad Sci U S A* **114**, E9618-E9625 (2017).

36. Hakkim, A., *et al*. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proc Natl Acad Sci U S A* **107**, 9813-9818 (2010).

37. Wada, N.*, et al.* Prevalence of serum anti-myeloperoxidase antineutrophil cytoplasmic antibodies (MPO-ANCA) in patients with Graves' disease treated with propylthiouracil and thiamazole. *Endocr J* **49**, 329-334 (2002).

38. Nakazawa, D.*, et al.* Abnormal conformation and impaired degradation of propylthiouracil-induced neutrophil extracellular traps: implications of disordered neutrophil extracellular traps in a rat model of myeloperoxidase antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* **64**, 3779-3787 (2012).

39. Lee, E.*, et al.* Inactivation of peroxidases of rat bone marrow by repeated administration of propylthiouracil is accompanied by a change in the heme structure. *Biochem Pharmacol* **37**, 2151-2153 (1988).

40. Sangaletti, S.*, et al.* Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood* **120**, 3007-3018 (2012).

41. Hurtado, P.R.*, et al.* CpG oligodeoxynucleotide stimulates production of anti-neutrophil cytoplasmic antibodies in ANCA associated vasculitis. *BMC Immunol* **9**, 34 (2008).

42. Nakazawa, D. *et al.* Enhanced formation and disordered regulation of NETs in myeloperoxidase-ANCA-associated microscopic polyangiitis. *J Am Soc Nephrol* **25**, 990-997 (2014).

43. Hiwa, R.*, et al.* Myeloperoxidase/HLA Class II Complexes Recognized by Autoantibodies in Microscopic Polyangiitis. *Arthritis Rheumatol* **69**, 2069-2080 (2017).

44. Xiao, H.*, et al.* Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* **110**, 955-963 (2002).

45. Little, M. A. *et al.* Experimental autoimmune vasculitis: an animal model of anti-neutrophil cytoplasmic autoantibody-associated systemic vasculitis. *Am J Pathol* **174**, 1212-1220 (2009).

46. Huugen, D.*, et al.* Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by

bacterial lipopolysaccharide: role of tumor necrosis factor-α. *Am J Pathol* **167**, 47-58 (2005).

47. Kanzaki, G.*, et al.* Impact of anti-glomerular basement membrane antibodies and glomerular neutrophil activation on glomerulonephritis in experimental myeloperoxidase-antineutrophil cytoplasmic antibody vasculitis. *Nephrol Dial Transplant* **31**, 574-585 (2016).

48. Primo, V.C.*, et al.* Anti-PR3 immune responses induce segmental and necrotizing glomerulonephritis. *Clin Exp Immunol* **159**, 327-337 (2010).

49. Little, M.A.*, et al.* Anti-proteinase 3 anti-neutrophil cytoplasm autoantibodies recapitulate systemic vasculitis in mice with a humanized immune system. *PLoS One* **7**, e28626 (2012).

50. Brouwer, E.*, et al.* Antimyeloperoxidase-associated proliferative glomerulonephritis: an animal model. *J Exp Med* **177**, 905-914 (1993).

51. Heeringa, P.*, et al.* Autoantibodies to myeloperoxidase aggravate mild anti-glomerular-basement-membrane-mediated glomerular injury in the rat. *Am J Pathol* **149**, 1695-1706 (1996).

52. Ruth, A.J.*, et al.* Anti-neutrophil cytoplasmic antibodies and effector $CD4^+$ cells play nonredundant roles in anti-myeloperoxidase crescentic glomerulonephritis. *J Am Soc Nephrol* **17**, 1940-1949 (2006).

53. Schreiber, A., Xiao, H., Falk, R.J. & Jennette, J.C. Bone marrow-derived cells are sufficient and necessary targets to mediate glomerulonephritis and vasculitis induced by anti-myeloperoxidase antibodies. *J Am Soc Nephrol* **17**, 3355-3364 (2006).

54. Kusunoki, Y.*, et al.* Peptidylarginine Deiminase Inhibitor Suppresses Neutrophil Extracellular Trap Formation and MPO-ANCA Production. *Front Immunol* **7**, 227 (2016).

55. Kinjoh, K., Kyogoku, M. & Good, R.A. Genetic selection for crescent formation yields mouse strain with rapidly progressive glomerulonephritis and small vessel vasculitis. *Proc Natl Acad Sci U S A* **90**, 3413-3417 (1993).

56. Jennette, J. C. & Falk, R. J. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat Rev Rheumatol* **10**, 463-473 (2014).

57. Falk, R.J., Terrell, R.S., Charles, L.A. & Jennette, J.C. Anti-neutrophil cytoplasmic autoantibodies

induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A* **87**, 4115-4119 (1990).

58. Charles, L.A., Caldas, M.L., Falk, R.J., Terrell, R.S. & Jennette, J.C. Antibodies against granule proteins activate neutrophils in vitro. *J Leukoc Biol* **50**, 539-546 (1991).

59. Kessenbrock, K.*, et al.* Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med* **15**, 623-625 (2009).

60. Kraaij, T.*, et al.* Excessive neutrophil extracellular trap formation in ANCA-associated vasculitis is independent of ANCA. *Kidney Int* (2018).

61. Yoshida, M., Sasaki, M., Sugisaki, K., Yamaguchi, Y. & Yamada, M. Neutrophil extracellular trap components in fibrinoid necrosis of the kidney with myeloperoxidase-ANCA-associated vasculitis. *Clin Kidney J* **6**, 308-312 (2013).

62. O'Sullivan, K.M.*, et al.* Renal participation of myeloperoxidase in antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis. *Kidney Int* **88**, 1030-1046 (2015).

63. Gupta, S. & Kaplan, M.J. The role of neutrophils and NETosis in autoimmune and renal diseases. *Nat Rev Nephrol* **12**, 402-413 (2016).

64. Futamata, E.*, et al.* Vanishing Immunoglobulins: The Formation of Pauci-Immune Lesions in Myeloperoxidase-Antineutrophil Cytoplasmic Antibody-Associated Vasculitis. *Nephron* **138**, 328-330 (2018).

65. Fussner, L.A.*, et al.* Factors Determining the Clinical Utility of Serial Measurements of Antineutrophil Cytoplasmic Antibodies Targeting Proteinase 3. *Arthritis Rheumatol* **68**, 1700-1710 (2016).

66. Fiedler, T.J., Davey, C.A. & Fenna, R.E. X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 A resolution. *J Biol Chem* **275**, 11964-11971 (2000).

67. Suzuki, K.*, et al.* Analysis of risk epitopes of anti-neutrophil antibody MPO-ANCA in vasculitis in Japanese population. *Microbiol Immunol* **51**, 1215-1220 (2007).

68. Gou, S. J., Xu, P. C., Chen, M. & Zhao, M. H. Epitope analysis of anti-myeloperoxidase antibodies in patients with ANCA-associated vasculitis. *PLoS One* **8**, e60530 (2013).

69. Yoshida, M.*, et al.* Two types of myeloperoxidase-antineutrophil cytoplasmic autoantibodies with a high affinity and a low affinity in small vessel vasculitis. *Clin Exp Rheumatol* **27**, S28-32 (2009).

70. Roth, A.J.*, et al.* Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. *J Clin Invest* **123**, 1773-1783 (2013).

71. Flint, S.M., McKinney, E.F. & Smith, K.G. Emerging concepts in the pathogenesis of antineutrophil cytoplasmic antibody-associated vasculitis. *Curr Opin Rheumatol* **27**, 197-203 (2015).

72. Abdulahad, W.H., Lamprecht, P. & Kallenberg, C.G. T-helper cells as new players in ANCA-associated vasculitides. *Arthritis Res Ther* **13**, 236 (2011).

73. Chavele, K.M.*, et al.* Regulation of myeloperoxidase-specific T cell responses during disease remission in antineutrophil cytoplasmic antibody-associated vasculitis: the role of Treg cells and tryptophan degradation. *Arthritis Rheum* **62**, 1539-1548 (2010).

74. Wilde, B., Hoerning, A., Kribben, A., Witzke, O. & Dolff, S. Abnormal expression pattern of the IL-2 receptor β-chain on $CD4^+$ T cells in ANCA-associated vasculitis. *Dis Markers* **2014**, 249846 (2014).

75. Xiao, H., Schreiber, A., Heeringa, P., Falk, R.J. & Jennette, J.C. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* **170**, 52-64 (2007).

76. Xiao, H.*, et al.* C5a receptor (CD88) blockade protects against MPO-ANCA GN. *J Am Soc Nephrol* **25**, 225-231 (2014).

77. Schreiber, A.*, et al.* C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol* **20**, 289-298 (2009).

78. Huang, Y.M., Wang, H., Wang, C., Chen, M. & Zhao, M.H. Promotion of hypercoagulability in antineutrophil cytoplasmic antibody-associated vasculitis by C5a-induced tissue factor-expressing microparticles and neutrophil extracellular traps. *Arthritis Rheumatol* **67**, 2780-2790 (2015).

79. Yuan, J.*, et al.* C5a and its receptors in human anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. *Arthritis Res Ther* **14**, R140 (2012).

80. Gou, S.J., Yuan, J., Chen, M., Yu, F. & Zhao, M.H. Circulating complement activation in patients with

anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int* **83**, 129-137 (2013).

81. Nakazawa, D., Tomaru, U. & Ishizu, A. Possible implication of disordered neutrophil extracellular traps in the pathogenesis of MPO-ANCA-associated vasculitis. *Clin Exp Nephrol* **17**, 631-633 (2013).

82. Kallenberg, C.G. Pathogenesis and treatment of ANCA-associated vasculitides. *Clin Exp Rheumatol* **33**, S11-14 (2015).

83. Nakazawa, D*., et al.* The responses of macrophages in interaction with neutrophils that undergo NETosis. *J Autoimmun* (2015).

84. Nishide, M*., et al.* Semaphorin 4D inhibits neutrophil activation and is involved in the pathogenesis of neutrophil-mediated autoimmune vasculitis. *Ann Rheum Dis* **76**, 1440-1448 (2017).

85. Nishide, M. & Kumanogoh, A. The role of semaphorins in immune responses and autoimmune rheumatic diseases. *Nat Rev Rheumatol* **14**, 19-31 (2018).

86. Wang, H*., et al.* Circulating Level of Neutrophil Extracellular Traps Is Not a Useful Biomarker for Assessing Disease Activity in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis. *PLoS One* **11**, e0148197 (2016).

87. Heeringa, P., Rutgers, A. & Kallenberg, C.G.M. The net effect of ANCA on neutrophil extracellular trap formation. *Kidney Int* **94**, 14-16 (2018).

88. Masuda, S*., et al.* NETosis markers: Quest for specific, objective, and quantitative markers. *Clin Chim Acta* **459**, 89-93 (2016).

89. Kallenberg, C.G., Stegeman, C.A., Abdulahad, W.H. & Heeringa, P. Pathogenesis of ANCA-associated vasculitis: new possibilities for intervention. *Am J Kidney Dis* **62**, 1176-1187 (2013).

90. Kantari, C*., et al.* Proteinase 3, the Wegener autoantigen, is externalized during neutrophil apoptosis: evidence for a functional association with phospholipid scramblase 1 and interference with macrophage phagocytosis. *Blood* **110**, 4086-4095 (2007).

91. Millet, A*., et al.* Proteinase 3 on apoptotic cells disrupts immune silencing in autoimmune vasculitis. *J Clin Invest* **125**, 4107-4121 (2015).

92. Nogueira, E*., et al.* Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in

patients with ANCA-associated vasculitis. *Nephrol Dial Transplant* **25**, 2209-2217 (2010).

93. Lepse, N., Abdulahad, W.H., Kallenberg, C.G. & Heeringa, P. Immune regulatory mechanisms in ANCA-associated vasculitides. *Autoimmun Rev* **11**, 77-83 (2011).

94. Csernok, E.*, et al.* Wegener autoantigen induces maturation of dendritic cells and licenses them for Th1 priming via the protease-activated receptor-2 pathway. *Blood* **107**, 4440-4448 (2006).

95. Pendergraft, W.F., 3rd*, et al.* Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med* **10**, 72-79 (2004).

96. Stegeman, C.A.*, et al.* Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med* **120**, 12-17 (1994).

97. Comarmond, C.*, et al.* Eosinophilic granulomatosis with polyangiitis (Churg-Strauss): clinical characteristics and long-term followup of the 383 patients enrolled in the French Vasculitis Study Group cohort. *Arthritis Rheum* **65**, 270-281 (2013).

98. Bremer, J.P., Csernok, E., Holle, J., Gross, W.L. & Moosig, F. Getting rid of MPO-ANCA: a matter of disease subtype. *Rheumatology (Oxford)* **52**, 752-754 (2013).

99. Khoury, P., Grayson, P.C. & Klion, A.D. Eosinophils in vasculitis: characteristics and roles in pathogenesis. *Nat Rev Rheumatol* **10**, 474-483 (2014).

100. Polzer, K.*, et al.* Eotaxin-3 is involved in Churg-Strauss syndrome--a serum marker closely correlating with disease activity. *Rheumatology (Oxford)* **47**, 804-808 (2008).

101. Vaglio, A., Buzio, C. & Zwerina, J. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss): state of the art. *Allergy* **68**, 261-273 (2013).

102. Sullivan, S., Salapow, M.A., Breen, R. & Broide, D.H. Eosinophil peroxidase differs from neutrophil myeloperoxidase in its ability to bind antineutrophil cytoplasmic antibodies reactive with myeloperoxidase. *Int Arch Allergy Immunol* **105**, 150-154 (1994).

103. Okubo, K.*, et al.* Lactoferrin Suppresses Neutrophil Extracellular Traps Release in Inflammation. *EBioMedicine* **10**, 204-215 (2016).

104. Grau, R.G. Drug-Induced Vasculitis: New Insights and a Changing Lineup of Suspects. *Curr Rheumatol*

Rep **17**, 71 (2015).

105. Olives, T.D., Kornas, R.L., Fujisawa, R. & Cole, J.B. Unexpected Complication of Cocaine-Associated Anti-Neutrophil Cytoplasmic Antibody Vasculitis Related to Persistent In-Hospital Cocaine Use. *J Addict Med* **11**, 157-160 (2017).

106. Lood, C. & Hughes, G.C. Neutrophil extracellular traps as a potential source of autoantigen in cocaine-associated autoimmunity. *Rheumatology (Oxford)* **56**, 638-643 (2017).

107. Irizarry-Caro, J.A.*, et al.* Brief Report: Drugs Implicated in Systemic Autoimmunity Modulate Neutrophil Extracellular Trap Formation. *Arthritis Rheumatol* **70**, 468-474 (2018).

108. Schneeweis, C.*, et al.* Increased levels of BLyS and sVCAM-1 in anti-neutrophil cytoplasmatic antibody (ANCA)-associated vasculitides (AAV). *Clin Exp Rheumatol* **28**, 62-66 (2010).

109. Bader, L., Koldingsnes, W. & Nossent, J. B-lymphocyte activating factor levels are increased in patients with Wegener's granulomatosis and inversely correlated with ANCA titer. *Clin Rheumatol* **29**, 1031-1035 (2010).

110. Lenert, A. & Lenert, P. Current and emerging treatment options for ANCA-associated vasculitis: potential role of belimumab and other BAFF/APRIL targeting agents. *Drug Des Devel Ther* **9**, 333-347 (2015).

111. Bontscho, J.*, et al.* Myeloperoxidase-specific plasma cell depletion by bortezomib protects from anti-neutrophil cytoplasmic autoantibodies-induced glomerulonephritis. *J Am Soc Nephrol* **22**, 336-348 (2011).

112. Novikov, P., Moiseev, S., Bulanov, N. & Shchegoleva, E. Bortezomib in refractory ANCA-associated vasculitis: a new option? *Ann Rheum Dis* **75**, e9 (2016).

113. Langford, C.A.*, et al.* An open-label trial of abatacept (CTLA4-IG) in non-severe relapsing granulomatosis with polyangiitis (Wegener's). *Ann Rheum Dis* **73**, 1376-1379 (2014).

114. Arimura, Y.*, et al.* Serum myeloperoxidase and serum cytokines in anti-myeloperoxidase antibody-associated glomerulonephritis. *Clin Nephrol* **40**, 256-264 (1993).

115. Ohlsson, S., Wieslander, J. & Segelmark, M. Circulating cytokine profile in anti-neutrophilic

cytoplasmatic autoantibody-associated vasculitis: prediction of outcome? *Mediators Inflamm* **13**, 275-283 (2004).

116. McAdoo, S.P. & Pusey, C.D. Is there a role for TNFα blockade in ANCA-associated vasculitis and glomerulonephritis? *Nephrol Dial Transplant* **32**, i80-i88 (2017).

117. Berti, A.*, et al.* Interleukin-6 in ANCA-associated vasculitis: Rationale for successful treatment with tocilizumab. *Semin Arthritis Rheum* **45**, 48-54 (2015).

118. Wechsler, M.E.*, et al.* Mepolizumab or Placebo for Eosinophilic Granulomatosis with Polyangiitis. *N Engl J Med* **376**, 1921-1932 (2017).

119. Chen, M., Jayne, D.R.W. & Zhao, M.H. Complement in ANCA-associated vasculitis: mechanisms and implications for management. *Nat Rev Nephrol* **13**, 359-367 (2017).

120. Huugen, D.*, et al.* Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int* **71**, 646-654 (2007).

121. Jayne, D.R.W.*, et al.* Randomized Trial of C5a Receptor Inhibitor Avacopan in ANCA-Associated Vasculitis. *J Am Soc Nephrol* **28**, 2756-2767 (2017).

122. de Luna, G.*, et al.* Plasma exchanges for the treatment of severe systemic necrotizing vasculitides in clinical daily practice: Data from the French Vasculitis Study Group. *J Autoimmun* **65**, 49-55 (2015).

123. Uechi, E., Okada, M. & Fushimi, K. Effect of plasma exchange on in-hospital mortality in patients with pulmonary hemorrhage secondary to antineutrophil cytoplasmic antibody-associated vasculitis: A propensity-matched analysis using a nationwide administrative database. *PLoS One* **13**, e0196009 (2018).

124. Espinasse, M.A.*, et al.* Glucocorticoid-Induced Leucine Zipper Is Expressed in Human Neutrophils and Promotes Apoptosis through Mcl-1 Down-Regulation. *J Innate Immun* **8**, 81-96 (2016).

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A.I. wrote the manuscript. D.N., S.M. and U.T. helped with researching data and made substantial contributions to discussions of the article content.

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The authors declare no competing interests.

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Key points

- Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a vasculitis that affects systemic small vessels and is accompanied by the presence of ANCA in the serum
- AAV includes microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), and drug-induced AAV.
- AAV can develop in patients with a genetically predisposing background who are exposed to causative environmental factors, such as infectious agents, drugs, and air pollutants.
- ANCAs have a central role in the pathogenesis of AAV because they induce excessive activation of neutrophils, which results in injury to small vessels.
- Other immune cells (such as dendritic cells, macrophages, B cells and T cells), the complement system and humoral factors are also involved in the pathogenesis of AAV.
- Elucidation of the aetiology and pathogenesis of AAV is needed to develop new biomarkers as well as therapeutic agents that target specific molecules involved in this disease.

Figure legends

Figure 1. Neutrophil extracellular traps are found in necrotizing lesions associated with microscopic polyangiitis but not polyarteritis nodosa. a | Formalin-fixed, paraffin-embedded tissue sections of vasculitic lesions from (top) a patient with microscopic polyangiitis (MPA), a disease associated with anti-neutrophil cytoplasmic antibodies (ANCAs); and (bottom) a patient with polyarteritis nodosa (PAN), an ANCA-unrelated disease. **b** | Immunofluorescence images of vasculitic lesions from these patients reveal neutrophil extracellular traps (NETs) in the patient with MPA (top) but not in the patient with PAN (bottom). Left panel, haematoxylin and eosin staining; right panel, immunofluorescent staining for citrullinated histone 3 (a NET marker); 4′,6-diamidino-2-phenylindole (DAPI), which binds strongly to adenine–thymine-rich regions in DNA, commonly used as a DNA

marker; and CD15, a neutrophil marker.

Figure 2. The common pathways in the pathogenesis of AAV. Dendritic cells present antigens, such as those derived from bacteria, to naive T cells, which differentiate into T helper type 17 (T_H17) cells and produce IL-17. Macrophages stimulated by IL-17 produce proinflammatory cytokines, such as tumour necrosis factor (TNF) and IL-1β, which prime neutrophils. At the same time, C5a generated by activation of the alternative complement pathway binds to the C5a receptor on the surface of neutrophils, which also primes neutrophils. Meanwhile, neutrophils stimulated by bacteria form NETs. In patients with low NET degradation activity, these NETs persist, and prolonged exposure to their contents disrupts tolerance to specific self-antigens, notably myeloperoxidase (MPO) and proteinase 3 (PR3). These antigens are presented to $CD4^+$ T cells by dendritic cells, resulting in production of anti-neutrophil cytoplasmic antibodies (ANCAs). Primed neutrophils express MPO and PR3 on their plasma membrane, to which PR3-ANCA and MPO-ANCA bind; at the same time, the Fc region of these ANCAs binds to the Fcγ receptor on neutrophils. This induces excessive activation of neutrophils, leading to abnormal cytokine production accompanied by the release of reactive oxygen species (ROS) and lytic enzymes, and further NET formation, which injure vascular endothelial cells. Humoral factors other than ANCAs, such as damage-associated molecular patterns (DAMPs), are also involved in NET formation in patients with ANCA-associated vasculitis (AAV). The major angiopathic molecules in NETs are MPO, histones dissociated from DNA and matrix metalloproteinases (MMPs) such as MMP2 and MMP9. Both BAFF (B-cell-activating factor, also known as B lymphocyte stimulator (BLyS) and as TNF ligand superfamily member 13B), which is produced by activated neutrophils, and $CD4^+$ T cells (via IL-21) stimulate B cells. This process enables continuous ANCA production. C, complement component; TGF, transforming growth factor.

Figure 3. A vicious cycle of neutrophil extracellular trap and anti-neutrophil cytoplasmic antibody formation is involved in the pathogenesis of AAV. The activity of DNase I in serum is considerably

lower in patients with microscopic polyangiitis (MPA) than in healthy individuals. In consequence, when these patients are exposed to precipitating environmental factors, such as infectious agents or drugs, breakdown of neutrophil extracellular traps (NETs) by DNase I is impaired and the persistence of NET contents *in vivo* results in the production of anti-neutrophil cytoplasmic antibodies (ANCAs). In turn, ANCAs and other humoral factors (such as damage-associated molecular patterns (DAMPs)) induce further NET formation; thus, a NET–ANCA vicious cycle is accomplished.

Table 1. Genes associated with AAV

Gene	Associated disease	Odds ratio	Refs.
HLA-DP	GPA	5.39	12,13
	PR3-AAV	7.03	
HLA-DQ	MPA	0.67	12
	MPO-AAV	0.65	
HLA-DR	MPA	1.56	15
	MPO-AAV	1.57	
PTPN22	PR3-AAV	1.63	16
SERPINA1	GPA	0.54	12
	PR3-AAV	0.53	
PRTN3	GPA	0.78	12
	PR3-AAV	0.73	
SEMA6A	GPA	0.74	13,14

HLA: human leukocyte antigen.

Table 2. Novel therapeutic targets in AAV

Target	Mechanism	Agent	Refs.
B-cells or plasma cells	BAFF antagonist	Blisibimod	110
	Proteasome inhibitor	Bortezomib	112
T cells	CD80 or CD86 Antagonist	Abatacept	113
Cytokines	IL-6-receptor antibody	Tocilizumab	117
	Anti-IL-5 antibody	Mepolizumab	118
Complement system	C5a receptor Antagonist	Avacopan	121
Humoral factors	Plasma exchange	NA	122, 123

NA, not applicable.

Figure 1

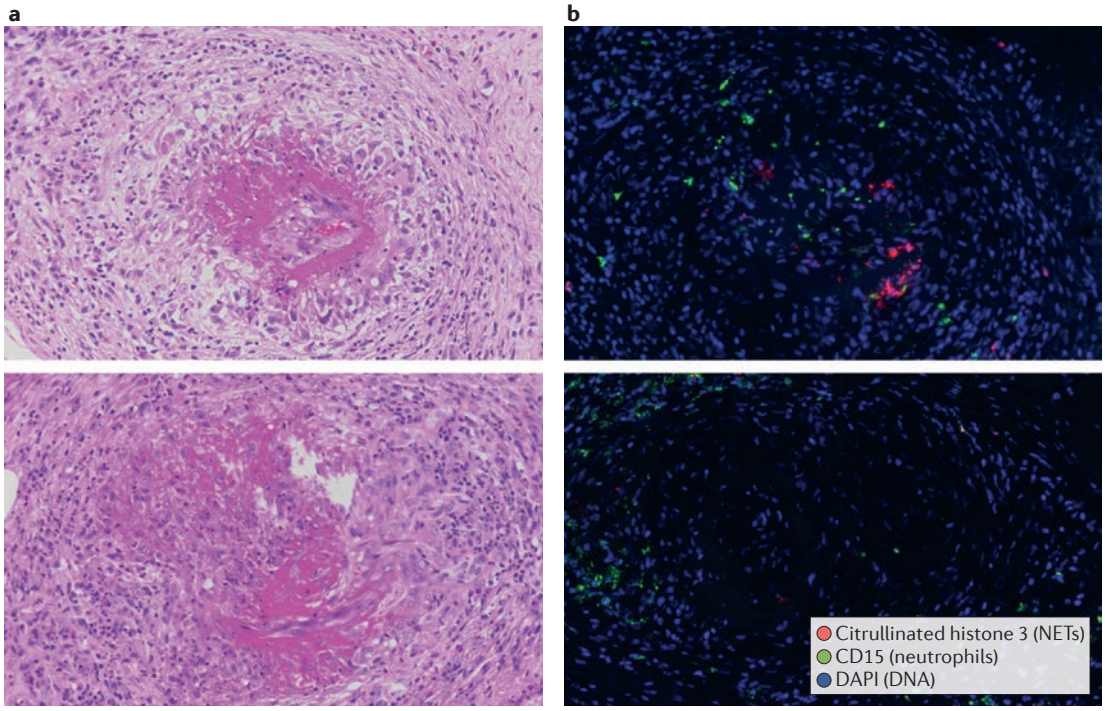


Figure 2

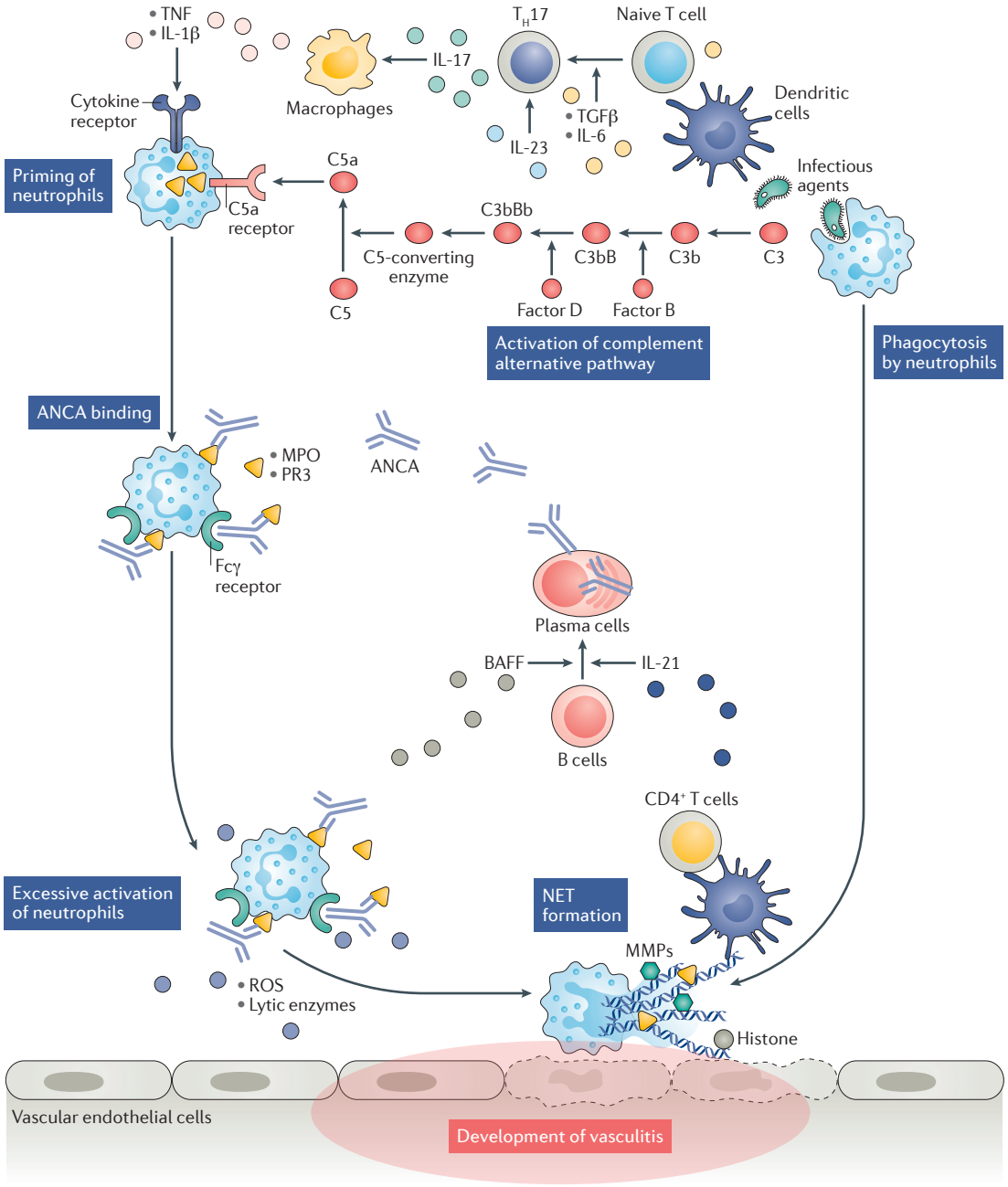
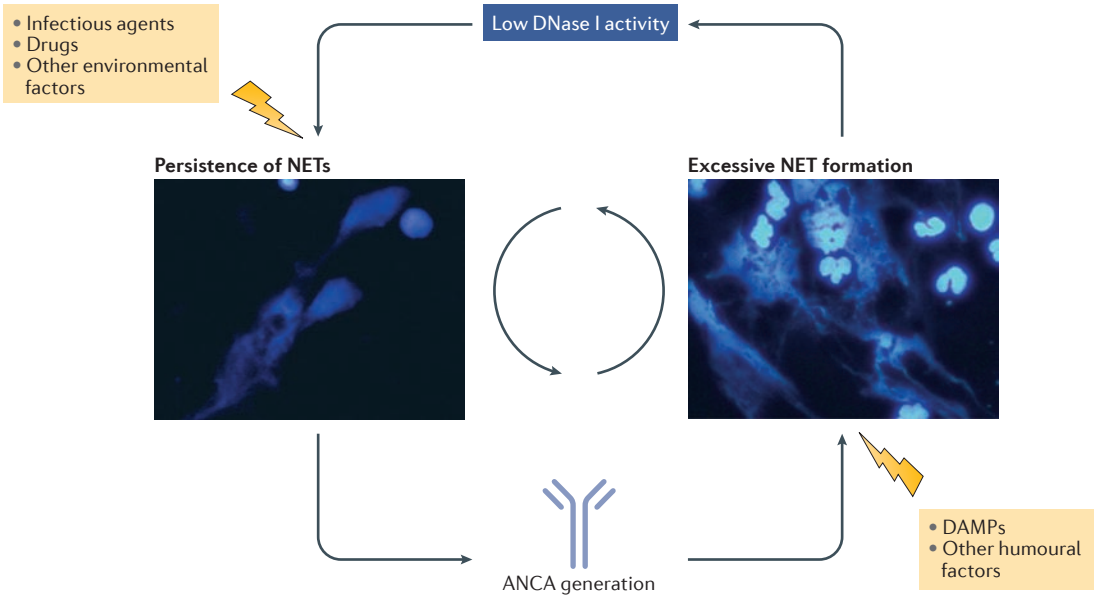

• TNF
• IL-1β
Cytokine receptor
Priming of neutrophils
Macrophages
IL-17
T_H17
Naive T cell
IL-23
• TGFβ
• IL-6
Dendritic cells
Infectious agents
C5a
C5a receptor
C5-converting enzyme
C3bBb
C3bB
C3b
C3
C5
Factor D
Factor B
Activation of complement alternative pathway
Phagocytosis by neutrophils
ANCA binding
• MPO
• PR3
ANCA
Fcγ receptor
Plasma cells
BAFF
IL-21
B cells
$CD4^+$ T cells
Excessive activation of neutrophils
NET formation
MMPs
• ROS
• Lytic enzymes
Histone
Vascular endothelial cells
Development of vasculitis

Figure 3


• Infectious agents
• Drugs
• Other environmental factors
Low DNase I activity
Persistence of NETs
Excessive NET formation
ANCA generation
• DAMPs
• Other humoural factors