Endothelium-Neutrophil Interactions in ANCA-Associated Diseases

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ABSTRACT

The two salient features of ANCA-associated vasculitis (AAV) are the restricted microvessel localization and the mechanism of inflammatory damage, independent of vascular immune deposits. The microvessel localization of the disease is due to the ANCA antigen accessibility, which is restricted to the membrane of neutrophils engaged in \( \beta 2 \)-integrin-mediated adhesion, while these antigens are cytoplasmic and inaccessible in resting neutrophils. The inflammatory vascular damage is the consequence of maximal proinflammatory responses of neutrophils, which face cumulative stimulations by TNF-\( \alpha \), \( \beta 2 \)-integrin engagement, C5a, and ANCA by the Fc\( \gamma \)RII receptor. This results in the premature intravascular explosive release by adherent neutrophils of all of their available weapons, normally designed to kill IgG-opsonized bacteria after migration in infected tissues.


The initial description of small-vessel vasculitides dates back to 1950, when Wainwright and Davson, by ocular examination of kidneys conserved in the Pathologic Museum of Manchester, were able to distinguish forms of vasculitis with enlarged and inflammatory glomeruli involving mostly vessels smaller than arteries.\(^1\) Their definition—of a microscopic form of periarteritis nodosa—was specified in 1994 by an international consensus committee,\(^2\) who changed the name to microscopic polyangiitis because of the localization of inflammatory lesions in arterioles, venules, but not in medium-sized or small arteries.\(^3\) The original observation that small-vessel vasculitis, including granulomatous polyangiitis (GPA) and microscopic polyangiitis, was associated with anti-proteinase 3 (PR3)\(^4\) or antimyeloperoxidase (MPO)\(^5\) ANCA, produced numerous studies that demonstrated the central role of neutrophils and their interaction with the endothelium in ANCA-associated vasculitis (AAV).\(^6\)–\(^8\)

Endothelium-neutrophil interactions are essential to allow neutrophils to move toward inflammatory sites and regulate spatially and temporally neutrophil recruitment. Neutrophils contain intracellular pools of toxic proteins aimed to kill microbes and digest tissues. To perform innate immune responses to infections, neutrophils must adhere and migrate toward the site of infection (Figure 1), while avoiding collateral damage caused by premature release of oxidants and proteolytic enzymes. This implies highly regulated neutrophil-endothelial cell interactions complying with the following demands: neutrophils must remain nonadhesive in the arterial and arteriolar circulation, independently of their recruitment in postcapillary venules of inflammatory organs; the 10-\( \mu \)m diameter neutrophil must squeeze through capillaries, smaller in diameter (7 \( \mu \)m), without activation that could result from neutrophil-neutrophil interactions, contact with endothelium, or distortion; and neutrophil adhesion to inflamed endothelium and diapedesis through the vessel wall should occur without release of toxic oxidants or proteases, which should be delayed until cells reach the inflammatory focus. This review examines current concepts of the ways ANCA disrupts these sophisticated regulatory mechanisms, leading to unwanted premature and improperly located neutrophil activation, almost exclusively in microvessels.

NEUTROPHILS IN THE BLOOD FLOW: PHYSIOLOGIC CONTROL AND ACTIVATION BY ANCA

Physiologic Control of TNF-Primed Neutrophils

The central role of TNF-\( \alpha \) in AAV is demonstrated \textit{in vitro}\(^9\)–\(^10\) and \textit{in vivo} by the striking effect of anti-TNF antibodies in experimental anti-MPO-induced GN\(^11\)–\(^12\) and in human AAV.\(^13\)–\(^15\) Circulating TNF-\( \alpha \) results in neutrophil priming, leading to weak degranulation, oxidative response, or adhesion...
and to hyperresponsiveness to subsequent stimuli such as chemoattractants or immune complexes.\textsuperscript{16,17} TNF-induced priming has normally limited consequences within the blood flow of circulation due to a strict control of neutrophil activation by the plasma itself (Figure 2A).

Under physiologic conditions, flowing neutrophils do not interact with the resting endothelium. They adhere only in response to the local expression of adhesion molecules, chemokines, and bioactive lipids on the endothelial surface due to tissue inflammation and release of cytokines along the basal side of endothelium. The recruitment of neutrophils is thus restricted locally and cell activation is delayed until they have migrated and reached the site of inflammation.

Early Misleading Signals and Homotypic Aggregation
In AAV, excessive amounts of fluid phase inflammatory stimuli in plasma may provide misguided information to neutrophils, suggesting an intravascular infection and leading to premature cell activation. Levels of circulating TNF-\(\alpha\) and IL-8 are indeed increased in ANCA vasculitis.\textsuperscript{18,19} High concentrations of Fc-reactive ANCA could provide these misleading signals as suggested by the glomerular neutrophil adhesion observed in naive LPS mice upon the injection of high doses of anti-MPO antibodies.\textsuperscript{20} C5a may also play an important role because neutrophils activate complement and release C5a when stimulated by inflammatory cytokines.\textsuperscript{21} C5a is a strong neutrophil agonist triggering homotypic aggregation,\textsuperscript{22} which is favored by the presence of cell-bound C3 fragments interacting with CR1 and CR3 (Mac-1) receptors, for C3b and iC3b, respectively, on bystander neutrophils. Moreover, TNF priming triggers the switching of the \(\alpha M\beta 2\)-integrin (Mac-1) to an active conformation, allowing its binding to intracellular adhesion molecule 3 (ICAM-3) on adjacent neutrophils.\textsuperscript{23}

It is thus reasonable to propose an initial step of low-grade activation of circulating neutrophils (Figure 2B) promoted by TNF-\(\alpha\) and complement, allowing neutrophil-neutrophil interactions through \(\beta 2\)-integrin binding of iC3b and/or ICAM-3. This would result in a transient homotypic aggregation, similar to that observed in the Shwartzmann phenomenon\textsuperscript{24} and to that recently described after \textit{in vivo} injection of granulocyte-colony stimulating factor.\textsuperscript{25} These neutrophil-neutrophil interactions might be stabilized by ANCA, as the \(\beta 2\)-integrin–induced cell adhesion.\textsuperscript{26} This implies that \(\beta 2\)-integrin–mediated homotypic interactions would promote low ANCA antigen expression on circulating neutrophils, accessible to ANCA autoantibodies in serum, as has been observed during \(\beta 2\)-integrin–mediated adhesion.\textsuperscript{27} This would allow ANCA to bind neutrophils and trigger a massive activation otherwise normally expected at infected sites in tissues.
levels are reported in AAV with a negative correlation between IL-8 serum levels and CXCR1/CXCR2 neutrophil expression.29,30 In these conditions of high systemic secretion of inflammatory stimuli, triggering β2-integrin activation, circulating neutrophils adhere to the endothelium while they squeeze into capillaries before reaching the usual site of neutrophil emigration, particularly postcapillary venules. For those neutrophils having passed through capillaries, they interact in the classic way along selectin-expressing postcapillary venules (Figure 1), but their rolling is converted by ANCA into firm adhesion even at minimal TNF-α concentration.26,31,32 Firm adhesion mainly involves neutrophil β2-integrins interacting with ICAM-1 with a likely participation of α4β1 on mouse neutrophils or α9β1 on human neutrophils.33,34 Increased ICAM-1 and de novo vascular cell adhesion molecule 1 (VCAM-1) expression are indeed observed on glomerular endothelia from ANCA patients.35 Moreover, increased levels of β2 and β1-integrins are described on circulating neutrophils from patients with ANCA+, active GPA that dissipates with treatment.36

Figure 2. Neutrophil activation promoted by TNF-α and amplified by ANCA. (A) Homeostatic control of TNF-induced neutrophil activation in the blood flow. Plasma proteins and oxidants prevent untimely intravascular activation of neutrophils. In particular, serum albumin prevents the shedding of leukosialin (CD43), a prerequisite for neutrophil adhesion and inhibits most neutrophil responses to low concentration of inflammatory stimuli.125 Neutrophil degranulation and oxidative responses are delayed by the endogenous ceramide, generated upon TNF-α priming.126 Finally, anti-PR3 ANCA do not react with membrane PR3 in the presence of plasma127 and particularly of α1-antitrypsin.95 (B) Initial step of low-grade activation of circulating neutrophils involving cell-cell homotypic interactions. TNF-stimulated neutrophils activate the complement alternative pathway via the secretion of endogenous properdin.22 This releases the powerful agonist C5a, able to promote homotypic aggregation of circulating neutrophils,22 via interactions of the TNF-activated αMβ2 (Mac-1)-integrins with ICAM-3 or iC3b on bystander neutrophils.23 TNF/β2-integrin joint signals would promote sufficient ANCA antigen membrane expression to overcome the plasma inhibitory effect22 and allow the access of ANCA Fab to their antigen, whereas their Fc portion interacts with FcγRIIA receptors. By doing so, ANCA give the final signals for the firm adhesion of neutrophils to the endothelium.31 (C) Explosive responses of neutrophils adherent to endothelial cells in the presence of ANCA. Neutrophils then adhere to ICAM-1–expressing endothelial cells. During this firm adhesion, TNF/β2-integrin joint signals result in degranulation and oxidative burst37,128 and a massive increase of ANCA antigen expression, particularly mPR3, on the neutrophil surface.27 Neutrophil-bound ANCAs trigger the complement classic pathway (CP), exclusively on adherent neutrophils and in synergy with the alternative pathway (AP) activated on TNF-stimulated cells.45 C5a fragments and C5b-9 soluble complexes, released close to endothelial cells, are able to activate these cells and to promote cell retraction and endothelium permeability.98,100,129 The synergy between signals promoted by TNF, β2-integrin engagement, and ANCA-bound FcγR leads to an intense degranulation and the release of proteases and oxidants highly toxic for the endothelium.
Proinflammatory Effects of Adhesion: Amplification by ANCA and Complement

During neutrophil adhesion and spreading, β2-integrin outside signaling synergizes with TNF-induced signals to trigger neutrophil responses such as degranulation and oxidative burst (Figure 2C). As well described by Carl Nathan, “in contrast to results with cells in suspension, the responses of TNF-activated adherent neutrophils last over an hour and lead to accumulation of nearly as much antimicrobial product as can be elicited by bacteria or phorbol esters” (p 178).37

These inflammatory responses of adherent neutrophils are further enhanced by the presence of ANCA and of complement. Indeed, an important consequence of adhesion is the appearance, on the neutrophil surface, of high levels of antigens such as mPR3 that is accessible to ANCA in plasma.27 ANCA binding to membrane antigens, through their Fab portion, and to FcγR receptors, through theirFc portion, results in a further synergy between TNF-α, β2-integrins, and FcγR signaling (Figure 3), leading to a striking increase of ANCA antigen membrane expression27 and to an explosive oxidative burst.38,39 Both FcγRIIa and FcγRIIIB seem to be involved,39,41 although the latter is not essential because polymorphonuclear neutrophils from FcγRII–deficient individuals showed a normal oxidative response to ANCA antibodies.42 Differences have been observed between ANCA IgG subclasses, with an IgG3 > IgG1 > IgG4 order of efficiency to induce neutrophil static adhesion.43 IgG glycosylation, critical for the interaction with FcγRs, is important in ANCA effects and treatment with endoglycosidase S significantly decreased ANCA’s ability to activate neutrophils without interfering with their antigen-binding capacity.34 The synergy between TNF-α, β2-integrins, and FcγR was confirmed in vivo in experimental anti-MPO–induced AAV.6

Complement further amplifies the proinflammatory responses of adherent neutrophils in the presence of ANCA. Neutrophil-bound ANCA triggers the complement classic pathway exclusively on adherent neutrophils, a condition allowing access of ANCA to their antigens in plasma46 and in synergy with the alternative pathway activated on TNF-stimulated cells.51 Mice treated with a C5-inhibiting mAb16 or deficient in C5 or in the C5a-receptor,47,48 but not in the downstream component C6,49 are protected against anti-MPO GN. This underscores the important role of C5 fragments, C5a, which are released on the neutrophil surface and enhance cell responses such as degranulation and oxidative burst, creating an inflammatory amplifying loop.21 One should point out that C5a significantly enhances the oxidative burst promoted by FcyRIIa cross-linking, which implies the existence of crosstalk between C5aR and FcyRIIa receptors, possibly important in ANCA-mediated neutrophil activation.50 Interestingly, analogous mechanisms have been elegantly dissected in experimental rheumatoid arthritis.51

Consequences of the Biphasic Expression of Membrane PR3

An important point in ANCA-induced amplification of neutrophil activation is the heterogeneous membrane distribution of one of the main ANCA antigens, PR3, due to its association with CD177 (NB-1). PR3 and CD177 are coexpressed on the membrane of a neutrophil subset and the proportion of mPR3+/CD177+ neutrophils, variable in the normal population, is increased in AAV, a high proportion being a risk factor for relapses.32–36 Positive correlations are also reported between a high percentage of mPR3+ neutrophils and abnormal renal function parameters in patients with GPA.57 Priming with TNF-α increases the mPR3 expression in both CD177− and CD177+ subpopulations35 and the low level of mPR3 then present on CD177− neutrophils appears to be sufficient for some of the effects promoted by anti-PR3 antibodies, such as actin polymerization and oxidative bursts.55,58 More recently, anti-PR3 ANCA were shown...
to specifically trigger degranulation and extracellular release of superoxide from the CD177+ neutrophil subset by a mechanism involving β2-integrins.59,60 The requirement of cytochalasin b in these observations implies a reorganization of the actin cytoskeleton similar to that induced by neutrophil adhesion and spreading. It is thus tempting to speculate that anti-PR3 ANCA preferentially promotes the release of oxidants and enzymes by mPR3+/CD177+ neutrophils adherent through β2-integrins to endothelial cells.

The potential pathogenicity of mPR3+/CD177+ neutrophils is, however, not only related to the effect of anti-PR3 ANCA, because a high proportion of mPR3+/CD177+ neutrophils is observed in AAV regardless of the ANCA anti-PR3 or anti-MPO specificity and is also observed in lupus patients.53,55 The major reported difference between mPR3+/CD177+ and mPR3−/CD177− cells is the higher transendothelial migration of mPR3/CD177 positive neutrophils.59,61 This difference could be related to the ability of CD177 to bind CD31 (PECAM-1) on endothelial cells and to trigger PECAM-1−induced signals, which modify the cell junctional integrity and facilitate neutrophil transmigration.61,62

In conclusion, ANCA antibodies lower the threshold of neutrophil responses to inflammatory cytokines and result in a vigorous response in the vascular bed before any diapedesis and migration. The cumulative effect of ANCA and chemokines has been shown in GPA, where heterozygous CCR5 deletion is never observed in ANCA-negative patients.63 To note, CCR5 is present on TNF-activated neutrophils.64

**SPECIFICITIES OF GLOMERULAR VERSUS PERITUBULAR MICROVESSELS**

In most tissues, leukocyte-endothelial interactions associated with inflammatory responses do not occur in capillaries, but are predominant in postcapillary venules, which are more favorable in terms of vessel size (15–50 μm) and rheology. Usually intravital models analyze such events by videomicroscopy acute preparations of mesenteric or cremasteric microvessels. Those models are partially relevant to AAV where renal and lung capillaries are primarily involved. New protocols of intravital imaging65 delineate neutrophil recruitment in capillaries in brain, lung, liver,66,67 and kidney, where unilateral ureteric ligation permits visualization of glomeruli in the hydronephrotic kidney.68

**Renal Capillaries**

AAV preferentially involves capillaries, venules, and arterioles (Figure 4). Two distinct types of renal lesions are seen: glomerular necrosis versus peritubular inflammatory capillaritis. Inflammatory tubulointerstitial lesions are more pronounced in AAV, where AKI is largely reversible, than in anti-glomerular basement membrane (GBM) GN, where AKI is mainly due to irreversible glomerular lesions. Interestingly, peritubular inflammatory capillaritis may be observed in AAV with few and even sometimes without any glomerular lesions.69

Thus, by analogy with the threshold model proposed in transfusion-related acute lung injury,70 we suggest that AAV capillary lesions evolve with increasing levels of neutrophil priming and activation (TNF-α, β2-integrins, chemokines, and ANCA levels). We hypothesize that the threshold is higher for glomerular sequestration than for peritubular inflammatory capillaritis.

**Nonconventional Pathways of Leukocyte Recruitment in Glomeruli**

Leukocyte adhesion is likely to be different in glomerular and lung capillaries compared with postcapillary venules. Because of the unique structure of glomerular capillaries, including the absence of P-selectin,71 leukocyte recruitment in glomeruli bypasses the requirement for initial rolling interactions. Both physical trapping and immediate arrest involving adhesion molecules have been demonstrated in different models (Figure 5). In particular, the trapping of neutrophils in microvessels (Figure 5A) may explain the ANCA-associated accumulation of neutrophils in the glomerulus6; it does not involve endothelial adhesion molecules and is reminiscent of selectin-independent neutrophil sequestrations observed in pulmonary capillaries.72 Participation of platelets in neutrophil adhesion to the endothelium has been observed in experimental anti-GBM GN (Figure 5B). This model, however, cannot be extrapolated to the ANCA situation, because the primary event is the endothelial lesions in the anti-GBM model and the neutrophil activation in AAV; a peculiar mechanism of glomerular leukocyte adhesion, mediated by the α4β1-integrin, has been observed in LPS-free mice injected with high doses of anti-MPO (Figure 5C) and is reminiscent of chronic vasculitis induced by *Mycobacterium butyricum* in rats73 and anti-MPO injection to LPS-treated mice results in a glomerular β2-integrin-dependent leukocyte adhesion (Figure 5D). These collected data indicate that ANCA can induce leukocyte adhesion in glomerular capillaries through multiple pathways and raise the possibility than antiadhesion molecule antibodies might represent potential therapies in AAV.

**Interstitial Postcapillary Venules and Peritubular Capillaries**

The ability of ANCA to promote conversion from rolling to adhesion in flow chambers in vitro,26,32 and to enhance chemokine-induced adhesion and transmigration both in mesenteric74 and cremasteric microvessels in vivo, suggests the canonic three-step neutrophil adhesion model (Figure 1) is operating in AAV in postcapillary venules (Figure 4). Peritubular capillaries are potentially recruited because they exhibit postcapillary venule-like transformation that enhances the influx of inflammatory cells after kidney allograft rejection,75,76 after renal ischemia-reperfusion,77,78 and in inflammatory and crescentic GN.79,80 The importance of neutrophil recruitment and transmigration into the kidney interstitium has been recognized in human AAV.81
UNWANTED NEUTROPHIL-ENDOTHELIUM INTERACTIONS LEAD TO ENDOTHELIAL LESIONS

Neutrophil-Mediated Endothelial Cell Lesions

One of the hallmarks of AAV is massive endothelial injury, mainly produced by neutrophils and resulting in necrotizing vasculitis (Figure 6A). Neutrophil firm adhesion transmits signals to the endothelium through β2-integrins/ICAM-1 interactions, leading to a rise of cytosolic free Ca²⁺ and cytoskeletal changes required for neutrophil diapedesis.⁸² This signaling produces a microvascular permeability, which is further enhanced by proteases and oxidants released at the immediate proximity of endothelial cells during TNF-induced adhesion in the presence of ANCA.⁸³ Vascular permeability leads to subendothelial edema that is observed in AAV.⁸¹ After these dissociations of cell-cell junctions, endothelial apoptosis and detachment are promoted by neutrophil-derived proteases and oxidants.⁸⁴⁻⁹⁰ Neutrophil secretion of these toxic oxidants and enzymes is greatly enhanced by ANCA⁹ and large numbers of circulating endothelial cells have been observed in patients, their levels decreasing with remission.⁹¹ Endothelial cell detachment results in denudation of the subendothelial matrix leading to fibrin deposition as seen by electron microscopy in AAV glomeruli,⁹² which implies platelet recruitment and thrombosis.

Plasma protease inhibitors, such as α₁ antitrypsin (α₁AT), regulate the toxicity of serine proteases released during neutrophil degranulation. This could, in part, explain the predisposition to ANCA-positive GPA reported for α₁AT deficiency alleles Z and S.⁹³,⁹⁴ Moreover, α₁AT prevents the anti-PR3–mediated neutrophil activation⁹⁵ and release of IL-8 by monocytes.⁹₆ Finally, α₁AT was recently shown to control neutrophil activation independently of ANCA by binding IL-8 and preventing its reaction with its receptor CXCR1.⁹⁷ All of these activities imply that α₁AT deficiency favors neutrophil-induced inflammation, particularly in the presence of ANCA.

Role of Complement

Although endothelial cells themselves do not trigger the complement cascade, their close contact with adherent neutrophils activates surface complement and may result in complement-mediated endothelial injury (Figure 2C). Indeed, C5a receptors are present on endothelial cells and C5a induces rapid P-selectin membrane expression,⁹₈ cell retraction and increased endothelial permeability,⁹⁹,¹⁰⁰ and the expression of different genes encoding for adhesion molecules, cytokines, chemokines, and related receptors.¹⁰¹

Role of Platelets

Neutrophil-platelet interactions play an important role during inflammation¹⁰² and provide a link between thrombosis and inflammation¹⁰³ and between inflammation and immune response.¹⁰⁴
During rolling and crawling steps, activated endothelium induces neutrophil polarization. Active \( \beta_2 \)-integrins, clustered at their leading edge, capture red cells and platelets resulting in cell aggregates.\(^{102} \) In addition, platelets bind exposed collagen during vascular injury and express very large amounts of P-selectin that avidly recruits neutrophils.\(^{104} \) Thus, additional platelets may accumulate in the glomerular microvasculature through neutrophil-platelet interactions.

The involvement of platelets in AAV is suggested by the increased frequency of venous thromboembolic events in GPA. In the Wegener’s Granulomatosis Eta-nercept Trial, including 180 patients, 7.0 venous thromboembolic events per 100 patient-years were reported with thrombosis occurring during a period of active disease.\(^{105} \) Plasma levels of platelet-derived soluble CD40L and P-selectin positively associated with the activity of disease in GPA, underscoring the participation of platelet activation in AAV.\(^{106} \)

Neutrophil-Platelet Microparticles

Membrane microparticles, released in vitro by fMLP or C5a-stimulated neutrophils, carry a large set of cell adhesion molecules and proteases such as PR3 or elastase\(^{107} \) that activate complement.\(^{21} \) These interactions suggest a dissemination of neutrophils’ toxic effects, although they were initially reported to have anti-inflammatory activities.\(^{108,109} \)

Microparticles released by TNF-primed neutrophils in the presence of ANCA bind endothelial cells and trigger expression of adhesion receptors and production of oxidants and inflammatory cytokines that generate thrombin in plasma.\(^{110} \) Microparticles released by activated or apoptotic neutrophils circulate in the plasma of patients with AAV and their level is strikingly increased during acute vasculitis.\(^{111} \) Most of these neutrophil microparticles form aggregates with platelet-derived microparticles and express neutrophil and platelet-adhesion receptors with potential prothrombotic and proinflammatory activities.

Figure 5. Nonconventional pathways of leukocyte recruitment in glomeruli. (A) Intravascular physical trapping independently of endothelial adhesion molecules. Neutrophil sequestration in capillaries results from ANCA-induced actin polymerization (green line) and increased cell rigidity\(^{28} \) and homotypic aggregation, mediated by TNF-activated \( \beta_2 \)-integrins interacting with ICAM-3 on bystander neutrophils.\(^{23} \) (B) Glomerular leukocyte adhesion induced by anti-GBM in mice and involving platelets. After anti-GBM antibody\(^{68} \) or immune complex\(^{136} \) deposition, platelets adhere to the glomerular endothelial lesions via platelet glycoprotein VI, fibrinogen being a potential ligand. Activated platelet express P-selectin, which recruits neutrophils by binding PSGL-1. The resulting neutrophil adhesion, which also involves \( \beta_2 \)-integrin/ICAM-1 interactions but bypasses the initial requirement for rolling, is restricted to glomerular capillaries and not observed in post-capillary venules. (C) Glomerular leukocyte adhesion induced by high doses of anti-MPO in mice. Infusion of high doses of anti-MPO antibody to LPS-free mice resulted in a glomerular leukocyte adhesion, which was \( \beta_2 \)-integrin independent, but instead required neutrophil \( \alpha_4 \)-integrin and an unknown ligand distinct from VCAM-1.\(^{20} \) Human neutrophils do not express \( \alpha_4 \beta_1 \), except in extreme septic conditions,\(^{33,34} \) but they express the \( \alpha_9 \beta_1 \)-integrin, which has similar specificities.\(^{33,34} \) (D) Anti-MPO–induced glomerular leukocyte adhesion in LPS-treated mice. In this AAV experimental model, the synergistic effect of LPS with anti-MPO antibodies is directly related to both LPS-induced TNF synthesis\(^{11} \) and to increased expressions of CXCL1, CXCL2 (homologs of human IL-8), which participate in neutrophil glomerular recruitment.\(^{138} \) TNF-primed neutrophils, bearing anti-MPO antibodies, adhere to the endothelium of glomerular capillaries, via the \( \beta_2 \)-integrin LFA-1 interacting with endothelial ICAM-1.\(^{20} \)
Transfer from Innate to Adaptive Immune Response

Neutrophils are a key component of innate immunity and contribute to the crucial decision to set up an adaptive immune response.\textsuperscript{112} As shown in Figure 6B, neutrophils recruit dendritic cells, monocytes, and lymphocytes, which are important in the promotion of granuloma formation\textsuperscript{113} and autoimmunity in AAV. A surprising number of patients with anti-GBM disease are known to express ANCA. The recent description of anti-PR3 and anti-MPO antibodies, long before the onset of anti-GBM injury, suggests that ANCA plays an antecedent role in glomerular pathophysiology.\textsuperscript{114} ANCA may induce low-grade intravascular activation, capillary lesions, disruption of the basement membrane with exposure of cryptic antigens, and possibly recruitment of immune cells involved in autoimmunization and epitope spreading. This epitope spreading driven by ANCA long before the onset of anti-GBM disease may be involved in AAV.

**NETosis and Endothelial Damage**

Upon activation, neutrophils release webs of DNA and histones, called neutrophil extracellular traps (NETs) (Figure 6C) that are designed to trap bacteria.\textsuperscript{115} Premature formation of NETs within capillaries induces endothelial cell injury\textsuperscript{116,117} and thrombosis by stimulating platelets.\textsuperscript{118}

The ANCA-mediated activation of TNF-primed neutrophils induces NETs formation, as shown \textit{in vitro} and in kidney biopsies of patients with AAV, where typical components of NETs, DNA, histones, and proteins from neutrophil granules are found in affected glomeruli and in the interstitium.\textsuperscript{119} This presence of NETs in blood vessels, in the absence of microbial infection, further confirms that neutrophils integrate the conjunction of signals from TNF, ANCA (Fc receptor), and \( \beta_2 \)-integrins as alarm signals similar to that sent by infectious microorganisms.

In this review, we favor a functional paradigm for the ANCA antigen: a neutrophil intracellular antigen, whose membrane expression is induced in an explosive manner by the autoantibody itself followed by four signaling pathways are recruited simultaneously: neutrophil preactivation by TNF-\( \alpha \); \( \beta_2 \)-integrin engagement during homotypic aggregation and adhesion to the microvascular endothelium; activation of the complement alternative pathway, releasing C5a, and; Fc\( \gamma \)RII receptor recruitment by ANCA.
autoantibodies. These events result in neutrophil misleading and maximal activation within microvessels leading to endothelial lesions, recruitment of adaptive immunity, and NETs formation.

Given these activation mechanisms and their consequences, the implications for therapy are diverse. The participation of neutrophil β2 and possibly α9β1-integrins raises the possibility that anti-integrin antibodies could represent potential therapies in AAV. The observation that anti-C5 antibodies or C5aR antagonists prevent anti-MPO–induced GN in mice suggests that eculizumab could also be an efficient treatment. Targeting the IgG3 subclass could be a therapeutic strategy because ANCA IgG3 is the most powerful neutrophil activators. The ability of neutrophils to recruit dendritic cells, monocytes, and lymphocytes to set up a physiologic antiinfectious adaptive immune response perpetuates a chronic autoimmune in patients with AAV. This may explain the favorable effect of anti-B cell therapy and suggests that a therapy directed at T cells may also improve the renal outcome in AAV. Finally, the fact that NET formation involves oxygen reactive species suggests that reactive oxygen species scavengers prevent or at least limit endothelial damage and chronic autoimmunity in AAV.

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DISCLOSURES

None.

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