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. 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 cells.
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 the disparity of organs affected pre-favoring each vasculitis subtype may be related to the differential expression levels, and in the ability of certain cytokines to influence their expression. The disparity of organs affected pre-favoring each vasculitis subtype may be related to the differential expression of this chemokine pattern would extend beyond its interaction with blood vessels, has been recently reviewed14-16 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 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. 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 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 reviewed14-16 and is not described here.
## TABLE 1 Clinical features of the different forms of vasculitis

<table>
<thead>
<tr>
<th>Vessel size</th>
<th>Vasculitis</th>
<th>Possible triggers</th>
<th>Age, sex, ethnicity</th>
<th>Incidence (new cases per year)</th>
<th>Genes</th>
<th>Predilection vessels</th>
<th>Target organs/main symptoms</th>
<th>Histology</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>ANCA-associated vasculitis: MPA, GPA, EGPA</td>
<td>Infectious agents (e.g., nasal carriage of <em>Staphylococcus aureus</em> in GPA), drugs, silica dust</td>
<td>GPA: northern Europe, MPA: northern Europe, Japan, Kuwait</td>
<td>GPA: 5–13/million</td>
<td>GPA/PR3-AAV: HLA-DR, SERPINA1, PR3, MPA/MPO- AAV: HLA-DQ, HLA-DR</td>
<td>Capillaries, venules, arterioles</td>
<td>GPA: upper and lower respiratory tract, necrotizing glomerulonephritis</td>
<td>GPA: granuloma, vasculitis, parenchymal necrosis</td>
<td>Induction: glucocorticoids and Cyc/RTX (organ-/life-threatening) or MTX/MMF (non-organ-threatening) Maintenance: AZA, MTX, MMF, RTX Novel: anti-C5a-receptor</td>
</tr>
<tr>
<td>Small</td>
<td>Immune complex vasculitis: SVV</td>
<td>IgA: Infections, drugs, cryoglobulinemia, vasculitis, HCV, connective tissue diseases, myeloproliferative disorders</td>
<td>IgA: &lt;17years</td>
<td>IgA: Adults: 1.18/million</td>
<td>IgA: HLA-DRB1</td>
<td>IgA: small vessels with skin predominance and gastrointestinal involvement</td>
<td>IgA: purpura, arthralgia/ arthritis, abdominal pain, glomerulonephritis</td>
<td>IgA: leukocytoclastic vasculitis with IgA deposits</td>
<td>IgA: analgesia, selected indications: glucocorticoids, nephritis ACEI, AZA, MMT, Cyc</td>
</tr>
<tr>
<td>Medium</td>
<td>PAN</td>
<td>Some are HBV-associated</td>
<td>Mean age in adults: 40–50 years</td>
<td>Children: 40–50 years</td>
<td>Subgroup: ADA2 deficiency</td>
<td>Medium vessels (and rarely small vessels with a muscular layer)</td>
<td>Constitutional symptoms, abdominal/testicular pain, myalgias, neuropathies, livedo reticularis/ skin ulcers</td>
<td>Necrotizing arteritis with mixed cell infiltrate, variable occlusion of the lumen</td>
<td>Induction: glucocorticoids, Cyc, antiviral treatment if HBV; Maintenance: MTX, AZA, MMT; DADA2: TNFi</td>
</tr>
<tr>
<td>Medium</td>
<td>Kawasaki disease</td>
<td>Infectious agents suspected, but not yet identified</td>
<td>Europe: 4.5–9/100000</td>
<td>Japan: 360/100000</td>
<td>Medium vessels, notably coronary arteries</td>
<td>Fever, rash, cervical lymphadenopathy, conjunctivitis, oral mucosal inflammation</td>
<td>Mixed cell infiltrate with neutrophils at disease onset in coronary artery lesion</td>
<td>IgVg: aspirin, IgVg: resistance: glucocorticoids, TNFi, Ciclosporin, IL-1RA</td>
<td></td>
</tr>
<tr>
<td>Vessel size</td>
<td>Vasculitis</td>
<td>Possible triggers</td>
<td>Age, sex, ethnicity</td>
<td>Incidence (new cases per year)</td>
<td>Genes</td>
<td>Predilection vessels</td>
<td>Target organs/main symptoms</td>
<td>Histology</td>
<td>Treatment</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>-------------------------------</td>
<td>-------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Large</td>
<td>TAK</td>
<td>Mycobacterial theory&lt;sup&gt;26,228&lt;/sup&gt;</td>
<td>&lt;50 years, Women &gt; men, Asia &gt; Europe&lt;sup&gt;310&lt;/sup&gt;</td>
<td>Japan: 1/million&lt;sup&gt;22,218&lt;/sup&gt;</td>
<td>HLA-B52&lt;sup&gt;210,322&lt;/sup&gt;</td>
<td>Aorta and its major branches, notably subclavian artery&lt;sup&gt;424&lt;/sup&gt;</td>
<td>Claudication of extremities, constitutional symptoms, arterial bruits (subclavian), decreased pulse, BP differences&lt;sup&gt;424&lt;/sup&gt;</td>
<td>Skip lesions, lymphoplasmytic cells, granulomas, giant cell formation&lt;sup&gt;44&lt;/sup&gt;</td>
<td>High-dose corticosteroids, MTX, Anti-TNFα, anti-IL6-RA&lt;sup&gt;298,335&lt;/sup&gt;</td>
</tr>
<tr>
<td>Large</td>
<td>GCA</td>
<td>Various infections such as Mycoplasma pneumonia suspected, but unconfirmed&lt;sup&gt;295&lt;/sup&gt;</td>
<td>&gt;50 years, Caucasians&lt;sup&gt;296&lt;/sup&gt;</td>
<td>15–35/100000 in northern Europe&lt;sup&gt;425&lt;/sup&gt;</td>
<td>HLA-DRB1*0401, PLG, PHHA2, (TNF, ICAM1, VEGF, TLR4)&lt;sup&gt;306,307,310–312&lt;/sup&gt;</td>
<td>Aorta, carotid, and vertebral artery, notably temporal artery&lt;sup&gt;426&lt;/sup&gt;</td>
<td>PMR, jaw claudication, headaches, constitutional symptoms, vision disturbances&lt;sup&gt;426&lt;/sup&gt;</td>
<td>Transmural lymphohistocytic infiltration, intima thickening, giant cells, usually not necrotizing&lt;sup&gt;294,315&lt;/sup&gt;</td>
<td>Induction: high-dose glucocorticoids, Maintenance: Anti-IL6-RA, MTX, other targeted therapies in trials&lt;sup&gt;298–304,335,427&lt;/sup&gt;</td>
</tr>
<tr>
<td>Variable</td>
<td>Behçet’s disease</td>
<td>Oral microorganisms suspected&lt;sup&gt;354&lt;/sup&gt;</td>
<td>Onset at 20–40 years, Between Mediterranean basin and China&lt;sup&gt;324&lt;/sup&gt;</td>
<td>0.05–3.9/100000&lt;sup&gt;24&lt;/sup&gt;</td>
<td>HLA-B5&lt;sup&gt;25,3&lt;/sup&gt;</td>
<td>Arteries and veins of all sizes&lt;sup&gt;428&lt;/sup&gt;</td>
<td>Oral and genital ulcers, uveitis, arthritis, venous thrombosis, erythema nodosum-like lesions&lt;sup&gt;428&lt;/sup&gt;</td>
<td>Mixed neutrophilic and monoclonal cell infiltrations, non-granulomatous&lt;sup&gt;356&lt;/sup&gt;</td>
<td>glucocorticoids, AZA, Cyclic citrullinated peptide, INFα, anti-TNFα&lt;sup&gt;355&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**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-1,4,5-triphosphate 3-kinase; IVIg, intravenous immunoglobulin; MAF, 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, leucocyte 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. 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. 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. 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. 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.

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. The re-entry of infiltrated neutrophils into systemic circulation is achieved by microvascular leakages. 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. 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 outermost 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 polyangitis, 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. 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. 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. 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.

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 FcRNs cooperate with complement receptors in the binding of soluble immunoglobulin G (IgG) ICs to mediate neutrophil activation. 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. 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.

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. 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.

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 (FcγRIIB), which preferentially bind complexes 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. 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. Clinically, anti-GBM is characterized by rapid progressive glomerulonephritis and pulmonary hemorrhage. 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. Patients present antibodies against epitopes on the α3 chain of type IV collagen, which is expressed in basement membranes in the glomeruli, alveoli, testis, inner ear, eye, and choroid plexus. Observations of antibodies deposited along the GBM are the gold-standard diagnostic feature. 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, who have a greater risk of relapse, but a higher rate of renal recovery.

The antibodies are directed more often towards myeloperoxidase (MPO) than proteinase 3 (PR3). 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 autoimmune process, leading to neutrophil recruitment and perform pro-inflammatory effects, including neutrophil recruitment. This may lead to neutrophil accumulation and tissue destruction during IgA-autoantibody-mediated vasculitis. 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. An association between interleukin (IL)-1 receptor antagonist allele 2 (ILRN*2) and renal involvement has also been shown. 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 and a history of infection is reported in 30–63% of cases. 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.

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). 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. Treatment of IgAV includes glucocorticoids and immunosuppressants for organ- or life-threatening vasculitis manifestations. 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. Abnormal glycosylation of the hinge region of IgA1 is suggested to cause aggregation to form macromolecular ICs. Because FcαRI-mediated cross-linking of neutrophils in vitro induces inflammatory processes such as ROS production and the release of NETs and leukotriene B4, 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, IgA1 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.

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. 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. 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. 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. 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.

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. 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. GPA is characterized histologically by necrotizing vasculitis and extravascular granulomatous. The main difference in EGPA is the involvement of eosinophils. Granuloma are not observed in MPA.

The two main target antigens of ANCs are the neutrophil granule proteases PR3 and MPO, both also expressed in monocytes. Anti-PR3 ANCs 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. Anti-MPO ANCs 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. 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, as both are able to bind and activate neutrophils, which is a key feature of this pathophysiology. 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.

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. 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. In contrast, no direct association with MPO-related genes was found in anti-MPO-associated vasculitis. 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-ANCA could initiate an immune response. 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 microbicidal 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 H₂O₂ 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 product proteins 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 plaques118 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 atherosclerosis. 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 proteinase126 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 cells127 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, therefore opening new fields of investigation based on membrane PR3 and particularly its partners and functions, which have been reviewed elsewhere.

The membrane protein CD177, also called NB1, has a glycosylphosphatidylinositol (GPI) anchor and comparable bimodal expression on human neutrophils. 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, 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. This is not the case for elastase. These four hydrophobic amino acids are essential for PR3 expression at the membrane and likely also for CD177 binding. 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. 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.

3.2.2 | Neutrophil function and their modulation by ANCA

The accessibility of the autoantigen (PR3 or MPO) to ANCA is a mechanistic prerequisite for cell activation by the ANCA. Using intravital microscopy techniques, ANCA have been shown to convert rolling neutrophils to firm stationary adhesion. 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. In addition, patients show large numbers of circulating endothelial cells, while remission is associated with decreasing numbers. Because the antigen is intracellular, measurement of neutrophil responses to ANCA in vitro requires a priming step with tumor necrosis factor (TNFα) 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 ANCA could activate NADPH oxidase and ROS generation in human neutrophils in vitro. Numerous studies have described the double engagement of ANCA with FcγR and the antigen (MPO or PR3) at the neutrophil surface to induce a functional response. Once ANCA 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 FcγRIIB (CD16B). FcγRIIA blockade was shown to abrogate ANCA-induced activation, whereas the role of the FcγRIIB blockade is somewhat more controversial. Intracellular signaling of Fc receptors has been discussed here with regard to IC-mediated vasculitis. Furthermore, the integrin and complement receptor CR3 (CD11b/CD18) was shown to be required for a complete response. Oxidative activity in response to anti-PR3 ANCA is increased in neutrophils expressing membrane PR3 compared to those not expressing PR3.

Despite numerous reports showing that anti-MPO or anti-PR3 ANCA can trigger a robust respiratory burst, overactivity of NADPH oxidase in neutrophils or monocytes is not observed in patients with GPA or MPA. Even more surprisingly, ROS production and disease severity are negatively associated, consistent with findings in other autoimmune diseases such as SLE. 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. Furthermore, in vivo 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.

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. 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 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. 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.
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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 ANCA vasculitis confirmed the in vivo pathogenic role of these antibodies in humans.\textsuperscript{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.\textsuperscript{169} In contrast, the pathogenicity of anti-PR3 ANCAs in vivo remains to be proven unequivocally.

Several studies have shown that the spontaneous apoptosis of neutrophils was delayed in patients with AAV.\textsuperscript{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.\textsuperscript{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 phos- pholipid 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\textsuperscript{185} and in G-CSF-treated donors.\textsuperscript{186} Although the clear identification and role of LDNs remain to be established, their function appears to vary greatly according to the pathological condition.\textsuperscript{187} LDNs are reportedly expanded in patients with acute AAV, but they are hy- poresponsive to MPO antibodies when compared to normal-density neutrophils.\textsuperscript{188,189} They have been associated with disease activity and show NET formation in vitro.\textsuperscript{190} However, their importance and function in disease pathogenesis are yet to be determined.
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 and during inflammation. 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. 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. 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. PR3 can also bind calreticulin (CRT), which is considered to be a potent "eat me" signal expressed on apoptotic neutrophils in patients with GPA.

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. Similarly, PR3 binds phosphatidylserine, another important "eat-me" signal, and this interaction depends on the hydrophobic patch responsible for membrane anchorage. 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. 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.

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.
4.1 Aneurysm as potential partners in crime

PR3 expression on apoptotic cells disrupted immune silencing in autoimmune vasculitis. 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. 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. This response is mediated by the IL-1R1/MyD88 signaling pathway as murine macrophages deficient for these pathways did not display a PR3-associated pro-inflammatory 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 in GPA pathophysiology (Figure 1).

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. 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. 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. 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. Deficiency of adenosine deaminase 2 (DADA2) is characterized by vasculopathy ranging from livedo reticularis to PAN and life-threatening ischemic and/or hemorrhagic stroke. 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. Currently, the American College of Rheumatology (ACR) criteria published in 1990 are still in use to classify PAN. However, new criteria are under investigation with the aim to increase sensitivity and specificity.

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. 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.

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. 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. 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. The direct link between neutrophils and aneurysm has yet to be established. Neutrophils are...
frequently associated with a significantly increased risk of aneurysm rupture because of the increased presence of neutrophil enzymes in the aneurysm wall (Figure 2). 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. 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. Accordingly, a decreased incidence of aortic aneurysm was associated with a lower number of infiltrated neutrophils in the vessels of mice. 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. 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.\(^{249-252}\) 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 and IL-1β may be considered 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 macrophages.\(^{253}\) Indeed in neutrophils secretion of IL-1β and gasdermin D pore formation were shown to be the result of serine protease activity, independently from NLRP3 inflammasome activation or caspase-1 activity.\(^{254-256}\) 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. As coronary arterial lesion biopsies showed a mixed infiltrate with macrophages in the later stages of the disease (Figure 2). 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. Neutrophil infiltration was also shown in the myocardium and neutrophilia is present in patients with KD. 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. Recently, one study showed that increased respiratory burst by neutrophils was associated with coronary artery lesions in KD. 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. 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. It is increased in different vasculitis forms such as AAV, IgAV, and KD.

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. 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. Spontaneous NET formation has been shown in vitro in neutrophils from patients with KD. Furthermore, it was shown to enhance pro-inflammatory cytokine production in peripheral blood mononuclear cells (PBMCs) from these patients. However, there are no reports of circulating NETs or histological NET formation in patients with KD. 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 and was predictive of coronary artery lesions. Interestingly, neutrophils have also been shown to express vascular endothelial growth factor (VEGF) in the acute phase, which may regulate early vascular responses.

With regard to neutrophil surface molecules, CD177 was found to be upregulated in patients with KD and this was independently confirmed via transcriptomic analysis. Furthermore, CD11b, an adhesion molecule on neutrophils, was upregulated in patients with KD and expression levels were related to coronary artery lesions. However, this finding was contradicted in another study. 

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. 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. GCA affects individuals over the age of 50 years and more often women than men. 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. 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. Furthermore there are ongoing trials for other targeted therapies such as blockade of IL-1 receptor, IL-17, IL-23, IL-12/23 p40 subunit, JAK1, and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α. 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. 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. 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. 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. 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. Treg immune responses were shown to improve after treatment with the IL-6 receptor antibody tocilizumab. 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. The presence of large numbers of neutrophils does not exclude the disease, but is unusual and increases the probability of another vasculitis type. 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. 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. 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.

As in other forms of vasculitis, the NLR was shown to be elevated in GCA. Furthermore, high NLR was shown to be associated with symptomatic and ruptured aortic aneurysm in patients with thoracic aortic aneurysm of different origin. In a mouse model, a decreased incidence of aortic aneurysm was associated with a reduction in neutrophil numbers in the vessel wall. 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. The soluble pattern recognition 1 receptor pentraxin 3, which is involved in vascular injury, is upregulated in GCA as in other types of vasculitis. 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. 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. 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. The leukocytosis 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. 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. Notably, G-CSF may also promote small vessel vasculitis including IgAV and AAV. 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. It affects more women than men and, in contrast to GCA, is found in younger adults. There is a genetic association with HLA-BS2, which also differentiates it from GCA, suggesting an important involvement of immune cells expressing HLA class I molecules on their surface. A recent study has also identified non-HLA susceptibility loci.

Although an infectious trigger has been reported (Mycobacterium tuberculosis), no causal relationship could be proven in other studies. Symptoms include claudication of the 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 differences may also be detected. Treatment resembles that for GCA, although glucocorticoid tapering can be slower because of differences in the artery wall, a phenotypic switch may occur in neutrophils, suggesting that following the development of inflammatory lesions neutrophils were also able to produce collagen I directly. Neutrophils 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. 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. Biopsy results are very similar to those of patients with GCA and include skin lesions, lymphoplasmacytic cells, granulomas, and giant cell formation. 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. 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. Interestingly, anti-endothelial cell antibodies have been reported in TAK, although their role in pathophysiology remains unclear.

The major histological difference observed on the vascular wall in TAK compared to GCA is the thickening of the adventitia. Since specimen usually originate from vascular surgery or are reported as incidental findings, in TAK late morphological stages are usually reported. 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. 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. 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. Importantly, neutrophils themselves undergo polarization after myocardial infarction as observed using a proteomic approach. In this study, seven days after myocardial infarction, neutrophils presented a reparative signature, including the expression of fibroactin and fibrinogen, which may contribute to ECM reorganization in the scarring process.

A recent study has shown that murine neutrophils provided components for fibrotic scar tissue and carried fibrotic matrix into the wound. Interestingly, this was independent of increased vascular permeability mediated by neutrophil proteases. 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. 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. It occurs most frequently in inhabitants in countries along the ancient Silk Road with incidence rates of approximately 0.05–3.9 per 100,000 and is associated with HLA-B5 and HLA-B5. Oral microorganisms and other infectious agents have been postulated as triggers, but no specific organism could be identified. 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. Histologically, it is characterized by neutrophil-dominated infiltration around the vasa vasorum, although there appears to be no specific histopathology.

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, 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, ROS generation, and NET release. Chemotactic factors such as CXCL-8 and S100A12, which orchestrate neutrophil activity similarly to that in KD and GCA, are elevated in the serum of patients with BD.

Increased ROS production was correlated with NET release in a study of patients with non-vascular BD. 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. 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. 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 in vitro. This controversy is also found in AAV. Interestingly, enhanced neutrophil NADPH oxidase activity correlated with fibrinogen clotting ability in BD. 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. 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. NETs have been shown to promote thrombin generation and play a role in immunothrombosis. Furthermore, NETs have been identified in the inflammatory aorta, within panniculitis and cutaneous vasculitis lesions, as well as in superficial venous thrombosis in BD. 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. 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. Conversely, platelets interact directly with neutrophils, and neutrophil–platelet aggregates foster their adhesion to the vascular wall.

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. 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. Several studies showed that neutrophils displayed functional abnormalities with regard to receptors, including TLRs, CD11b, CXCR2, FcγRII, and CXCR2. 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. 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, 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. Indeed, high concentrations of CD62P (P-selectin) platelet-derived microvesicles and CD62P+ platelets were observed in BD, independent of the patient clinical activity. 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, but this may also be dependent on TLRs. Platelet TLR4 activates NETs in patients with sepsis. 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, as observed in other immune-mediated diseases such as SLE or other forms of vasculitis. 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). 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. 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, removing 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). Therefore, inhibition of MAPK, MPO, or PAD4 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 and to reduce glomerular microvascular endothelial cell damage in vitro. 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. Nanoparticles are also under investigation to target neutrophils and macrophages in inflammatory diseases.

B lymphocytes play an important role in pathogenesis of ANCA. There is now evidence of atypical autoreactive PR3+ memory B cells accumulating through the maturation process in patients with PR3-AAV. The crucial role of B cells in AAVs is highlighted with the successful therapy using anti-CD20 depleting B-cells. 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 neutrophils can communicate with B cells through production of BAFF (B cell-activating factor), a molecule known to sustain B cell survival and responsiveness. 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. 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.

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.W.S. 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.

ACKNOWLEDGEMENTS

Editorial support (English language editing) was provided by Lucidity Editing. The laboratory of Véronique Witko-Sarsat was supported by several grants from Fondation pour la Recherche Médicale FRM (EQU202003010155); from the Investissements d’Avenir Programme (ANR-11-IDEX-0005-02, Sorbonne Paris Cite, LabEx INFLAMEX); from patient associations such as Vaincre la Mucoviscidose (RC20180502225); ABCF-Proteïne; Arthritis foundation. Jennifer Amsler has obtained a postdoc mobility fellowship from the Swiss National Science Foundation.

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 available.

ORCID

Karen Aymonnier https://orcid.org/0000-0002-9587-8822
Jennifer Amsler https://orcid.org/0000-0001-7049-2926
Peter Lamprecht https://orcid.org/0000-0001-5929-868X
Alan Salama https://orcid.org/0000-0002-9255-9092
REFERENCES


Véronique Witko-Sarsat https://orcid.org/0000-0002-5296-9601

129. Hajjar E, Broemstrup T, Kantari C, Witko-Sarsat V, Reuter N. Structures of human proteinase 3 and neutrophil elastase--so similar yet so different. FEBs J. 2010;277:2238-2254.


214. Freire AL, Bertolo MB, de Pinho AJ, Samara AM, Fernandes SRM.


222. Freire AL, Bertolo MB, de Pinho AJ, Samara AM, Fernandes SRM.


228. Freire AL, Bertolo MB, de Pinho AJ, Samara AM, Fernandes SRM.


300. University Hospital, Caen. Randomized, controlled, double-blind study of anakinra against placebo in addition to steroids in giant cell arteritis. 2020. https://clinicaltrials.gov/ct2/show/NCT03725202


344. AYMONNIER et al.
369. Bettiol A, Becatti M, Silvestri E, et al. Neutrophil-mediated mecha-
nisms of damage and in-vitro protective effect of colchicine in non-
density neutrophils are increased in patients with Behçet’s disease but do not explain differences in neutrophil function. J Inflamm
(Lond). 2022;19:5.
372. Becatti M, Emmi G, Silvestri E, et al. Neutrophil activation pro-
motes fibrinogen oxidation and thrombus formation in Behçet’s
375. Kimball AS, Obi AT, Diaz JA, Henke PK. The emerging role of NETs
in venous thrombosis and Immunothrombosis. Front Immunol.
2016;7:236.
extracellular traps in superficial venous thrombosis of Behçet’s
377. Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps pro-
2014;123:2768-2776.
379. Page C, Pitchford S. Neutrophil and platelet complexes and their
relevance to neutrophil recruitment and activation. Int Immunopharmacol. 2013;17:1176-1184.
380. Jorch SK, Kubes P. An emerging role for neutrophil extracellular
381. Papayannopoulos V. Neutrophil extracellular traps in immunity
382. Matsumura N, Mizushima Y. Leucocyte movement and colchicine
383. Carmona-Rivera C, Kaplan MJ. Low-density granulocytes: a
distinct class of neutrophils in systemic autoimmunity. Semin
385. Houben E, Penne EL, Voskuyl AE, et al. Cardiovascular events in
2018;57:555-562.
386. Salama AD. Relapse in anti-neutrophil cytoplasm antibody
387. Jayne DRW, Merkel PA, Schall TJ, Bekker P. ADVOCATE Study
388. McAdoo SP, Prendecki M, Tanna A, et al. Spleen tyrosine kinase
inhibition is an effective treatment for established vasculitis in a
389. van der Steen LP, Bakema JE, Sesarman A, et al. Blocking Fcγ rece-
regulates neutrophil elastase release and Actin dynamics during
391. Los AM, Ma FY, Tesch GH, et al. ASK1 inhibitor treatment sup-
ameliorates acute inflammatory renal injury in rat anti-GBM glo-
393. O’Sullivan KM, Gan P, Kittchong R, Holdsworth S. 209. Inhibition of
PEPTIDYLARGININE deiminase 4 limits neutrophil extracellular
trap formation and inflammation IN experimental anti-MPO-
394. Korkmaz B, Caughhey GH, Chapple I, et al. Therapeutic targeting of
cathepsin C: from pathophysiology to treatment. Pharmacol Ther.
2018;190:202-236.
395. Jerke U et al. 196. Characterization of cathepsin c as a treat-
ment target in ANCA-associated vasculitis. Rheumatology (Oxford).
gets for development of Nanotherapeutics in inflammatory dis-
398. Oleinik N, Mauri C, Salama AD. Effector and regulatory B cells in
of the B cell receptor repertoire in six immune-mediated diseases.
400. Berti A, Hillion S, Hummel AM, et al. Circulating autoreactive pro-
teinase 3+ B cells and tolerance checkpoints in ANCA-associated
vasculitis. JCI Insight. 2021;6:e150999.
401. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclo-
2010;363:221-232.
411. Guillenvin L, Pagnoux C, Karras A, et al. Rituximab versus azathio-
2014;371:1771-1780.

412. Tieu J, Smith R, Basu N, et al. Rituximab for maintenance of remis-

413. Costa S, Bevilacqua D, Cassatella MA, Scapini P. Recent advances
on the crosstalk between neutrophils and B or T lymphocytes. Immunology. 2019;156:23-32.

414. Thai L-H, le Gallou S, Robbins A, et al. BAFF and CD4+ T cells are
major survival factors for long-lived splenic plasma cells in a B-cell-


416. Yates M, Watts RA, Bajema IM, et al. EULAR/ERA-EDTA recom-

417. Gardner-Medwin JMM, Dolezalova P, Cummins C, Southwood TR. Incidence of Henoch-Schönlein purpura, Kawasaki disease,

418. Rutgers A, Slot M, van Paassen P, van Breda Vriesman P, Heeringa P, Tervaert JWC. Coexistence of anti-glomerular basement mem-
brane antibodies and myeloperoxidase-ANCAs in crescentic glo-


421. Mohammad AJ, Jacobsson LTH, Mahr AD, Sturfelt G, Segelmark M. Prevalence of Wegener’s granulomatosis, microscopic poly-
angitis, polyarteritis nodosa and Churg-Strauss syndrome within a

422. Chung SA, Gorelik M, Langford CA, et al. 2021 American College of Rheumatology/Vasculitis Foundation guideline for the classification of Takayasu ar-

423. Arend WP, Michel BA, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of Takayasu ar-

424. Brekke LK, Diamantopoulos AP, Fevang BT, Aijms J, Esperø E, Gjesdal CG. Incidence of giant cell arteritis in Western Norway