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INVITED REVIEW

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The neutrophil: A key resourceful agent in immune-mediated vasculitis

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Summary

The term "vasculitis" refers to a group of rare immune-mediated diseases characterized by the dysregulated immune system attacking blood vessels located in any organ of the body, including the skin, lungs, and kidneys. Vasculitides are classified according to the size of the vessel that is affected. Although this observation is not specific to small-, medium-, or large-vessel vasculitides, patients show a high circulating neutrophil-to-lymphocyte ratio, suggesting the direct or indirect involvement of neutrophils in these diseases. As first responders to infection or inflammation, neutrophils release cytotoxic mediators, including reactive oxygen species, proteases, and neutrophil extracellular traps. If not controlled, this dangerous arsenal can injure the vascular system, which acts as the main transport route for neutrophils, thereby amplifying the initial inflammatory stimulus and the recruitment of immune cells. This review highlights the ability of neutrophils to "set the tone" for immune cells and other cells in the vessel wall. Considering both their long-established and newly described roles, we extend their functions far beyond their direct host-damaging potential. We also review the roles of neutrophils in various types of primary vasculitis, including immune complex vasculitis, anti-neutrophil cytoplasmic antibody-associated vasculitis, polyarteritis nodosa, Kawasaki disease, giant cell arteritis, Takayasu arteritis, and Behçet's disease.

KEYWORDS
neutrophils, vascular damage, vasculitis

1 | INTRODUCTION

Neutrophils are no longer considered to be a highly differentiated and uniform population whose functions are solely confined to antimicrobial activities. This former restricted view of neutrophils as fixed effectors and that lack of plasticity was partly due to their characteristic short lifespan in circulation and very low transcriptional activity.[1–3] However, there has been increasing appreciation of the plasticity of neutrophils with the ongoing categorization of their subsets, including myeloid-derived suppressor cells and low-density neutrophils. Newly recognized neutrophil functions include the ability to communicate and modulate with almost all immune

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cells and cells in the tissues. Furthermore, studies of neutrophil cell death, both apoptotic and non-apoptotic, have revealed remarkable differences with that in other cell types. Over the last decade, the multifaceted functions of neutrophils have been explored, and it is hoped that these insights will pave the way for new approaches in the treatment of vasculitis in which the importance of neutrophils was previously disregarded but now realized.[4]

Neutrophils have largely been described as the archetypal effector of vessel damage in vasculitis, principally due to their ability to generate deleterious mediators such as reactive oxygen species (ROS), granule proteases, and cationic proteins able to damage endothelial cells and alter the permeability of the vessel wall.[5] More recently, neutrophil extracellular traps (NETs) were reported to be increased in the plasma and vascular lesions of patients with vasculitis and may be responsible for the increased thrombotic risk associated with these diseases.[6,7] However, the role of neutrophils cannot be restricted to their host-damaging capacity or to the release of NET, which appears extremely ubiquitous. Rather, the sophisticated immunomodulatory potential of viable neutrophils and ability to "set the tone" for other cells in the vessel wall or immune system should be considered.

The term vasculitis refers to a group of rare immune-mediated diseases characterized by the dysregulated immune system attacking blood vessels located in any organ of the body, including the skin, heart, lungs, and kidneys. A common feature of patients with vasculitis is the high neutrophil-to-lymphocyte ratio (NLR) in circulation. This is predictive of the disease severity and highlights the essential role of neutrophils in these diseases. The exact etiology of many forms of vasculitis remains unclear but it is thought that infection or environmental factors may often be a trigger.[8] Currently, the treatment is non-specific and therefore takes little account of diversity of the pathways activated in most forms of vasculitis. The clinical features and treatment for each type of primary vasculitis discussed here are detailed in Table 1. Although there is a real movement away from this strategy, current global treatment for vasculitis is primarily based on corticosteroid medication, thus silencing any immune response even though the clinical and pathological features differ between types of systemic vasculitis. In addition, the treatment includes other immunosuppressive drugs such as cyclosporin, azathioprine, methotrexate, mycophenolate, and cyclophosphamide. Targeted immune-modulating therapy using anti-cytokine or anti-CD20 depleting B-cells approaches can also be combined with additional drugs treating specific symptoms.[9] In some types of vasculitis, intravenous immunoglobulin therapy (IVIG) and plasma exchange have been successful.[10,11] Considering the recent advances made in neutrophil biology, there is an urgent need to better understand the specific mechanisms through which neutrophils are involved in these diseases, as there is a significant unmet clinical need in this area.[9]

The involvement of neutrophils in immune complex-induced vasculitis has historically been recognized and scrutinized in vasculitis associated with anti-neutrophil cytoplasmic antibodies (ANCAs). In contrast, the importance of neutrophils in other types of vasculitis has been largely neglected, especially in medium- and large-vessel vasculitis, partly because clinicians were not aware of the potential impact of neutrophils in the pathophysiology of these diseases. As vasculitides are named and defined based on immunopathological features and according to the size of the vessel involved (small, medium, or large),[12,13] neutrophil involvement may be considered using the same nosology. Accordingly, we highlight the functions of neutrophils, both well-characterized and recently discovered, in selected immune-mediated vascular diseases to define the range of immunomodulatory roles that can affect vessels and trigger vascular remodeling. We also examine recent discoveries made thanks to available animal models in order to position the respective role of the neutrophil in each form of these prototypical diseases. However, the role of neutrophils in vasculitis associated with systemic autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus (SLE), two diseases in which the role of neutrophils extends beyond its interaction with blood vessels, has been recently reviewed[14–16] and is not described here.

2 | NEUTROPHILS AND VESSELS: AN OLD PARTNERSHIP WITH REMAINING MYSTERIES

As the predominant type of immune cell in the circulation, neutrophils patrol and monitor any signals of danger emitting from acutely damaged tissue. Their migration from the bloodstream to the site of injury is a finely orchestrated stepwise process that has been already thoroughly described.[17,18] Briefly, neutrophils undergo tethering and rolling until they firmly adhere to the luminal vessel wall, transmigrate through the endothelial layer, and ultimately reach the inflammatory site.[18] Once in the tissue, neutrophils fight invading pathogens by deploying defenses such as the secretion of their cytotoxic granular contents, phagocytosis, or their own death. Excessive and unrestrained activation is responsible for the destruction of normal tissue architecture and uncontrolled inflammation.[3]

Neutrophil trafficking is remarkably well coordinated by localized directional cues and factors that induce changes in blood vessels to promote neutrophil vascular attachment and diapedesis. We refer the reader to reviews detailing the molecules involved in neutrophil recruitment.[18,19] These molecules differ in their cellular localization, expression levels, and in the ability of certain cytokines to influence their expression. The disparity of organs affected preferentially in each vasculitis subtype may be related to the differential distribution of adhesion and chemokine molecules.[19,20] In general, chemokines and cytokines are considered to display redundancy for effective elimination of pathogens. In contrast, in the context of leukocyte migration, every chemoattractant and chemokine might play a unique and non-redundant role that contributes to the precise choreography of leukocyte trafficking.[21] Indeed, in vivo murine models of sterile inflammation revealed that chemoattractants collaborate sequentially in temporal and spatial cascades.[22] Any dysregulation in the spatiotemporal expression of this chemokine pattern would

TABLE 1 Clinical features of the different forms of vasculitis

Vessel size	Vasculitis	Possible triggers	Age, sex, ethnicity	Incidence (new cases per year)	Genes	Predilection vessels	Target organs/main symptoms	Histology	Treatment
Small	ANCA-associated vasculitis: MPA, GPA, EGPA	Infectious agents (e.g., nasal carriage of *Staphylococcus aureus* in GPA), drugs, silica dust[83,84]	GPA: northern Europe MPA: northern Europe, Japan, Kuwait[203,224,415]	GPA: 5–13/million MPA: 10.4/million EGPA: 0.14–4/million[203,224,415]	GPA/PR3-AAV: HLA-DP, SERPINA1, PR3, MPA/MPO-AAV: HLA-DQ, HLA-DR[97,98,100,101]	Capillaries, venules, arterioles[104–106]	GPA: upper and lower respiratory tract, necrotizing glomerulonephritis MPA: necrotizing glomerulonephritis, pulmonary capillaritis EGPA: asthma and eosinophilia[88–91,104–106]	GPA: granuloma, vasculitis, parenchymal necrosis EGPA: with eosinophilic infiltrates MPA: no granulomatous inflammation; leukocytoclastic vasculitis[66]	Induction: glucocorticoids and Cyc/RTX (organ−/life-threatening) or MTX/MMF (non-organ-threatening) Maintenance: AZA, MTX, MMF, RTX Novel: anti-C5a-receptor 1[393,395,416]
Small	Immune complex SVV: cryoglobulinemic, anti-GBM, IgA (Henoch-Schönlein purpura), hypocomplementemic	IgA: Infections, drugs, cryoglobulinemic vasculitis, HCV, connective tissue diseases, myeloproliferative disorders[60–64]	IgA: <17 years[417]	IgA: Adults: 1-18/million Children: 20/100000 (4–6 years: 70/100000)[417,418] Anti-GBM: 1.5/Mio[419–421]	IgA: HLA-DRB1[58,59] Anti-GBM: HLA-DRB1*15[45]	IgA: small vessels with skin predominance and gastrointestinal involvement[12]	IgA: purpura, arthralgia/arthritis, abdominal pain, glomerulonephritis[12,65] Anti-GBM: GBM (glomerulonephritis), the alveolar BM (pulmonary hemorrhage), BM in the testis, the inner ear, eye, and the choroid plexus[12,49,52]	IgA: leukocytoclastic vasculitis with IgA deposits[66,67] Anti-GBM: Anti-GBM ab deposits along the GBM[46–48]	IgA: analgesia, selected indications: glucocorticoids, nephritis: ACEI, AZA, MMF, Cyc[68] Anti-GBM: Corticosteroids, Cyc, Plasma exchange[50]
Medium	PAN	Some are HBV-associated[422]	Mean age in adults: 40–50 years Children: 9 years Alaskan Indians (but all HBV-positive)[107,422]	<1.6/million in western Europe[107,422]	Subgroup: ADA2 deficiency[204,210]	Medium vessels (and rarely small vessels, but only vessels with a muscular layer)[12]	Constitutional symptoms, abdominal/testicular pain, myalgias, neuropathies, livedo reticularis/ skin ulcers[202,207,215]	Necrotizing arteritis with mixed cell infiltrate, variable occlusion of the lumen ➔ major organ infarction[66]	Induction: glucocorticoids, Cyc, (antiviral treatment if HBV); Maintenance: MTX, AZA, MMF[423] DADA2: TNFi[205,206]
Medium	Kawasaki disease	Infectious agents suspected, but not yet identified[226,228–232]	<5 years Boys > girls Asia[224]	Europe: 4.5–9/100000 Japan: 360/100000[224,225]	FcGR2A, Casp3, HLA class 2, BLK, IPTKC, CD40[234]	Medium vessels, notably coronary arteries[12]	Fever, rash, cervical lymphadenopathy, conjunctivitis, oral mucosal inflammation[225]	Mixed cell infiltrate with neutrophils at disease onset in coronary artery lesions[66,262]	IVIg, aspirin, IVIg-resistance: glucocorticoids, TNFi, Ciclosporin, IL1-RA[235–239,242]

Vessel size	Vasculitis	Possible triggers	Age, sex, ethnicity	Incidence (new cases per year)	Genes	Predilection vessels	Target organs/main symptoms	Histology	Treatment
Large	TAK	Mycobacterial theory[226,228]	<50 years Women > men Asia > Europe[330]	Japan: 1/million[329,330]	HLA-B52[331,332]	Aorta and its major branches, notably subclavian artery[424]	Claudication of extremities, constitutional symptoms, arterial bruits (subclavian), decreased pulse, BP differences[424]	Skip lesions, lymphoplasmacytic cells, granulomas, giant cell formation[66]	High-dose corticosteroids, MTX, Anti-TNFα, anti-IL6-RA[298,335]
Large	GCA	Various infections such as *Mycoplasma pneumonia* suspected, but unconfirmed[305]	>50 years Caucasians[296]	15–35/100000 in northern Europe[425]	HLA-DRB1*0401, PLG, P4HA2, (TNF, ICAM1, VEGF, TLR4)[306,307,309–312]	Aorta, carotid, and vertebral artery, notably temporal artery[426]	PMR, jaw claudication, headaches, constitutional symptoms, vision disturbances[426]	Transmural lymphohistiocytic infiltration, intima thickening, giant cells, usually not necrotizing[66,315]	Induction: high-dose glucocorticoids Maintenance: Anti-IL6-RA, MTX, other targeted therapies in trials[298–304,335,427]
Variable	Behçet's disease	Oral microorganisms suspected[354]	Onset at 20–40 years Between Mediterranean basin and China[224]	0.05–3.9/100000[224]	HLA-B51[353]	Arteries and veins of all sizes[428]	Oral and genital ulcers, uveitis, arthritis, venous thrombosis, erythema nodosum-like lesions[428]	Mixed neutrophilic and mononuclear cell infiltrations, non-granulomatous[356]	glucocorticoids, AZA, Cyc, ciclosporin-A, INFα, anti-TNFα[355]

Abbreviations: AAV, anti-neutrophil cytoplasmic antibody-associated vasculitis; ACEI, angiotensin-converting enzyme inhibitor; ADA2, adenosine deaminase 2; ANCA, anti-neutrophil cytoplasmic antibody; AZA, azathioprine; BLK, tyrosine-protein kinase; BP, blood pressure; Cyc, cyclophosphamide; DADA2, deficiency of ADA2; EGPA, eosinophilic granulomatosis with polyangiitis; FcGR2A, Fc fragment of IgG receptor 2A; GBM, glomerular basement membrane; GCA, giant cell arteritis; GPA, granulomatosis with polyangiitis; HBV, hepatitis B virus; HCV, hepatitis C virus; HLA, human leukocyte antigen; ICAM1, intracellular adhesion molecule 1; IFN, interferon; IgA, immunoglobulin A; IL1-RA, interleukin-1 receptor antibody; IPTKC, inositol-triphosphate 3-kinase; IVIg, intravenous immunoglobulin; MMF, mycophenolate mofetil; MPA, microscopic polyangiitis; MPO myeloperoxidase; MTX, methotrexate; P4HA2, Prolyl 4-hydroxylase subunit alpha-2; PAN, polyarteritis nodosa; PLG, plasminogen; PMR, polymyalgia rheumatica; PR3, proteinase 3; RTX, rituximab; SERPINA1, serpin family A member 1; SVV, small-vessel vasculitis; TAK, Takayasu arteritis; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFi, TNF inhibitor; VEGF, vascular endothelial growth factor.

therefore presumably affect vasculitis development. Therefore, leukocyte entry into the tissue via the chemokine system represents a major therapeutic target whose blockade may be developed in the treatment of vasculitis. Of note is also the fact that the endothelium can also be the target of the immune system. Whether neutrophils could modulate this process is currently unknown. Anti-endothelial cell antibodies (AECAs) can bind to endothelial cells (ECs) via variable region-specific interactions.[23] AECAs have been described in almost all primary systemic vasculitis diseases but also in many secondary vasculitis diseases, with the identification of various antigens. To date, their pathogenic role is still unclear but there is some evidence showing that they may play a pathogenic role in vasculitis both in vitro and in vivo. AECAs will not be discussed since several reviews have recently underlined their importance in vasculitis.[23,24] Vasculitides are diseases in which the neutrophil-endothelial cell interaction plays a significant role. In addition to functioning as a highway for blood, the vascular system is also the first physical barrier and the primary partner for circulating neutrophils. Deciphering the dialog between neutrophils and vessel walls in all types of vasculitis may improve our therapeutic approach.

To date, the reasons why systemic inflammation results in targeting a specific vascular bed remain unclear.[8] However, some clues may be obtained from the study of Toll-like receptors (TLRs), which are specifically expressed in different vascular territories, suggesting dedication to a selected spectrum of TLR ligands in each vascular region.[25] Interestingly, in atherosclerosis, neutrophils co-localize with TLR2 on endothelial cells to cause endothelial cell stress and apoptosis, an effect that may be abolished by TLR2 deficiency, neutropenia, or blockade of neutrophil adhesion.[20,26,27]

More recent studies showed that neutrophil migration is not only unidirectional, but that neutrophils may also return into the vascular lumen from the inflammatory tissue.[28] The re-entry of infiltrated neutrophils into systemic circulation is achieved by microvascular leakages.[29,30] This new concept, also known as reverse transmigration, may contribute to the dissemination of systemic inflammation, as well as alert and modulate the adaptive immune system. However, regulation of this process in different types of vasculitis has not yet been explored.

Finally, although the interplay between immune and vascular cells has been well studied in atherosclerosis, this has been neglected in different types of vasculitis, especially in large-vessel vasculitis. Indeed, the artery wall comprises different layers that perform different functions and contain different cell types.[31] The intima is the innermost lining layer in which longitudinally oriented endothelial cells are grounded on a connective tissue basal membrane with elastic fibers. The middle layer or media comprises primarily vascular smooth muscle cells (VSMCs) that provide support for the vessel and allow changes in diameter to regulate flow and pressure. The adventitia is the outmost layer that possesses its own vasculature, known as the vasa vasorum, and is composed of matrix proteins. Vascular cells may be affected by inflammatory neutrophils; therefore, it would be useful to decipher these cellular interactions, which may be specific to each type of vasculitis and which may provide novel insights for further therapeutic approaches.

3 | SMALL-VESSEL VASCULITIS (SVV)

Small-vessel vasculitis refers to a particular group of systemic disorders predominantly involving small intraparenchymal arteries, arterioles, capillaries, or venules, leading to different levels of vascular obstruction, tissue ischemia, and infarction. These can be divided into immune complex (IC) vasculitides (IgAV, formerly known as Henoch-Schönlein purpura, cryoglobulinemic vasculitis, anti-glomerular basement membrane disease, and hypocomplementemic urticarial vasculitis) and pauci-immune ANCA-associated vasculitis (granulomatosis with polyangiitis, microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis). Other causes of secondary SVV include drug-induced vasculitis, paraneoplastic vasculitis, and infection-associated vasculitis (hepatitis C) and are not discussed here.

3.1 | IC vasculitis: a misdirected activation of the prototypic and potent Fc receptor

Immune complex small vessel vasculitis (IC-SVV) is an umbrella term for SVV with IC deposits in IgAV, cryoglobulinemic vasculitis, and hypocomplementemic urticarial vasculitis.[32] Anti-glomerular basement membrane (GBM) disease is caused by anti-GBM deposition and thus in situ IC formation that is why it is included in the IC-SVV group.[32] Although the precise etiology of these diseases remains unclear, their common feature is circulating antibody which forms IC in situ to activate neutrophils via binding to Fc receptor (FcR). This prototypic FcR, together with complement receptors, belongs to an "old" family of opsonin receptors that are crucial for the effector response of neutrophils against microorganisms.[33] Opsonization of a microbe with antibodies or complement components is a potent mechanism in their recognition and engulfment, via binding to FcR or complement receptors expressed on the neutrophil surface. Engagement of these receptors triggers their powerful killing mechanisms, including phagocytosis, degranulation, and ROS generation via the NADPH oxidase. Additionally, they can orchestrate the adaptive immune system by secreting cytokines and chemokines. In addition, activation of the complement system is a major inflammatory event, and all complement pathways lead to cleavage of C5 to form C5a and C5b. C5a is involved in the alternative complement pathway and exerts pro-inflammatory effects on both immune and non-immune cells. C5a is thought to be a major neutrophil-activating molecule that promotes proteinase 3 (PR3) expression at the neutrophil surface.[34,35]

Fundamental to an appreciation of the role of neutrophils in IC-induced injury is the principle that the toxic mediators mobilized to destroy microorganisms also play significant roles in tissue damage.

Immunoglobulin binding in situ or IC deposition is a key pathogenic factor in numerous clinical conditions such as glomerulonephritis, immune vasculitis, arthritis, and SLE. It has long been recognized that FcRs cooperate with complement receptors in the binding of soluble immunoglobulin G (IgG) ICs to mediate neutrophil activation.[36] The signaling pathways controlling the activation of FcRs in immune cells and its coordination with activation of the complement receptors (CR3:CD11b/CD18) in IC-mediated diseases are reviewed elsewhere and are not discussed in detail here.[37,38] As numerous reviews of the structure and biology of FcRs in the control of humoral and innate immunity are available, these are not discussed here.[39–42]

However, it is important to highlight a few notions that are essential to understanding this review. First, FcRs belong to the immunoreceptor tyrosine-based activation motif (ITAM)-associated receptor family and can therefore perform both activating and inhibiting functions.[43] Each receptor has specialized functions that are strongly modulated by the inflammatory state. Accordingly, FcR can bind opsonized particles or antibodies as monomeric, aggregated immunoglobulins, or ICs depending on the inflammatory context. Finally, there is functional cooperation between the different FcRs and complement receptor (CR3:CD11b/CD18) that serves to amplify the inflammatory response, which is beneficial in the case of phagocytosis but which may exacerbate the inflammatory process in IC-mediated vasculitis.[44]

Human neutrophils can express three different types of IgG FcR (FcγR), although their respective levels of expression vary depending on the state of activation. Neutrophils express mainly activating FcγR receptor, highlighting the strong potential of this pathway. Under homeostatic conditions, neutrophils express high levels of the low-affinity FcγR CD32 (FcγRIIA) and CD16 (FcγRIIIB), which preferentially bind complexed IgG. The physiological function of the latter is the removal of IC from the vasculature through a non-inflammatory process. Neutrophils poorly express the high-affinity Fc receptor CD64 (FcγRI), which binds monomeric IgG. However, this expression pattern is totally different under inflammatory conditions, as CD64 is strongly upregulated by interferon (IFN)-γ and granulocyte colony-stimulating factor (G-CSF). Subsequently, CD64 ligation by IC or opsonized bacteria may trigger phagocytosis, ROS generation, and antibody-dependent cell cytotoxicity. As mentioned previously, expression of the inhibitory FcγRIIb is very low under basal or inflammatory conditions. Dysregulation of IgG clearance may be involved in IC-mediated vasculitis, highlighting the pathophysiological importance of these pathways in inducing SVV.

Anti-GBM disease is a prototype of autoimmune disease in which the patients develop autoantibodies that bind in situ to the glomerular and pulmonary basement membranes.[45] This anti-GBM will not bind other vascular beds. IC formed in situ activate the classical pathway of the complement system, thus triggering neutrophil-dependent inflammation.[46] Clinically, anti-GBM is characterized by rapid progressive glomerulonephritis and pulmonary hemorrhage.[12] The eponym "Goodpasture's syndrome" refers to the combined pulmonary and renal vasculitis due to anti-GBM antibody deposition. Given the frequent involvement of alveolar basement membranes, the term anti-GBM disease actually is a misnomer.[13,47] Patients present antibodies against epitopes on the α3 chain of type IV collagen,[48] which is expressed in basement membranes in the glomeruli, alveoli, testis, inner ear, eye, and choroid plexus.[49] Observations of antibodies deposited along the GBM are the gold-standard diagnostic feature.[47] Renal biopsies show crescent formations which is often of similar ages in different glomeruli. Interestingly, a subset of patients presents an overlap with ANCA-vasculitis. This double positivity for anti-GBM and ANCA is observed in 21–47% of patients,[50] who have a greater risk of relapse, but a higher rate of renal recovery.[51] The antibodies are directed more often towards myeloperoxidase (MPO) than proteinase 3 (PR3).[52] These findings suggest that neutrophils display effector functions in anti-GBM disease resembling that seen in other IC diseases. However, in anti-GBM and ANCA double-positive patients neutrophils are both effector and target of the autoimmunity, similarly to ANCA-associated vasculitis as discussed below in more detail. Despite the immunosuppressive treatment, mortality remains very high (9–36%) and less than one-third of patients only retain kidney function.[50]

Among the experimental models developed to study the pathogenesis of antibody-mediated glomerulonephritis, the most extensively studied is that of anti-GBM nephritis induced by injection of heterologous anti-GBM antibodies.[53,54] IC deposits under the fenestrated endothelium are easily accessible to circulating cells. It has therefore been suggested that the initial accumulation of neutrophils may be driven by neutrophil FcR engagement with immobilized ICs in the glomerular capillary walls.[55] The putative role of the complement in the pathogenesis of the heterologous phase of anti-GBM disease is suggested by the deposition of complement components in a distribution matching that of the antibody.[56] The use of an oral C5a receptor antagonist is under investigation to reduce corticosteroid reliance. The critical role of FcR-mediated damage in anti-GBM disease is exemplified by the early benefit of using imlifidase (IdeS endoprotease), a streptococcal enzyme that cleaves immunoglobulin, leaving the Fab fragment bound to the target antigen, but without the Fc portion, making neutrophil engagement and inflammation impossible.[57]

Immunoglobulin A (IgA) is the most abundant antibody at mucosal sites and performs multiple functions in homeostasis and immunity. Neutrophils and IgA can interact via the IgA FcR (FcαRI or CD89), leading to pro- or anti-inflammatory responses. Crosslinking of FcαRI by monomeric IgA has an inhibitory effect, whereas IgA-ICs result in potent neutrophil activation and perform pro-inflammatory effector functions, including neutrophil recruitment. This may lead to neutrophil accumulation and tissue destruction during IgA-autoantibody-mediated vasculitis.[41] IgAV is the most common form of vasculitis in childhood and frequently involves joints, kidneys, the gastrointestinal tract, and skin. IgAV is thought to be associated with the human leukocyte antigen (HLA) class 2 region HLA-DRB1, as supported by a genome-wide association study.[58] An association between interleukin (IL)-1 receptor antagonist allele 2 (ILRN*2) and renal involvement has also been shown.[59] As IgA is produced in response to external stimuli, many bacteria and viruses as well as drugs

are thought to act as triggers for IgAV[60] and a history of infection is reported in 30–63% of cases.[61–63] Although vaccination has been proposed as a trigger for IgAV, a recent study could not confirm this as a major etiological factor in childhood IgAV.[64]

IgAV is characterized by IgA1 immune deposits and neutrophil infiltrates damaging the small vessels. It is clinically characterized by palpable purpura, polyarthralgia or arthritis, abdominal pain, and renal involvement (glomerulonephritis).[12,65] Histologically, leukoclasia and ICs may be observed in skin biopsies, as well as fibrinoid necrosis of the small vessels. In early lesions, neutrophils are the predominant cell type and vascular damage can be unapparent in contrast to late lesions in which fibrinoid vascular changes and lymphocytes can be observed and immunofluorescence shows the presence of IgG.[66,67] Treatment of IgAV includes glucocorticoids and immunosuppressants for organ- or life-threatening vasculitis manifestations.[68] For IgAV nephritis, angiotensin-converting enzyme inhibitors and immunosuppressive agents may be used depending on the severity of the disease.

Although the antigen-recognition sites of IgA1 in IgAV have not yet been identified, it is proposed that these antibodies recognize epitopes on endothelial cells as binding of IgA1 antibodies to endothelial cells induces the release of IL-8, thereby promoting neutrophil recruitment.[69] Abnormal glycosylation of the hinge region of IgA1 is suggested to cause aggregation to form macromolecular ICs.[70] Because FcαRI-mediated cross-linking of neutrophils in vitro induces inflammatory processes such as ROS production and the release of NETs and leukotriene B4,[71,72] it is hypothesized that FcαRI-mediated activation of neutrophils results in vessel damage and leakage of red blood cells into the skin, causing typical cutaneous hemorrhage. More recently, IgA was shown to trigger neutrophil death when primed with inflammatory mediators in phosphoinositide 3-kinase (PI3K)-, p38 mitogen-activated protein kinase (MAPK)-, and c-Jun N-terminal kinase (JNK)-dependent pathways.[73]

FcαRI (CD89) has been shown to be lower in the urine of children with active IgAV, suggesting that the deposits remain in the kidneys and are not excreted.[74,75] In skin biopsies of patients with IC-SVV such as IgAV, NETs were present in the early stages of the disease and were significantly more abundant than in patients with urticarial vasculitis.[76] Their abundance was also correlated with the production of ROS. The presence of NETs was furthermore confirmed in renal and gastrointestinal tissues obtained from patients with IgAV but not from controls.[77] The same study also showed that the amount of circulating free DNA was higher in the plasma of patients with IgAV than that of controls and was correlated with the amount of MPO-DNA and neutrophil elastase. Another study has shown that the plasma of patients with IgAV contains high levels of MPO, which is secreted by activated neutrophils and endothelial cells and forms hypochlorous acid ($HOCl^-$), thus amplifying oxidative stress and promoting damage to endothelial cells.[78] Regarding ICs and NET release, there are few in vitro studies of murine and human neutrophils releasing NETs in response to preformed ICs with conflicting results reported concerning FcγR involvement.[79–81]

3.2 | ANCA-associated vasculitis: a neutrophil-targeted auto-immune disease

Neutrophils are key players in the pathophysiology of ANCA-associated vasculitis (AAV), as they are both the target of autoimmunity and effector cells responsible for endothelial damage.[82] AAV includes granulomatosis with polyangiitis (GPA), previously called Wegener's granulomatosis, microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA), previously called Churg-Strauss syndrome. Infectious agents, drugs, and silica dust have been postulated as potential triggers for AAV.[83,84] GPA is characterized histologically by necrotizing vasculitis and extravascular granulomatosis. The main difference in EGPA is the involvement of eosinophils. Granuloma are not observed in MPA.[66,85]

The two main target antigens of ANCAs are the neutrophil granule proteins PR3 and MPO, both also expressed in monocytes.[86] Anti-PR3 ANCAs are found in sera from more than 90% of patients with GPA, which is characterized by granulomatous inflammation of the upper or lower respiratory tract as well as pauci-immune glomerulonephritis. The unique feature of anti-PR3 AAV is the formation of granuloma, which may be related to PR3 functions and respiratory barrier dysfunction.[87] Anti-MPO ANCAs are present in sera from 60–70% of patients with MPA and less frequently in those with EGPA (30–40% of patients), which associates with late-onset asthma, hypereosinophilia, and SVV. In the latter, although neutrophils are present in lesions, abundance of eosinophils is the hallmark of this type of vasculitis as described in recent reviews.[88–91] The selectivity and exclusivity of ANCA target antigens (either PR3 or MPO) is surprising considering the wide range of proteins stored within the cytosolic granules of neutrophils. Although the respective functions of PR3 and MPO are extremely different, the pathophysiological mechanisms of AAV share similarities for both anti-PR3 and anti-MPO ANCA,[92] as both are able to bind and activate neutrophils, which is a key feature of this pathophysiology.[93,94] Although neutrophils are equally important in GPA and MPA, further studies suggest that these are different clinical entities with different underlying mechanisms depending on whether the target antigen is MPO or PR3.[95,96]

Genetic variants are more strongly associated with the ANCA serotype than with the clinical diagnosis and may be major histocompatibility complex (MHC)- or non-MHC-associated.[97,98] Both GPA and MPA show specific HLA dependence-implicating autoreactive T cells as being critical. The association of anti-PR3 ANCA positivity with genes coding for HLA-DP, PR3 itself, and its inhibitor, SERPINA1 (also called alpha-1-antitrypsin) may relate to the levels of target antigen on the neutrophils and the ability of alpha-1 antitrypsin to regulate these levels.[99] In contrast, no direct association with MPO-related genes was found in anti-MPO-associated vasculitis.[100] Recently, the association between risk allele HLA-DPB1*04:01 and PR3-ANCA was further confirmed by functional studies showing that binding between HLA-DPB1*04:01 and $PR3_{225-239}$ could initiate an immune response.[101] Accordingly, the new vasculitis classification criteria published this year corroborate this notion as the

presence of ANCAs was given greater importance than in the former classification criteria of 1990.[12,102-107]

3.2.1 | MPO and PR3 as autoantigens

Both MPO and PR3 are granule proteins whose primary store is within azurophil granules. The distinct biochemical properties of MPO and PR3 are exploited empirically in the indirect immunofluorescence test for ANCAs. Perinuclear and cytoplasmic fluorescence is observed with anti-MPO and anti-PR3 ANCAs, respectively. Ethanol treatment used to fix and permeabilize isolated neutrophils before performing indirect immunofluorescence triggers an artefactual translocation of MPO to the nuclear membrane whereas PR3, which is anchored to the granule membrane via a strong hydrophobic patch,[108,109] remains in place within the granule. Importantly, these two types of antibodies are mutually exclusive and very rarely detected in the same patient, except following polyclonal B cell stimulation with drugs such as hydralazine, carbimazole, or cocaine.[110]

Because MPO and PR3 are cationic, as are most granular proteins, they are associated with NET structure. Indeed, the formation of NETs, which are composed of DNA expelled by dying neutrophils, has been described in situ within glomerulonephritis lesions in AAV.[111] However, the formation of NETs is reported in other autoimmune diseases such as SLE, as well as in almost all other inflammatory diseases.[79] It is therefore questionable whether such a ubiquitous mechanism could explain the tight specificity of the autoimmune mechanisms observed in MPA and GPA characterized by ANCAs directed only against MPO and PR3, respectively. Nevertheless, there is undoubtedly an association between NETs released by neutrophil lysis and inflammation intensity in all neutrophil-associated diseases, including AAV. Specificity likely could be related to particular HLA type presenting oxidatively modified antigen to T cells.[112]

Both MPO, a powerful oxidant-generating protein, and PR3, a neutral serine protease, share proinflammatory properties,[113-115] because they are able to modify proteins by oxidation or cleavage, respectively. They can modulate the inflammatory process during which they may perform synergistic activities. PR3 and MPO are both involved in the microbicidal activities of neutrophil.[33] However, their structures and functions differ dramatically. As a key player of the intracellular microbicide oxygen-dependent system,[116] MPO may be targeted by the immune system partly due to its high abundance (up to 5% dry weight). MPO produces HOCl from H_2O_2 and Cl^-, which exerts cytotoxic effects on both microorganisms (bacteria, fungi, and parasites) and host cells. Furthermore, HOCl also reacts with endogenous amines (R-NH2) to generate chloramines (SHR-Cl), also called long-lived oxidants in opposition to free oxygen radicals whose lifespan is no more than one second. HOCl oxidizes a wide variety of molecules including plasma proteins and generates advanced oxidized protein products that possess proinflammatory activities and can act as neoantigen.[117] The role of MPO in inflammatory mechanisms occurring in the absence of infection has also been investigated. Indeed, MPO has been detected in atherosclerotic plaques[118] and may oxidize low-density lipoproteins and proteins in vascular extracellular matrix (ECM).[119] These results suggest that MPO may be considered a key element for atherogenesis. Moreover, patients with AAV present accelerated atherosclerosis as well as an increased ability to develop cardiovascular disease.[120]

It is generally admitted that MPO translocates to the cell surface during neutrophil degranulation and may bind to the cell membrane (of either neutrophils or endothelial cells) via cationic interactions. MPO may be transferred to endothelial cells ("planted antigen"), thereby allowing ANCA binding and amplification of the inflammatory process.[121] MPO may bind to platelets,[122] fibroblasts,[123] and macrophages,[124] thereby contributing to tissue injury. In addition, active MPO has been described in microparticles released by activated neutrophils, thereby contributing to the endothelial cell damage observed in vasculitis.[125]

PR3 differs from MPO in many aspects and is much less abundant than MPO. PR3 has been cloned both as a granular serine proteinase[126] and as a protein involved in granulocytic differentiation, a process in which it is known as myeloblastin.[127] In fact, inhibition of PR3/myeloblastin expression at the promyelocytic stage triggers the differentiation of these cells[127] and is involved in myeloid leukemia.[128] In contrast to the unique biological activity of MPO, which is the only enzyme able to generate chlorinated oxidants at physiologic pH, PR3 has homologous proteins called serprocidins. Antibiotic serine proteinases, namely elastase, azurocidin, and cathepsin G, are also stored in azurophilic granules.[114] Interestingly, elastase, which shares 56% of homology with PR3, is not a specific target of ANCAs. Indeed, the enzymatic specificities of PR3 and neutrophil elastase are very similar, but not identical, which allows the possibility of developing inhibitors specific for each serine protease.[129]

Several studies in animal models have demonstrated that serine proteinases exert pro-inflammatory effects.[130,131] These animal models include mice that are genetically deficient in elastase or cathepsin G, double-deficient in elastase and PR3, or deficient in dipeptidyl peptidase enzyme, an enzyme essential for serine protease maturation. Likewise, a model of vasculitis associated with anti-MPO antibodies revealed the injurious role of neutrophil serine proteases.[132] PR3 may exert pro-inflammatory activities by cleaving anti-inflammatory proteins such as annexin-A1,[133] by activating cytokine pro-forms such as pro-IL-1β,[134,135] or by inducing kinin pathway activation via kininogen cleavage.[136]

Besides its enzymatic activity, the structural features of PR3 distinguish it from other neutrophil serine proteases, especially its capacity to anchor to the neutrophil plasma membrane in the absence of activation.[108,129] Furthermore, PR3 shows bimodal surface protein expression and a high proportion of neutrophils expressing PR3 on their surface is a risk factor for the development of vasculitis in patients.[137] We showed in a study of two representative families that neutrophil surface expression of PR3 is genetically determined,[137] which was confirmed in a study of twins.[138] Unexpectedly, we showed that PR3 was present in the secretory vesicles, an easily mobilizable neutrophil compartment that increases membrane

receptor expression on neutrophils. Considering this last observation, PR3 should not formally follow the rules of compartmentalization "in use" in the neutrophil,[139] therefore opening new fields of investigation based on membrane PR3 and particularly its partners and functions, which have been reviewed elsewhere.[140]

The membrane protein CD177, also called NB1,[141,142] has a glycosylphosphatidylinositol (GPI) anchor and comparable bimodal expression on human neutrophils.[143] CD177 is implicated in neutrophil migration and has been suggested to act as a receptor for PR3. However, the mechanism by which PR3 may be released and then retained at the neutrophil surface in the absence of activation remains unclear. Several research groups showed PR3 localization in membrane microdomains called lipid rafts,[144] which may explain its binding to the membrane. Unlike its homologs, PR3 may fit into lipid vesicles. In addition, molecular modeling studies using dynamic simulation demonstrated that PR3 binds to neutral and anionic membranes via a hydrophobic patch comprising four hydrophobic amino acids.[108,109] This is not the case for elastase. These four hydrophobic amino acids are essential for PR3 expression at the membrane[109] and likely also for CD177 binding.[145] Interestingly, recent studies showed that a competing peptide and alpha-1 antitrypsin could disrupt the interaction between PR3 and CD177 and that this could inhibit anti-PR3-induced activation, thus highlighting the importance of the PR3-CD177 complex.[99,146] Importantly, we have shown that PR3 can be expressed in the membrane of microvesicles and that these PR3-expressing vesicles could enhance the activity of NADPH oxidase in bystander neutrophils at the site of inflammation to further promote inflammation.[147]

3.2.2 | Neutrophil function and their modulation by ANCAs

The accessibility of the autoantigen (PR3 or MPO) to ANCAs is a mechanistic prerequisite for cell activation by the ANCA. Using intravital microscopy techniques, ANCAs have been shown to convert rolling neutrophils to firm stationary adhesion.[148,149] Vascular permeability leads to the subendothelial edema observed in AAV. The mechanisms affecting the complex sequence of steps regulating transmigration in patients with AAV remains unclear and this knowledge would contribute to understanding vascular damage. Studies have shown that endothelial cell apoptosis is promoted by neutrophil proteases.[150] In addition, patients show large numbers of circulating endothelial cells, while remission is associated with decreasing numbers.[151] Because the antigen is intracellular, measurement of neutrophil responses to ANCAs in vitro requires a priming step with tumor necrosis factor (TNF)α and the use of cytochalasin B, presumably to mimic neutrophil adhesion and induce translocation of the antigen to the membrane. Several studies have shown that both anti-MPO and anti-PR3 ANCAs could activate NADPH oxidase and ROS generation in human neutrophils in vitro.[152] Numerous studies have described the double engagement of ANCAs with FcγR and the antigen (MPO or PR3) at the neutrophil surface to induce a functional response.[153] Once ANCAs have bound to their neutrophil-expressed antigens, signaling and activation are initiated. Both the antigen-binding and Fc parts are needed. ANCA IgG bind to FcγRIIA (CD32A) and FcγRIIIB (CD16B). FcγRIIA blockade was shown to abrogate ANCA-induced activation,[154] whereas the role of the FcγRIIIB blockade is somewhat more controversial. Intracellular signaling of Fc receptors has been discussed here with regard to IC-mediated vasculitis.[39,40] Furthermore, the integrin and complement receptor CR3 (CD11b/CD18) was shown to be required for a complete response.[155] Oxidative activity in response to anti-PR3 ANCAs is increased in neutrophils expressing membrane PR3 compared to those not expressing PR3.[138]

Despite numerous reports showing that anti-MPO or anti-PR3 ANCAs can trigger a robust respiratory burst, overactivity of NADPH oxidase in neutrophils or monocytes is not observed in patients with GPA or MPA.[156] Even more surprisingly, ROS production and disease severity are negatively associated, consistent with findings in other autoimmune diseases such as SLE.[157] This was also observed in an anti-MPO murine model in which the transplantation of NADPH oxidase (Phox) protein-deficient bone marrow (gp91phox- and p47phox-) accelerated disease.[158] Furthermore, in vitro challenge with anti-MPO antibodies from gp91phox- and gp47phox-deficient monocytes enhanced caspase-1 activity and IL-1β generation, thus suggesting that NADPH oxidase restrains the inflammasome by downregulating caspase-1. Whether the cross-talk between NADPH oxidase and the inflammasome is relevant in patient neutrophils will require further investigations.

ANCAs have been shown to induce degranulation and NET release under various experimental settings. Most studies report NET release associated with neutrophil death after stimulation with phorbol myristate acetate, which may not be relevant to physiological settings. In vivo, patients with AAV show higher levels of circulating NET remnants as well as free MPO during active disease, which is indicative of neutrophil degranulation, compared to patients in remission and to healthy controls.[82,159,160]

In addition to death-inducing NET release, activated viable neutrophils can also produce NETs, which contain mitochondrial DNA able to bind granule proteins and microorganisms. The formation of NETs requires the availability of increased amounts of adenosine triphosphate (ATP) as it is an active, and therefore energy-dependent, cellular process.[161] This NET release is part of the activation and communication process during physiological neutrophil activation. Some studies have reported that NETs from ANCA-stimulated neutrophils can cause endothelial damage *in vitro*[162] and can thus activate the alternative complement pathway, which plays a role in amplifying the inflammatory process in AAV in a vicious circle involving neutrophil recruitment and activation.[163–166] NETs are also found in the thrombi and in glomeruli of patients with AAV and are thought to contribute to thrombosis by expressing tissue factor.[167,168]

The pathogenic role of anti-MPO was demonstrated in vivo 20 years after the description of ANCAs. The transfer of purified

serum IgG containing anti-MPO antibodies or spleen cells from mice immunized with murine MPO into *recombinase-associated gene type-2*-deficient mice that have no B-cells, T-cells, or antibodies, resulted in the occurrence of extracapillary glomerulonephritis.[160] This demonstrated the pathogenic role of anti-MPO ANCAs in vivo as well as the involvement of different signaling pathways, including the alternative complement pathway involving C5a, in the disease. In human pathology, the occurrence of pulmonary-renal syndrome in the newborn of a mother with anti-MPO ANCA vasculitis confirmed the in vivo pathogenic role of these antibodies in humans.[164] Alternatively, one study points to the fact that there are dominant pathogenic epitopes for anti-MPO ANCAs and that the non-detection of ANCAs in patients with MPA may result from the fact that anti-MPO ANCAs were associated with a fragment of ceruloplasmin, a natural inhibitor of MPO, and were eliminated during purification of IgG from serum.[169] In contrast, the pathogenicity of anti-PR3 ANCAs in vivo remains to be proven unequivocally.

Since their discovery, ANCAs and clinical correlates have been studied extensively.[92] Although they are a useful diagnostic tool, using ANCAs as predictive biomarkers for relapse is questionable. First, there is a small (5–10%) proportion of patients with biopsy-proven MPA or GPA that have no detectable ANCAs.[170]Second, serum ANCA detection may reflect disease activity in some but not all patients with AAV. These observations raise questions about the pathogenicity of anti-PR3 ANCA in vivo. A study showed that more than 20 epitopes of MPO were recognized by anti-MPO ANCAs in patients with AAV.[169] Interestingly, in this study, anti-MPO IgG ANCAs from patients with active AAV recognized a large number of epitopes, including epitopes that were never recognized by anti-MPO IgG ANCAs from patients with inactive disease and from healthy controls. Some patients with clinical and pathological features of AAV who were negative for ANCAs using conventional clinical assays reacted to a specific MPO epitope. IgG ANCA binding to this MPO epitope was blocked by a ceruloplasmin fragment and bound to the portion of the MPO molecule containing this epitope. This suggests that the epitope specificity of ANCAs may influence their biological activity in a manner that is independent of ANCA titers.

In accordance with this concept, anti-PR3 antibodies can exert a dual biological effect depending on their epitope specificity, thus raising the possibility of "good" and "bad" anti-PR3 ANCAs. The former would limit inflammation, whereas the latter would potentiate it. A similar concept has been proposed with regard to the existence of altered Fc N-glycosylation patterns of IgG, as agalactosylation is proposed to be proinflammatory and galactosylation and sialylation of IgG antibodies are proposed to be anti-inflammatory.[171] Agalactosylation of total IgG is associated with AAV and sialylation of anti-PR3 antibodies is inversely correlated with disease activity.[172,173] Another research group investigated the value of IgG galactosylation and sialylation levels as prognostic markers for AAV relapse and showed an inverse correlation with total IgG1 only.[174] In situ, the number of activated neutrophils in renal biopsies from patients with PR3-ANCA glomerulonephritis correlates with ANCA titer and renal damage,[82,175] and MPO deposition correlates with the extent of renal dysfunction.[176]

Gene expression profiling studies on peripheral blood leukocytes identified more than 200 genes overexpressed in neutrophils from patients with AAV.[177] More recently, transcriptomic analysis demonstrated that neutrophil degranulation may be used to define different disease endotypes in small- and medium-vessel vasculitides.[178] Interestingly, synthesis of the autoantigens MPO and PR3 was shown to be disturbed in neutrophils from patients with AAV at the epigenetic level, showing that the chromatin modification H3K27me3, which is associated with gene silencing, was depleted at PR3 and MPO loci in patients with ANCA compared with healthy controls.[179] Interestingly, this DNA methylation abnormality was associated with disease severity.[180] This is even more complex in the case of PR3 as the transcriptional dysregulation reported in patient neutrophils resulted in a variety of mRNA processing events from the PR3 gene locus. In addition, leukocyte RNA from patients contained PR3 transcripts with an alternative 3′ untranslated region leading to the synthesis of different forms of PR3.[181] This newly synthesized PR3 in mature neutrophils may have different functions and localization, as targeting of granules only occurs at the promyelocytic stage during differentiation.[182]

Several studies have shown that the spontaneous apoptosis of neutrophils was delayed in patients with AAV.[183] A global proteomic analysis performed on the cytosol of neutrophils isolated from patients with GPA and healthy controls showed that the proteomes of controls and patients (even those in remission) were very different.[184] These results suggest that treatment may not completely resolve the intrinsic defects participating in the pathogenesis of the disease. Interestingly, this difference was more noticeable under apoptosis than in a basal state and many of the proteins identified are involved in cell survival/death pathways such as annexin A1, a phospholipid-binding protein crucial in apoptotic cell clearance and associated with PR3. The correlation was stronger in patients with severe disease such as that with renal involvement. This differential expression of annexin A1 was also correlated with phospholipid scramblase 1 (PLSCR1) in an apoptotic state. Therefore, in the context of inflammation, a delay in the phagocytosis of apoptotic cells may play a crucial role in the development of autoimmune manifestations.

Interestingly, there is a subpopulation of granulocytes in peripheral blood that has recently attracted growing interest: the low-density neutrophils (LDNs). These have been well characterized in autoimmune diseases such as rheumatoid arthritis or SLE[185] and in G-CSF-treated donors.[186] Although the clear identification and role of LDNs remain to be established, their function appears to vary greatly according to the pathological condition.[187] LDNs are reportedly expanded in patients with acute AAV, but they are hyporesponsive to MPO antibodies when compared to normal-density neutrophils.[188,189] They have been associated with disease activity and show NET formation in vitro.[190] However, their importance and function in disease pathogenesis are yet to be determined.

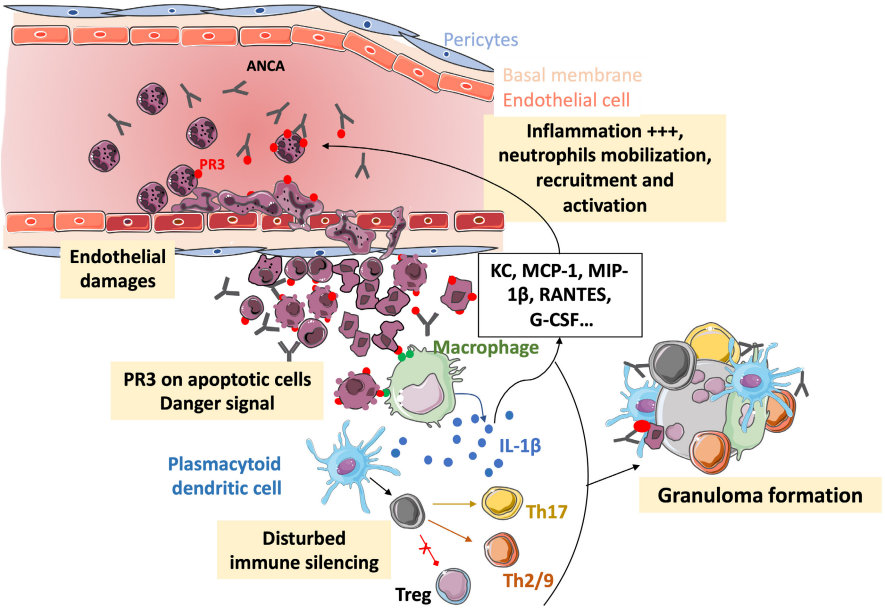

FIGURE 1 Defective resolution of inflammation observed in anti-PR3-associated vasculitis. Under normal conditions, neutrophils adhere weakly to endothelial cells and roll along the vessel wall. Pathogenesis begins with an infection that preactivates neutrophils via the release of pro-inflammatory cytokines, leading to the expression of integrins and allowing adhesion to the endothelium. This also promotes the increased expression of ANCA autoantigens (proteinase 3 [PR3] or myeloperoxidase [MPO]) on the surface of neutrophils, making them accessible to circulating ANCAs. Activated neutrophils then degrade the endothelium by releasing reactive oxygen species and proteases. Antigen–antibody complexes may be formed locally with ANCAs amplifying the inflammatory reaction and the recruitment of neutrophils. Membrane PR3-expressing neutrophils show a defect in apoptosis regulation as well as in their phagocytosis by macrophages. PR3 expressed at the membrane of apoptotic neutrophils acts as a danger signal thereby inducing macrophage activation. These two mechanisms result in the persistence of apoptotic and/or necrotic neutrophils at the inflammatory site and in the disruption of the immune silencing normally induced by the phagocytosis of apoptotic cells through the activation of plasmacytoid dendritic cells (pDC). This defect in the resolution of inflammation can promote autoimmunity evidenced by an imbalance in different T cell sub-populations, in particular a decrease in regulatory T cells and a disturbed activity of T-helper 17 (TH17) lymphocytes. In this inflammatory context, neutrophils might dialog with activated B and T lymphocytes, macrophages, and dendritic cells to favor granuloma formation.

3.2.3 | Disturbance of death pathways in GPA: a PR3-driven process

In addition to delayed apoptosis, apoptotic neutrophils from patients with GPA show increased expression of PR3, which interferes with phagocytosis by macrophages and inhibits the resolution of inflammation. The engulfment of apoptotic neutrophils results in the reprogramming of the engulfing cells, which is a key step in limiting their activation and restoring tissue homeostasis[191] and during inflammation.[192] If neutrophils fail to undergo apoptosis or are not cleared properly, inflammation prevails and can proceed to a deleterious and damaging chronic phase that may lead to progressive autoimmune disorders.[193–195] This scenario is likely to occur in the context of GPA, which is an example of unresolved inflammation partly due to the delayed apoptosis observed in neutrophils combined with the increased expression of PR3 at the surface of apoptotic neutrophils.[112] As previously mentioned, PR3 may interact with different proteins involved in the clearance of apoptotic cells, thereby hampering this phenomenon. For instance, PR3 can interact with PLSCR1, which plays a key role in membrane flip-flop to externalize phosphatidylserine during apoptosis.[196] PR3 can also bind calreticulin (CRT), which is considered to be a potent "eat me" signal expressed on apoptotic neutrophils in patients with GPA.[197]

This precludes signaling by the CRT receptor lipoprotein receptor-related protein (LRP) 1 by reversing the production of anti-inflammatory cytokines from macrophages towards more inflammatory cytokines such as IL1, IL6, and TNFα.[197] Similarly, PR3 binds phosphatidylserine, another important "eat-me" signal, and this interaction depends on the hydrophobic patch responsible for membrane anchorage.[147] Moreover, PR3 may also directly interact with the complement component C1q, acting as a bridging molecule between the phagocytic and apoptotic cells to favor their elimination.[198] Finally, in neutrophils from patients with GPA showing severe disease with renal involvement, PR3 was associated with annexin A1, which normally promotes the phagocytosis of apoptotic cells.[184]

Structural and molecular analyses of PR3 have provided new insights demonstrating that PR3 participates in an apoptosis-induced membrane complex through very specific interactions, which negatively modulate proteins involved in the clearance of apoptotic cells. Moreover, the presence of PR3 at the membrane of apoptotic neutrophils has a direct effect on the anti-inflammatory response of macrophages. As a result, macrophages exposed to apoptotic PR3-expressing neutrophils expressed increased levels of the M1 marker

inducible NO synthase (iNOS) and secreted higher levels of inflammatory cytokines and chemokines. PR3 expression on apoptotic cells disrupted immune silencing in autoimmune vasculitis.[199]

The pro-inflammatory responses of PR3 required both its enzymatic activity and membrane anchorage, suggesting that recognition of PR3 at the surface of apoptotic cells involves multi-molecular interactions.[109] In patients with GPA, the phagocytosis of apoptotic PR3-expressing neutrophils by macrophages generates substantial amounts of pro-inflammatory mediators, especially IL-1, thereby creating a pro-inflammatory microenvironment leading to autoimmunity.[199,200] This response is mediated by the IL-1R1/MyD88 signaling pathway as murine macrophages deficient for these pathways did not display a PR3-associated proinflammatory response. Plasmacytoid dendritic cell activation and polarization of Th2, Th9, and Th17 accompanied this dysregulated immune process. Taken together, these results indicate that PR3 disrupts immune silencing associated with the clearance of apoptotic cells and highlight the pivotal role of PR3 as both auto-antigenic and auto-inflammatory. This sheds light on PR3 involvement in GPA pathophysiology (Figure 1).[200]

A human PR3 transgenic mouse model (hPR3Tg) has provided evidence supporting the hypothesis that PR3 can affect the resolution of inflammation and exacerbate systemic inflammation.[201] During zymosan-induced peritonitis, neutrophil peritoneal recruitment was increased in hPR3Tg mice compared to wildtype mice, with no differences in the recruitment of macrophages or B or T lymphocytes. After cecum ligation and puncture, a model used to induce peritoneal inflammation through infection, hPR3Tg mice displayed decreased survival rates in acute sepsis with increased neutrophil extravasation associated with the cleavage of annexin A1. Moreover, hPR3Tg neutrophils displayed enhanced survival during apoptosis compared with controls, likely contributing to their increased accumulation in the peritoneal cavity. Overall, this human PR3 transgenic mouse strain may be a suitable model to examine PR3-dependent proinflammatory mechanisms and may be used to develop a murine model of GPA.

4 | MEDIUM-VESSEL VASCULITIS

4.1 | Polyarteritis nodosa: neutrophils and aneurysm as potential partners in crime

Polyarteritis nodosa (PAN) is a rare necrotizing form of vasculitis that preferentially affects medium-sized vessels and leads to organ infarction. It may also target small vessels but spares vessels without a muscular layer, such as arterioles, capillaries, or venules, and thus does not cause glomerulonephritis and is not associated with ANCA.[12] Patients may present a wide constellation of constitutional symptoms with abdominal or testicular pain, myalgia, neuropathy, and skin involvement such as livedo reticularis. PAN is known to cause pseudoaneurysm formation, particularly in the mesenteric and renal arteries.[202] PAN was previously frequently associated with hepatitis B virus infection, although this has become very uncommon since the introduction of vaccination programs and screening of blood products.[203] Deficiency of adenosine deaminase 2 (DADA2) is characterized by vasculopathy ranging from livedo reticularis to PAN and life-threatening ischemic and/or hemorrhagic stroke.[204] DADA2 is a well-described systemic inflammatory vasculopathy caused by mutations in the *CERC1* gene that encodes the ADA2 protein and often, but not always, clinically resembles PAN. DADA2 is the first molecularly described monogenic vasculitis syndrome. It is important to distinguish primary PAN from DADA2 as different treatment regimens may be applicable, such as TNFα inhibitors in the vasculitis-predominant phenotype of DADA2 instead of corticosteroids and immunosuppressors in PAN.[205,206] Currently, the American College of Rheumatology (ACR) criteria published in 1990 are still in use to classify PAN.[207] However, new criteria are under investigation with the aim to increase sensitivity and specificity.[208,209] Over 60 disease-associated mutations have been identified in all domains of ADA2, affecting its catalytic activity, protein dimerization, and secretion. ADA2 is highly expressed in myeloid cells but its role in vasculitis pathogenesis has not yet been elucidated.[210] Interestingly, in a case study of patients with DADA2, transcriptomic analysis of the leukocytes revealed overexpression of neutrophil-derived genes, suggesting ADA2 may regulate neutrophil activity and endothelial damage. A reduction in ADA2 activity resulted in significant endothelial damage via a neutrophil-driven process. This was suggested because *CERC1* is not expressed in endothelial cells and thus additional cell types involved in disease pathology were investigated. Although ADA2 was suggested to modulate NET formation in neutrophils, further studies are required to elucidate its function in neutrophils.[210–212]

Fibrinoid necrosis is frequently observed in active lesions with a high frequency of infiltrated neutrophils. Neutrophil involvement in PAN was suggested in one study showing the investigating the role of lysosomal-associated membrane protein-2 (LAMP-2), a glycoprotein expressed on the membranes of neutrophils and endothelial cells, which was increased in the serum of patients with PAN and AAV.[213] Neutrophil involvement was also suggested because of elevated IL-8 levels in the serum of patients with Behçet's disease (BD) and PAN compared to that in controls.[214] As IL-8 is a potent chemoattractant and activator of neutrophils found in the inflamed vessel walls in PAN, this finding may suggest that neutrophils play a role in initiating PAN pathogenesis, although this remains to be proven definitively.

The presence of aneurysms and pseudoaneurysms in medium-sized vasculature is a hallmark of PAN. Aneurysm refers to an abnormal bulge in a blood vessel that is caused by a weakness in the blood vessel wall, usually at a branch point. Pseudoaneurysms are bulges bounded only by the tunica adventitia. These vascular balloons, observed as characteristic "rosary signs" on imaging, are formed by (i) intimal proliferation, (ii) medial degeneration marked by VSMC depletion and disorganization, and (iii) elastic lamina disruption and excessive deposition of collagen and proteoglycans. The rupture of an aneurysm can cause life-threatening hemorrhage and the treatment of PAN is therefore of prime importance.[215] The direct link between neutrophils and aneurysm has yet to be established. Neutrophils are

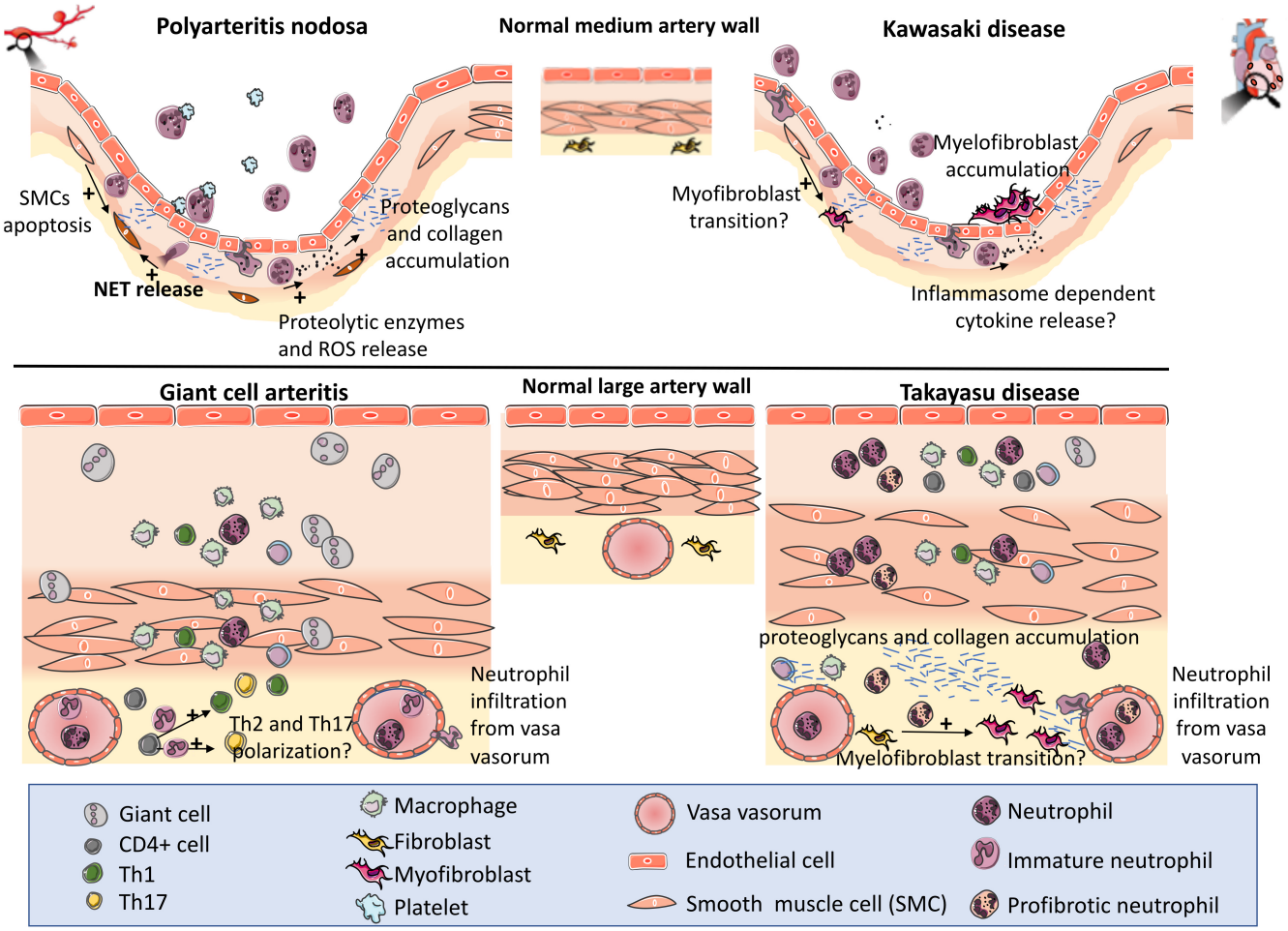

FIGURE 2 Neutrophil functions hypothesized to contribute to vascular damage in medium- and large-vessel vasculitis. Diagrammatic representation of the cross-section of a medium- and large-vessel artery under healthy conditions and in the indicated disease. In polyarteritis nodosa (PAN), medium-vessel arteries are affected by the initiation of aneurysm formation, which is responsible for the "rosary sign" observed on imagery. By infiltrating the media, neutrophils may disorganize this layer by promoting smooth muscle cell death and releasing enzymes and reactive oxygen species (ROS) responsible for the remodeling of the extracellular matrix. In Kawasaki disease, coronary arteries are also often affected by aneurysms. Neutrophils may destroy the intima, media, and some portions of the adventitia of the coronary artery. These cells may also participate in the release of pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF), which contribute to luminal myofibroblast proliferation, in which myofibroblasts (mainly derived from smooth muscle cells) and their matrix products progressively obstruct the coronary lumen. Giant cell arteritis is histologically characterized by thickening of the intima, infiltration of immune cells (mostly $CD4^+$ lymphocytes and giant cells), and disorganized media. Increased infiltration of neutrophils from the vasa vasorum in the early stages of vascular inflammation may direct the lymphocyte response, because of the specific immunosuppressive function of low-density and immature neutrophil subtypes. In Takayasu disease, vascular damage is predominantly characterized by adventitial fibrosis as well as significant infiltration of neutrophils and other immune cells from the vasa vasorum. A certain neutrophil subtype may promote fibrosis by interacting directly with fibroblasts and stimulating their conversion into myofibroblasts.

frequently associated with a significantly increased risk of aneurysm rupture because of the increased presence of neutrophil enzymes in the aneurysm wall (Figure 2).[216–218] Indeed, metalloproteinases, such as MMP9 and MMP2, as well as neutrophil serine proteases may influence arterial remodeling by cleaving several components of the vascular ECM such as collagen and elastin. In a mouse model of aneurysm triggered by elastase perfusion, neutrophil depletion inhibited the development of abdominal aortic aneurysm in a non-MMP2/9-dependent manner.[219] These results suggest that circulating neutrophils may be important players in the initial formation of aneurysms. Furthermore, a high NLR indicated the potentially pathogenic role of neutrophils in the development of thoracic aortic aneurysm.[220] Accordingly, a decreased incidence of aortic aneurysm was associated with a lower number of infiltrated neutrophils in the vessels of mice.[221] These studies involved only large vessels and in the case of PAN, the mechanisms by which neutrophils may remodel the vascular wall of medium-sized vessels and whether they may contribute to the destabilization of the media remain to be elucidated (Figure 2).

A direct interaction between neutrophils and VSMCs may also contribute to VSMC loss.[222] In different murine models of atherosclerosis, authors demonstrated that VSMCs, which are found in

the injured vessel, attracted neutrophils and triggered NET release, causing VSMC lysis and ultimately leading to atheroma plaque destabilization.[222] Another study showed that neutrophils impaired VSMC recovery preceding allograft vasculopathy.[223] Interactions between neutrophils and VSMCs remain to be elucidated and should be explored further with regard to PAN.

4.2 | Kawasaki disease: shedding light on inflammasome activation by neutrophils

Kawasaki disease (KD), also known as infantile polyarteritis nodosa, is a medium-vessel vasculitis primarily affecting children under the age of 5 years with a male predominance. Its incidence is highest in Japan with approximately 360 cases per 100 000.[224,225] Patients present with fever, rash, cervical lymphadenopathy, conjunctivitis, and oral mucosal inflammation, and may develop coronary artery aneurysm if untreated, which may lead to thrombosis and myocardial infarction. An environmental trigger such as an infection is thought to be involved because of the age of patients and seasonal appearance of the disease, but no infectious agent has been identified to date.[226] Interestingly three well-established murine models are currently used to mimic the disease and they all employ intraperitoneal injection of infectious-like stimuli, thus supporting the hypothesis of a microbial initial trigger for KD. Those three models consist of injection of cell wall components from *Lactobacillus casei*, *Candida albicans*, or nucleotide-binding oligomerization domain containing 1 (Nod1) ligand associated with LPS, their characteristics are reviewed in.[227]

During the COVID-19 pandemic, interest in the potential infectious triggers of KD was renewed because of the Kawasaki-like disease observed after COVID-19 infection named multi-system inflammatory syndrome in children (MIS-C) thereafter COVID-KD.[228–232] Indeed, both KD and MIS-C are postinfectious inflammatory diseases with some overlapping clinical and immunological features, but with different inflammatory signatures.[233] Therefore, they are probably mediated by different but related inflammatory pathways and possibly different but related pathogenic mechanisms. A genetic component is also suspected because of familial links between cases and several single-nucleotide polymorphisms (SNPs) have been identified, many of which are also implicated in other immune-mediated diseases, suggesting a common pathway in the pathophysiology of these diseases.[234] Because of its anti-inflammatory and anti-platelet activities, aspirin is used for treatment.[235] To reduce the prevalence of coronary artery abnormalities, the first treatment of choice is intravenous immunoglobulin (IVIG), whose mechanism of action is unclear although it appears to have generalized anti-inflammatory effects.[236] Glucocorticoids and cyclosporin[237,238] are used to treat IVIG-resistant KD patients[239] and immune-mediating agents such as TNF inhibitors may also be administered. Because serum levels of IL-1β and IL-1 related genes are upregulated in KD peripheral blood during the acute phase of illness,[240,241] clinical trials have investigated the IL-1 receptor antagonist anakinra following promising results in mouse models[242,243] and now anakinra is recommended for the treatment of KD.[244]

Regarding molecular mechanisms leading to this increase of IL-1β, several studies have highlighted the importance of inflammasome signaling as key in the pathogenesis of various cardiovascular disorders, including KD. Inflammasomes are multiprotein signaling platforms that mediate pivotal inflammatory responses of innate immunity by gathering in the cytosol after detecting pathogen- and danger-associated molecular patterns (PAMPs and DAMPs, respectively). In immune cells, a sensor protein assembles with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) upon activation. Subsequently, the complex recruits procaspase-1, resulting in its cleavage/activation and thereby inducing the maturation and secretion of the important inflammatory cytokine IL-1β and gasdermin D resulting in cell death via pyroptosis.[245] The mouse model of KD involving injection of *Lactobacillus casei* cell wall extract demonstrated the critical role of caspase-1 and IL-1β in the development of coronary lesions.[246] The murine model of KD induced by *Candida albicans* water-soluble fraction confirmed these results by showing that the NLR family pyrin domain-containing 3 (NLRP3) inflammasome was essential for KD development.[247] Furthermore, researchers found significant epigenetic hypomethylation, increased transcripts, and upregulation of NLR family CARD domain-containing protein 4 (NLRC4) and NLRP12 sensor in patients with KD.[248] Remarkably, both NLRC4 and NLRP12 sensors are also expressed by human neutrophils and neutrophil NLRC4 derived caspase-1 and IL-1β activation upon *Salmonella* activation without leading to the pyroptotic death.[249]

For a long time, the study of inflammasomes was primarily restricted to monocytes and macrophages with little interest in neutrophils. More recently, researchers have shown a growing interest in the neutrophil inflammasome, which is involved in various infections and immune-mediated diseases.[249–252] Considering their prominent presence in the blood compared with other types of leukocytes, neutrophils may be a major location of inflammasome activation and dysregulation. In KD, neutrophils might be a relevant source of IL-1β the origin of which could be the inflammasome activation. Nonetheless, inflammasome machinery in neutrophils has been described to be different from that in monocytes and in macrophages.[253] Indeed in neutrophils secretion of IL-1β and gasdermin D pore formation were shown to be the result of serine protease activities, independently from NLRP3 inflammasome activation or caspase-1 activity.[254,255] For instance, PR3 can cleave and mature IL-1β intracellularly.[256] Those recent discoveries confirm the growing necessity of delineating the functions of the different inflammasomes in neutrophils, particularly in KD. Interestingly, IL-1β-expressing neutrophils were reduced in the circulation of patients with KD after administration of IVIG, which promotes neutrophil cell death independently of caspase activation.[257,258] Furthermore, neutrophil apoptosis was shown to be delayed in the acute phase of KD compared to that in controls.[259] Conversely, pyroptosis expression was increased in the leukocytes of patients with KD.[260] Because pyroptosis is an inflammatory form of cell death and apoptosis is not,

an increase in pyroptosis and a decrease in apoptosis in neutrophils from patients with KD may maintain inflammation during disease progression.

Several other studies corroborate the pathogenic role of neutrophils beside their potential contribution to the production of cytokines. First, neutrophils have been shown to play a central role in the early stages of KD,[261] as coronary arterial lesion biopsies showed a mixed infiltrate with macrophages in the later stages of the disease (Figure 2).[66,262] Although most studies show neutrophils to play a role in the beginning of the disease, neutrophil elastase concentrations were shown to remain elevated in the circulation of patients with KD three months after disease onset.[263,264] Neutrophil infiltration was also shown in the myocardium and neutrophilia is present in patients with KD.[262,265] The involvement of neutrophils in the early stages of the disease is also suggested by the fact that a peak of NO production by neutrophils is observed at the time of diagnosis, which changes to a similar peak produced by monocytes two weeks after onset of the disease. In general, NOS is higher in patients with coronary lesions who also show a higher number of circulating endothelial cells.[266,267] Recently, one study showed that increased respiratory burst by neutrophils was associated with coronary artery lesions in KD.[268] Interestingly, the rate of circulating platelet–neutrophil aggregates was shown to be higher in patients with KD compared to those with bacterial infections and healthy controls, and even higher in patients with coronary artery abnormalities than in those without.[269] These aggregates decreased after treatment with IVIG and prednisolone. This finding suggests that the crosstalk between neutrophils and platelets in vasculitis may be distinct from that in bacterial infections. In addition, high-mobility group box 1 (HMGB1), which is secreted after tissue damage and activates vascular endothelial cells and neutrophils, is increasingly thought to play a role in vasculitis.[270] It is increased in different vasculitis forms such as AAV, IgAV, and KD.[270–272]

Another study has demonstrated that semaphorin 4D (SEMA4D) derived from neutrophils is elevated in the serum of patients with KD and correlates with concentrations of IL-1β, IL-6, and IL-8.[273] This protein is bound to the neutrophil membrane under normal conditions and negatively regulates ROS generation and NET formation. In patients with AAV, it is cleaved from the surface of neutrophils and shed by ADAM17 and was shown to be elevated in serum. Soluble SEMA4D exerts pro-inflammatory effects on endothelial cells.[274] Spontaneous NET formation has been shown in vitro in neutrophils from patients with KD.[275] Furthermore, it was shown to enhance pro-inflammatory cytokine production in peripheral blood mononuclear cells (PBMCs) from these patients.[276] However, there are no reports of circulating NETs or histological NET formation in patients with KD.[5] Another interesting aspect is the NLR, which is thought to be implicated in many different immune-mediated diseases. High NLR was associated with failing treatment response to IVIG[277,278] and was predictive of coronary artery lesions.[279] Interestingly, neutrophils have also been shown to express vascular endothelial growth factor (VEGF) in the acute phase, which may regulate early vascular responses.[280]

With regard to neutrophil surface molecules, CD177 was found to be upregulated in patients with KD[281] and this was independently confirmed via transcriptomic analysis.[282] Furthermore, CD11b, an adhesion molecule on neutrophils, was upregulated in patients with KD and expression levels were related to coronary artery lesions.[283] However, this finding was contradicted in another study.[284] Fcγ-receptor CD64, which recognizes the Fc portion of IgG and forms a link between humoral and cellular immunity, is another surface protein of neutrophils that was shown to be increased in KD.[285,286] Its decreased expression after IVIG administration implicates a possible role as a biomarker for diagnosis and therapy evaluation. S100A12, another proinflammatory neutrophil-derived protein, is increased in the early stages of acute KD.[287] Its soluble receptor (sRAGE), which neutralizes S100A12, was shown to be decreased in KD and correlates negatively with S100A12.[288] The ratio of S100A12 to sRAGE has been shown to differ between responders and non-responders after IVIG treatment, suggesting this may serve as a novel biomarker for treatment response. S100A12 was also identified as a hub gene in a genomic analysis comparing patients with KD to healthy controls.[289] This finding was further confirmed in a study comparing DNA methylation in gene expression assays from total white blood cells of patients with KD and demonstrated in vitro that S100A family proteins enhance leukocyte transendothelial migration.[290] Pentraxin-3, another inflammatory protein, was shown to be released by neutrophils in cardiovascular disease and sepsis[291] and is associated with neutrophil activation, vascular inflammation, and endothelial cell dysfunction.[292] Pentraxin-3 was increased in the acute phases of KD with the highest concentrations observed in patients with coronary artery lesions and was correlated with neutrophilia in patients with KD.[293] MMP-9, which may also be released by activated neutrophils, was shown to be elevated in KD and was an independent predictor of coronary artery lesions,[294] although this was not confirmed in another study.[263] Taken together, these findings indicate beyond doubt that neutrophils participate in the pathogenesis of KD, although the exact role they play in this disease remains to be elucidated.

5 | LARGE-VESSEL VASCULITIS (LVV)

In large vessels such as the aorta, the adventitia contains a heterogeneous cell population that includes dendritic cells, T cells, B cells, macrophages, progenitor cells, and fibroblasts, which can differentiate into myofibroblasts embedded in a matrix rich in collagen and elastin. The adventitia also contains an adrenergic nervous system, a lymphatic network, and the vasa vasorum, which is a specialized microvasculature. Traditional concepts of vascular inflammation are considered "inside-out" responses centered on leukocyte infiltration in the vessel wall from the luminal side. However, it is becoming increasingly apparent that vascular inflammation of large vessels is initiated in the adventitia and progresses toward the intima.[295] In this "outside-in" theory, leukocytes (including neutrophils) transmigrate through the vasa vasorum to invade the vascular wall. Thus, we

can hypothesize that vascular inflammation in large-vessel vasculitis (LVV) springs from direct crosstalk between neutrophils and adventitial components, resulting in vascular remodeling and ultimately in an increased risk of cardiovascular disease.

5.1 | Giant cell arteritis: miscommunication between different neutrophil subsets and T cells?

Giant cell arteritis (GCA) is the most common primary vasculitis in adults. The term "temporal arteritis" is obsolete since not all GCA patients have temporal artery involvement, and other categories of vasculitis can affect the temporal artery.[12] GCA affects individuals over the age of 50 years and more often women than men.[296] This form of granulomatous vasculitis predominantly affects large vessels such as the aorta and its branches. While the diagnosis of GCA can be made without additional biopsy in patients in whom there is a high clinical suspicion of GCA and a positive imaging test, a biopsy is recommended for those patients with suspected GCA and inconclusive imaging test.[297] Clinically, patients present with polymyalgia rheumatica, jaw claudication, headaches, vision disturbances, and constitutional symptoms. Vision loss is one of the most serious complications, but may be prevented by prompt administration of high-dose glucocorticoids. Anti-IL-6R antibody therapy with tocilizumab is recommended as targeted therapy in refractory or relapsing disease. MTX may be used as an alternative.[298] Furthermore there are ongoing trials for other targeted therapies such as blockade of IL-1 receptor, IL-17, IL-23p19 subunit, IL-12/23p40 subunit, JAK1, and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α.[299–304]

Various infections such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* were suspected as potential triggers of GCA, but this has not been proven.[305] A genetic link has been made to HLA-class II region (HLA-DRB1*04 alleles) and confirmed in a genome-wide association study, which is in line with the predominant presence of CD4+ T cells in inflammatory lesions and justifies the use of IL-6 blockade to inhibit differentiation of T-helper cells.[306] Indeed, IFNγ produced by type 1 T-helper cells (Th1) and IL-17A produced by Th17 cells are primarily responsible for the systemic and vascular manifestations in GCA, thus making this a T-cell-mediated disease.[307,308] Plasminogen and prolyl4-hydroxylase subunit α2 (P4Hα2), a key enzyme in collagen synthesis, were also identified as genes contributing to GCA risk because of their involvement in vascular remodeling and angiogenesis.[306] Furthermore, the role of genes encoding cytokines such as TNFα, molecules associated with endothelial function such as intracellular adhesion molecule 1 (ICAM1) and VEGF, and regulators of innate immunity such as TLR4 have also been described.[309–312] Finally, age-associated decline of regulatory T cells (Tregs) and deficiencies in the checkpoint inhibitory pathway may contribute to a loss of tolerance, which induces the disease.[313] Treg immune responses were shown to improve after treatment with the IL-6 receptor antibody tocilizumab.[314]

Histological observations include transmural lymphohistiocytic infiltrate combined with intimal thickening, presence of giant cells, and to a lesser extent laminar necrosis along the internal elastic lamina.[315] The presence of large numbers of neutrophils does not exclude the disease, but is unusual and increases the probability of another vasculitis type.[66] Although neutrophils are not observed to a great extent in histological samples, pro-inflammatory S100 proteins, which activate endothelial cells and are specific for phagocytes such as neutrophils, are abundant in the adventitia of affected vessels. S100A12 is restricted to the vasa vasorum and its concentrations are increased in the serum of patients with GCA.[316] Recently, NETs have been identified in temporal artery biopsies from patients with GCA and, in agreement with the former studies, were located close to the vasa vasorum.[317] Therefore, neutrophils may play an important role in the vasa vasorum, which is the site of entry for inflammatory cells. Neutrophil counts were higher in patients with GCA than in healthy controls and remained elevated in cases of treatment-free remission.[318]

As in other forms of vasculitis, the NLR was shown to be elevated in GCA.[319] Furthermore, high NLR was shown to be associated with symptomatic and ruptured aortic aneurysm in patients with thoracic aortic aneurysm of different origin.[220] In a mouse model, a decreased incidence of aortic aneurysm was associated with a reduction in neutrophil numbers in the vessel wall.[320] In addition, whole-transcriptome analysis of blood samples revealed that the IL-1 signaling pathway was upregulated in patients with LVV compared to that in healthy donors and remained elevated after treatment with corticosteroids. However, it is unclear whether this would affect neutrophil function.[321] The soluble pattern recognition 1 receptor pentraxin 3, which is involved in vascular injury, is upregulated in GCA as in other types of vasculitis.[322] Taken together, these results provide proof of concept that neutrophils may participate in the pathogenesis of GCA.

Different neutrophil phenotypes have been defined via flow cytometry throughout disease progression, showing responses to glucocorticoid administration.[323] At week 1 of glucocorticoid treatment, an annexinA1(high)CD62L(low)CD11b(low) phenotype was observed with minimal adhesion to endothelial monolayers under flow, while an annexinA1(high)CD62L(high)CD11b(high) phenotype was evident at week 24 (lowest glucocorticoid dose). The phenotype at week 24 did not show a suppressive effect on T-cell proliferation, suggesting the importance of the neutrophil-T-cell crosstalk in disease pathology.[323]

Immature neutrophils, defined as CD66b(positive)CD15(positive) CD10(low)CD64(negative), were found to be enriched in the blood of patients with GCA and are resistant to apoptosis, thus remaining in the circulation for a prolonged period of time and interacting with platelets. Platelets are activated in LVV and hetero-aggregates of platelets with leukocytes have been identified in GCA, possibly contributing to the ischemic risk.[324,325] The leakiness of the endothelial barrier, which contributes to the disease, may be in part due to ROS production from neutrophils, enabling them to breach the endothelial barrier as shown in an in vitro co-culture system using immature neutrophils.[326] Interestingly, there have been several case reports about isolated aortitis following administration of G-CSF, which

stimulates the proliferation and differentiation of neutrophil precursors and enhances neutrophil chemotaxis. It is unclear whether the effect of G-CSF on vessel inflammation is a result of regulating T-cell responses rather than neutrophils, or whether the pathogenesis in these cases is the same as that in LVV.[327] Notably, G-CSF may also promote small vessel vasculitis including IgAV and AAV.[328] Taken together, these findings indicate that the existence of different neutrophil subtypes with immunosuppressive functions may contribute to the pathophysiology of GCA, which is a T-cell-mediated disease, via subversive dialog with typical adaptive immune cells.

5.2 | Takayasu arteritis: when neutrophils participate in arterial adventitial fibrosis

Takayasu arteritis (TAK) is a LVV initially described in Japan with an incidence of 1–3 cases per million people worldwide.[296,329,330] It affects more women than men and, in contrast to GCA, is found in younger adults. There is a genetic association with HLA-B52, which also differentiates it from GCA, suggesting an important involvement of immune cells expressing HLA class I molecules on their surface.[331] A recent study has also identified non-HLA susceptibility loci.[332]

Although an infectious trigger has been reported (*Mycobacterium tuberculosis*), no causal relationship could be proven in other studies.[333,334] Symptoms include claudication of extremities, constitutional symptoms, and arterial bruits especially over the subclavian artery. Decreased pulse and blood pressure differences may also be detected. Treatment resembles that for GCA, although glucocorticoid tapering can be slower because of a higher relapse rate. Methotrexate therapy is also recommended and TNFα or IL-6 inhibitors may be used in cases of relapsing or refractory disease.[298,335]

Neutrophil counts are elevated in the circulation of patients with TAK and are positively correlated with disease activity. NLR is also reported to be elevated, as seen in GCA.[336,337] The concentrations of cytokines that play major roles in neutrophil recruitment, activation, and survival, such as IL-17, IL-8, IFNγ, and TNFα, are significantly increased in TAK.[338–341] Biopsy results are very similar to those of patients with GCA and include skin lesions, lymphoplasmacytic cells, granulomas, and giant cell formation.[66] However, biopsies are not regularly performed as the affected branches are not as easily reached as the temporal artery, which is typically affected in GCA. However, one study has described massive accumulation of granulocytes within the adventitia of aortal biopsies from patients with TAK.[342] As described previously, the IL-1 signaling pathway was shown to be upregulated in patients with TAK and GCA and remained elevated after corticosteroid treatment. Whether this affects neutrophil function remains to be elucidated.[321] Interestingly, anti-endothelial cell antibodies have been reported in TAK, although their role in pathophysiology remains unclear.[343,344]

The major histological difference observed on the vascular wall in TAK compared to GCA is the thickening of the adventitia.[295,345] Since specimen usually originate from vascular surgery or are reported as incidental findings, in TAK late morphological stages are usually reported.[346] TAK is an inflammatory fibrotic arteritis with unknown mechanisms, rendering its treatment very challenging. Fibrosis is defined as the thickening and scarring of connective tissues resulting from an injury. In TAK, increased collagen, ECM remodeling, and fatty acid oxidation are hallmarks of adventitial fibrosis. These are a consequence of fibroblast proliferation and stimulation, as well as an imbalance between matrix metalloproteases and their tissue inhibitors.[329,347,348] The primary effectors of fibrosis are fibroblasts, which communicate with other types of cells present in the tissue.

Neutrophils are thought to be involved in the fibrotic process taking place in the adventitia in TAK, but this remains to be proven. Pertinently, the wide spectrum of neutrophil proteases, collagenase, and other enzymes contained in their granules likely participate in ECM remodeling, and neutrophils have also been shown to modulate fibroblasts.[349] For instance, during healing after myocardial infarction, murine neutrophils were shown to modulate ECM protein production and transforming growth factor (TGF)-β expression by fibroblasts. Consequently, neutrophils may contribute to the conversion of fibroblasts into myofibroblasts.[349] Importantly, neutrophils themselves undergo polarization after myocardial infarction as observed using a proteomic approach.[350] In this study, seven days after myocardial infarction, neutrophils presented a reparative signature, including the expression of fibronectin and fibrinogen, which may contribute to ECM reorganization in the scarring process.[350] A recent study has shown that murine neutrophils provided components for fibrotic scar tissue and carried fibrotic matrix into the wound.[230] Interestingly, this was independent of increased vascular permeability mediated by neutrophil proteases.[351] In the same study, single-cell transcriptomic analysis was used to distinguish specific sub-populations of neutrophils without providing their precise phenotypic characterization. Finally, a murine model of renal fibrosis showed that a new Siglec-F-expressing neutrophil subtype promoted collagen I production by fibroblasts.[352] Siglec-F-positive neutrophils were also able to produce collagen I directly.

These recent findings highlight the newly discovered pro-fibrotic roles of neutrophils, which may impact vascular remodeling as observed in TAK-associated adventitial fibrosis. Indeed, the presence of neutrophils in the adventitia of biopsies from patients with TAK suggests that following the development of inflammatory lesions in the artery wall, a phenotypic switch may occur in neutrophils, thereby worsening the development of fibrous lesions (Figure 2).

6 | VARIABLE VESSEL VASCULITIS

6.1 | Behçet's disease (BD): a vicious cycle involving neutrophils, platelets, and endothelial cells

BD is a unique type of vasculitis that can affect veins and arteries of all sizes. It is clinically characterized by recurrent vascular events, such as venous thrombosis, oral and genital ulcers, uveitis, skin involvement, and arthritis, and may also include central

nervous system involvement.[12] It occurs most frequently in inhabitants in countries along the ancient Silk Road with incidence rates of approximately 0.05–3.9 per 100000[224] and is associated with HLAB51 and HLA-B5.[353] Oral microorganisms and other infectious agents have been postulated as triggers, but no specific organism could be identified.[354] Treatment depends on the organs involved and includes colchicine for ulcers and arthritis, immunosuppressors and glucocorticoids for uveitis, thrombosis, and pulmonary artery aneurysms, as well as anti-TNF antibodies for refractory cases.[355] Histologically, it is characterized by neutrophil-dominated infiltration around the vasa vasorum, although there appears to be no specific histopathology.[356]

BD is considered to be a typical clinical model for thromboinflammation, particularly venous thromboembolism which shows a prevalence of 15–40% in patients with BD. It is unclear why BD is the only vasculitis that affects mostly veins. As reviewed recently,[357] pathological thrombogenesis in BD is related to the coexistence of endothelial dysfunction disrupting blood flow and abnormality in blood components (i.e., platelets, neutrophils, and the plasma coagulation system). Indeed, neutrophils must play a major role in the pathology of BD as they show significant infiltration in the vessels of affected tissue and a hyperactivated state, as evidenced by increased chemotaxis,[358–362] ROS generation,[363–365] and NET release.[6,365,366] Chemotactic factors such as CXCL-8 and S100A12, which can orchestrate neutrophil activity similarly to that in KD and GCA, are elevated in the serum of patients with BD.[367,368]

Increased ROS production was correlated with NET release in a study of patients with non-vascular BD.[369] Neutrophils from patients with BD exhibited spontaneous NET formation in vitro, which was inhibited by colchicine and dexamethasone as well as specific PAD4 and ROS inhibitors.[6] In the same study, endothelial cells cultured in the presence of NETs from patients with BD showed an increase in apoptosis and cell proliferation. This may be explained by the fact that citrullinated histones released exhibit high toxicity and disrupt the endothelial barrier.[370] The finding of increased ROS production is controversial, as another group showed decreased ROS production in both normal and low-density neutrophils from patients with BD patients in vitro.[371] This controversy is also found in AAV.[156] Interestingly, enhanced neutrophil NADPH oxidase activity correlated with fibrinogen clotting ability in BD.[372] ROS can modify the secondary structures of fibrinogen by promoting its carbamylation, which renders it less accessible to plasmin-induced lysis, thereby stabilizing and consolidating the thrombus.[373] Neutrophils may interact directly with plasmatic hemostasis components. Patients with active BD show elevated concentrations of circulating free DNA and MPO-DNA complexes compared to patients with inactive BD and healthy controls.[366] In addition, thrombin generation in plasma from patients with BD was significantly increased and positively correlated with NET markers. NETs have been shown to promote thrombin generation and play a role in immunothrombosis.[374,375] Furthermore, NETs have been identified in the inflammatory aorta, within panniculitis and cutaneous vasculitis lesions,[6] as well as in superficial venous thrombosis in BD.[376] BD-derived NETs were also found to stimulate macrophages to produce higher amounts of IL-8 and TNFα compared to those stimulated by NETs from healthy controls.[365] To date, mechanisms of NET release from neutrophils in BD remain to be elucidated. Despite this knowledge gap, it is hypothesized that decondensed DNA associated with neutrophil granule proteins offers a platform for platelet activation and aggregation, thus laying the basis for thrombus formation.[377,378] Conversely, platelets interact directly with neutrophils, and neutrophil–platelet aggregates foster their adhesion to the vascular wall.[378,379]

The damaging role of NETs and their increased presence in BD have been described in other vascular immune-mediated diseases such as AAV and SLE.[380,381] However, thrombotic risk is not the primary complication associated with these diseases. Enhanced neutrophil chemotaxis has been detected in vitro at the onset of BD and has led to treatment of colchicine, which inhibits their migration.[382] Several studies showed that neutrophils displayed functional abnormalities with regard to receptors, including TLRs, CD11b, CXCR2, FcγRII, and CXCR2.[383–386] TLR-1, TLR-3, TLR-4, and TLR-6 were concentrated on the surface of neutrophils from patients with BD compared to those from healthy controls.[383] In this study, authors hypothesized that HMGB1, an agonist of TLR-2, TLR-4, and TLR-5 that is increased in serum from patients with BD,[387] may contribute to the deleterious cutaneous inflammatory responses observed.

As observed in KD and other forms of vasculitis, neutrophil activation may originate from HMGB1 contained in platelet-derived microvesicles.[270] Indeed, high concentrations of CD62P (P-selectin) platelet-derived microvesicles and CD62P+ platelets were observed in BD, independent of the patient clinical activity.[384] These data suggest that a CD62P+ signature may be associated with BD. Although monocyte-platelet aggregates did not differ between healthy donors and patients with BD, neutrophil–platelet aggregates were significantly higher in the inactive BD patient cohort but not in patients with active BD. Platelets usually interact with neutrophils through P-selectin and P-selectin glycoprotein ligand-1 recognition,[388] but this may also be dependent on TLRs.[389,390] Platelet TLR4 activates NETs in patients with sepsis.[390] A phenotypic study of membrane receptors expressed on BD neutrophil subtypes and associated platelet interaction would help to further delineate the prothrombotic functions of neutrophils.

Interestingly, elevated numbers of LDNs were recorded in patients with BD compared to healthy donors,[371] as observed in other immune-mediated diseases such as SLE or other forms of vasculitis.[391] In psoriasis, which is another chronic inflammatory immune-mediated skin disease with increased cardiovascular risk, LDNs present higher chemotactic migration in comparison to normal-density neutrophils (NDNs).[392] LDNs also upregulated the transcription of genes related to low-density granulocyte-platelet aggregation. More importantly, platelet adherence occurred only with LDNs but not with NDNs, and these LDN-platelet aggregates were correlated with psoriasis severity.[392] In addition, the authors showed that LDNs exerted cytotoxic effects on endothelial cells.

By analogy, this crosstalk between platelets, NDNs, and LDNs may take place in the context of BD. Future studies could therefore examine whether LDNs present in the disease also preferably aggregate with platelets and thereby increase thrombotic risk (Figure 3).

7 | NEUTROPHILS AS TARGETS FOR INNOVATIVE THERAPEUTIC STRATEGIES

The recent developments made in our understanding of the pathogenesis of (ANCA)-associated vasculitides (AAV) and in particular the role of neutrophils in these conditions are good examples to highlight the potential of targeting neutrophil functions and mediators.[9,82] Treatment of AAV is challenging and can be accompanied by multiple side effects, and novel therapeutic approaches are therefore required. Cardiovascular disease is currently the most common cause of mortality in patients with AAV, whose cardiovascular risk is 65% higher than that in the general population.[393] As neutrophil involvement has been demonstrated in the development of cardiovascular disease, this must be considered when considering novel treatment options. Neutrophils may be a suitable target for disease management although specific attention has to be paid also with regard to the increased risk of infection and the risk of relapses.[394]

Therapies involving novel targets are continually evolving and blockade of C5aR1 with the small-molecule avacopan was successful in a phase 3 randomized controlled trial in patients with AAV.[395] When neutrophils are stimulated by ligation of neutrophil receptors, for instance the FcR, several kinases are activated to initiate neutrophil degranulation, ROS generation, and NET formation. Some of these pathways have been used to develop new therapeutic targets.[82] The cytoplasmic spleen tyrosine kinase (SYK) showed increased phosphorylation in renal biopsies of patients with AAV, and SYK inhibition reduced glomerular inflammation in an MPO-ANCA rat model.[396] Considering that the signaling cascade underlying FcR-mediated neutrophil activation is extremely well characterized, several therapeutic options are available or currently under study for the treatment of AAV and also IC-induced vasculitis. It is worth mentioning the endopeptidase IdeS (imlifidase) here as it shows how critical Fc mediated-mechanisms are currently crucial to neutrophil activation in anti-BGM disease. This endopeptidase specifically cleaves human IgG in the lower hinge region, producing an F(ab')2 fragment and two half-Fc fragments. Prior studies in experimental anti-GBM disease showed that IdeS can cleave GBM-bound IgG in vivo, removing the Fc portion that harbors the effector functions; as a result, IdeS-treated mice had significantly lower complement C1q and C3 deposition and reduced influx and activation of neutrophils in glomeruli.[57]

Several studies have provided direct evidence that monoclonal antibodies or mimetic peptides that block the IgA FcR (FcαRI) have a beneficial effect on IgA vasculitis.[397] These studies suggest the important role of neutrophil-mediated vascular injury in IgA vasculitis and indicate that neutrophils should be considered when developing novel treatment options for IC-SVV.

Regarding signaling pathways in AAV, phosphorylation of p38 MAPK and extracellular signal-regulated kinase have been shown to induce ROS generation.[398] This was shown to be associated with the formation of NETs, which was promoted by MPO and

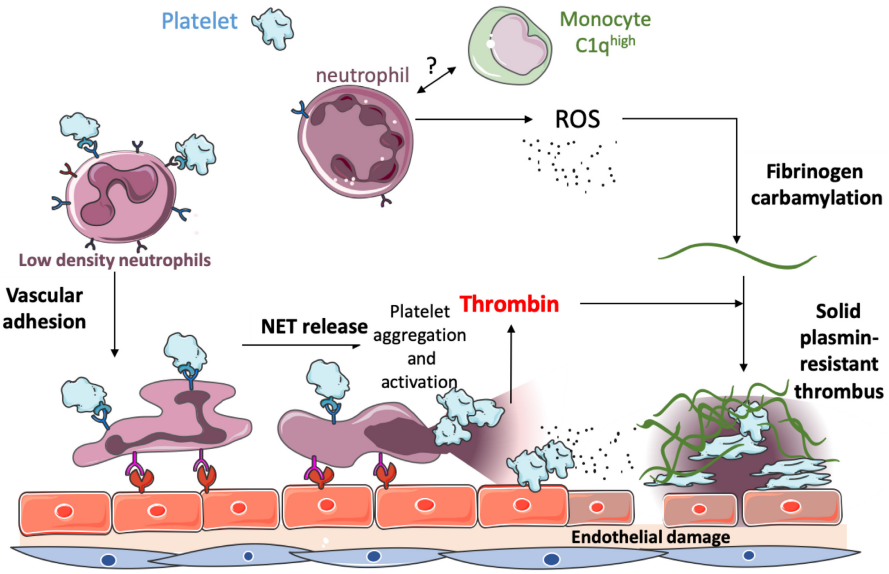

FIGURE 3 Pro-thrombotic function of neutrophils in Behçet's disease. The hypercoagulability and thrombotic risk observed in Behçet's disease may be a consequence of the actions of a specific subset of highly chemoattracted and immature neutrophils. These neutrophils may show greater interaction with platelets, which would facilitate their adhesion to endothelial cells. Adherent immature neutrophils may release NETs and build a highly pro-coagulant platform for platelet activation and aggregation. Specific neutrophil subsets may then promote thrombin generation by producing ROS and NETs and render the fibrin fibrils resistant to fibrinolytic enzymes, thus resulting in a highly stabilized and solid thrombus.

protein-arginine deiminase type 4 (PAD4).[399] Therefore, inhibition of MAPK,[400,401] MPO,[176] or PAD4[402] may attenuate neutrophil activation and crescentic glomerulonephritis in animal models. Neutrophil serine proteases (NSPs) have also been implicated in glomerular injury. These must be proteolytically activated by cathepsin C in the bone marrow. Accordingly, cathepsin C inhibition has been shown to reduce NSP expression and proteolytic activity in mice[403,404] and to reduce glomerular microvascular endothelial cell damage in vitro.[405] Finally, stimulation of innate immunity by the defective clearance of PR3-expressing neutrophils may also be harnessed in future therapies targeting macrophage activation or plasmacytoid dendritic cells.[200] Nanoparticles are also under investigation to target neutrophils and macrophages in inflammatory diseases.[406]

B lymphocytes play an important role in pathogenesis of ANCA.[407,408] There is now evidence of atypical autoreactive PR3+ memory B cells accumulating through the maturation process in patients with PR3-AAV.[409] The crucial role of B cells in AAVs is highlighted with the successful therapy using anti-CD20 depleting B-cells.[410–412] Of particular interest in the context of AAV, is the dialogue between neutrophils and B cells that is yet to be fully characterized. Nonetheless, it has been already demonstrated that that neutrophils can communicate with B cells through production of BAFF (B cell-activating factor), a molecule known to sustain B cell survival and responsiveness.[413] Conversely, whether anti-CD20 therapy could induce specific neutrophil subsets is not known yet but would warrant attention. Recently, a study has suggested a potential contribution of neutrophils to the lack of response to B-cell depletion therapy.[413,414] In this study, BAFF production by splenic neutrophils could sustain the differentiation of long-lived splenic plasma cells in lupus-prone mice receiving anti-CD20 antibody treatment.[414]

8 | CONCLUSION

Neutrophils are implicated in vascular damage associated with immune-mediated vasculitis, and this has been extensively explained by their capacity for the excessive release of powerful mediators that are initially tailored for efficient antimicrobial defense activities, including ROS and proteases, as well as granule proteins and DNA, known as NETs. Although these findings are irrefutable, it appears that the roles of neutrophils may be much more complex than previously anticipated if we consider results from the last decades of research. More recent studies have supported the theory that neutrophils may adapt and modulate their pro-inflammatory roles towards immunosuppressive, profibrotic, or pro-thrombotic effects, or to exert as-yet-unknown activities, thus impacting the vessel wall in different ways.

The diverse pathogenic processes that promote vasculitis exemplify the *polyvalence* of neutrophils as they encounter abnormal or injured vessel walls. In fact, we considered using the word *polyvalent* in our title but elected not do so out of concern that the term would be unfamiliar to many readers. In French, polyvalent connotes something or someone that is simultaneously useful and practical as an adaptation to meet a need or overcome a challenging situation. A *"agent polyvalent"* is a man whose extensive repertoire of aptitudes can meet the needs of any critical situation. In like fashion, neutrophils display flexibility, adaptability, and versatility both in their response to endothelial warning signals and later in their resourceful communications with their partners in the inflammatory response, such as macrophages, lymphocytes, platelets, fibroblasts, or smooth muscle cells, to influence their phenotypes.

In the future, studies should focus on molecular mechanisms and on characterizing different subtypes of neutrophils in the various forms of vasculitis. A better understanding of how neutrophils switch their phenotypes and how this affects the microenvironment in which they are—or will be—recruited is fundamental if we want to develop new therapeutic strategies for these incurable diseases. Taken together, these findings support an important role for neutrophils in vasculitis pathogenesis that is not restricted to NET release and which, in many situations, seems to require a conversation between neutrophils and other cellular partners. Molecular analysis of the role of neutrophils in immune-mediated vasculitis may pave the way towards the development of novel neutrophil-targeted treatments.

AUTHOR CONTRIBUTIONS

K.A., A.S., and V.WS. wrote the manuscript. K.A. and J.A. generated the figures and the table, respectively. P.L. and A.S. provided critical feedback and edited the review. All the authors contributed to the final manuscript.

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CONFLICT OF INTEREST

The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

We agree on the data availability statement. All our data are vailable.

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