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Pathogenesis of Antineutrophil Cytoplasmic Autoantibody–Associated Small-Vessel Vasculitis

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Abstract

Clinical, in vitro, and experimental animal observations indicate that antineutrophil cytoplasmic autoantibodies (ANCA) are pathogenic. The genesis of the ANCA autoimmune response is a multifactorial process that includes genetic predisposition, environmental adjuvant factors, an initiating antigen, and failure of T cell regulation. ANCA activate primed neutrophils (and monocytes) by binding to certain antigens expressed on the surface of neutrophils in specific inflammatory microenvironments. ANCA-activated neutrophils activate the alternative complement pathway, establishing an inflammatory amplification loop. The acute injury elicits an innate inflammatory response that recruits monocytes and T lymphocytes, which replace the neutrophils that have undergone karyorrhexis during acute inflammation. Extravascular granulomatous inflammation may be initiated by ANCA-induced activation of extravascular neutrophils, causing tissue necrosis and fibrin formation, which would elicit an influx of monocytes that transform into macrophages and multinucleated giant cells. Over time, the neutrophil-rich acute necrotizing lesions cause the accumulation of more lymphocytes, monocytes, and macrophages and produce typical granulomatous inflammation.

Keywords

autoimmunity; inflammation; immunopathology; microscopic polyangiitis; granulomatosis with polyangiitis; eosinophilic granulomatosis with polyangiitis

INTRODUCTION

Antineutrophil cytoplasmic autoantibodies (ANCA) bind to antigens in the primary granules of neutrophils and the peroxidase-positive lysosomes of monocytes (1). Myeloperoxidase (MPO) and proteinase 3 (PR3) are two major antigens recognized by ANCA in patients with vasculitis and glomerulonephritis (1–3). Lysosomal-associated membrane protein 2 (LAMP2) has also been proposed as a major target for ANCA (4), but this hypothesis remains controversial (5). ANCA are associated with a pathologically and immunopathologically distinctive form of vasculitis that is characterized in the acute phase

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by necrotizing inflammation with a paucity of vessel wall immunoglobulin detectable by immunohistologic methods (6, 7). ANCA-associated vasculitis (AAV) affects predominantly small vessels in any organ of the body, including small arteries, arterioles, venules, and veins. Although single-organ AAV occurs in, for example, renal-limited disease, most patients have systemic disease that can be classified on the basis of clinical and pathologic features as microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA; also known as Wegener's granulomatosis), or eosinophilic granulomatosis with polyangiitis (EGPA; also known as Churg–Strauss syndrome) (8, 9). Acute renal-limited disease is characterized by glomerulonephritis with fibrinoid necrosis, crescent formation, and the absence or paucity of immunoglobulin deposition. This pauci-immune necrotizing and crescentic glomerulonephritis (NCGN) also frequently occurs as a component of MPA and GPA and, less frequently, as a component of EGPA.

Current clinical analytical methods have revealed that at least 80% to 90% of MPA, GPA, and renal-limited pauci-immune NCGN patients have ANCA, as do approximately 40% of EGPA patients. However, more than 90% of patients with EGPA who have NCGN have ANCA (10).

Each clinicopathologic variant of AAV can be associated with either MPO-ANCA or PR3-ANCA. In North America and Europe, PR3-ANCA cases are more frequent than MPO-ANCA in GPA patients, whereas MPO-ANCA are more frequent than PR3-ANCA in MPA, EGPA, and renal-limited pauci-immune NCGN patients (11). In the same regions, the frequency of MPO-ANCA relative to that of PR3-ANCA increases from north to south (11). In Asia, MPO-ANCA is much more frequent relative to PR3-ANCA than in Europe and North America (11). The pathologic features of acute AAV suggest that neutrophils play an important pathogenic role (6). Clinical, in vitro, and animal model observations strongly support a role for ANCA in the pathogenesis of AAV.

PATHOLOGY OF ANCA-ASSOCIATED VASCULITIS

AAV is a necrotizing small-vessel vasculitis (SVV) that affects predominantly capillaries, venules, arterioles and small arteries, and (less often) medium arteries and veins (8, 9, 12). Large-vessel vasculitis (LVV) (e.g., giant cell arteritis and Takayasu arteritis) affects predominantly the aorta and its major branches (9). Medium-vessel vasculitis (e.g., polyarteritis nodosa and Kawasaki disease) affects predominantly the main visceral arteries leading to major organs and their initial branches within an organ or tissue (9). SVV affects predominantly small vessels (often microscopic) that are within organs and tissues, with a predilection for capillaries and venules. In addition to AAV, which typically has a paucity of immunoglobulin deposited in vessel walls, the SVV category also includes various vasculitides that have conspicuous vessel wall immunoglobulin and complement deposits, such as Henoch–Schönlein purpura vasculitis (IgA vasculitis), cryoglobulinemic vasculitis, and anti–glomerular basement membrane disease (anti-GBM disease) (8). The extensive vessel wall immunoglobulin and complement deposits, which may be antibody-complexed with antigens and activated complement components, appear to cause vasculitis by mediating humoral and cellular inflammatory processes. The paucity of immunoglobulin in AAV suggests a different pathogenic mechanism. Importantly, however, most patients with

AAV have at least some focal immunoglobulin and complement deposition at sites of vasculitis. In addition, some AAV patients have concurrent immune complex SVV or anti-GBM disease, in which case there is extensive vessel wall immunoglobulin associated with ANCA (13). The histopathologic patterns of injury in AAV have shed light on pathogenic mechanisms, and pathogenic mechanisms should explain pathologic observations.

Microscopic Polyangiitis

MPA is pauci-immune SVV in the absence of evidence for GPA or EGPA (8, 9). Small vessels in any organ or tissue can be affected. Frequent examples are dermal venulitis causing purpura, pulmonary alveolar capillaritis causing hemoptysis, NCGN causing rapidly progressive glomerulonephritis, and epineural arteritis causing mononeuritis multiplex. Patients may initially have single-organ involvement that may or may not progress to systemic disease. Renal-limited NCGN occurs in up to one-quarter of patients with AAV. For a diagnosis of MPA, there should be no evidence for GPA or EGPA.

The acute vascular lesion has similar features in all vessels and is characterized by localized in-flux of neutrophils with leukocytoclasia, as well as vessel wall necrosis, often with accumulation of material containing fibrin (Figure 1*a–c*) that has formed from the activation of coagulation factors in plasma that have spilled from the lumen and contacted thrombogenic substances, including tissue factor, in necrotic vessel walls and adjacent tissue. Within a week, acute lesions are transformed into lesions that contain predominantly monocytes, macrophages, and T lymphocytes, which progress to fibrotic (sclerotic) lesions. Thus, in most biopsy specimens that are obtained within days of disease onset, the lesions of AAV contain mostly monocytes, macrophages, and T cells, although the preceding acute lesion contained predominantly neutrophils. In a given tissue, AAV often has multiple vasculitic lesions ranging from acute necrotizing lesions with neutrophilic leukocytoclasia to mononuclear leukocyte-rich lesions to fibrotic lesions. Pathologically, these observations are readily made in kidney tissue with glomerular lesions ranging from acute lesions with segmental fibrinoid necrosis to chronic lesions with segmental sclerosis. A Masson trichrome stain is useful to distinguish between fibrinoid necrosis (fuchsinophilic) (Figure 1*c*) and sclerosis (blue or green) (Figure 1*d*). Clinically, the different times of onset are easily observed in the sequential crops of dermal angiitis and result in lesions of different ages, ranging from acute hemorrhagic raised purpuric lesions to chronic pigmented macules.

The characteristic pulmonary lesion of MPA is hemorrhagic alveolar capillaritis (Figure 2*a,b*). Histologically, there are focal areas with increased neutrophils in alveolar capillaries and areas of lysis of capillaries with residual neutrophils and leukocytoclastic debris. Special stains that highlight alveolar capillary basement membranes, such as Jones silver stain, demonstrate focal lysis (Figure 2*b*). As in other tissues, pulmonary vasculitic lesions can be of different ages and may contain, for example, focal acute capillaritis along with focal alveolar septal fibrosis.

Granulomatosis with Polyangiitis

The vasculitis of GPA can be pathologically identical to that of MPA. GPA differs from MPA by the presence of necrotizing granulomatous inflammation that can affect vessels or

appear exclusively in extravascular tissue (Figure 2*c,d*). Recently, the preferred name for this category of vasculitis changed from Wegener's granulomatosis to GPA (14).

GPA granulomatous inflammation is most common in the upper or lower respiratory tract but can occur anywhere, including the orbit, skin, and meninges. Such granulomatous inflammation of GPA, and EGPA, is pathologically very different from that typically observed with sarcoidosis, mycobacterial infection, and fungal infection (15). The acute lesions have intense neutrophilic infiltration that resembles abscess formation, rather than a monocyte- and T cell-rich cell-mediated immune response (7, 15). The primary granulomatous feature in the acute phase is the presence of multinucleated giant cells (Figure 2*c*). Acute lesions may have focal accumulations of fibrinoid material, indicating substantial vascular exudation or vascular disruption, even though necrotic vessels are not identifiable in the lesions. This pattern of extravascular necrosis in the absence of an identifiable associated vessel has been termed pathergic granulomatosis (15, 16).

As the lesions progress, they develop more classic features of granulomatous inflammation; there are palisading macrophages and giant cells at the margins of zones of necrosis that are composed of amorphous necrotic debris (Figure 2*d*) (15). At low magnification, larger zones of necrosis have an irregular outline that is referred to as geographic necrosis. The term granulomatosis has been frequently used in the medical literature to refer to this characteristic pattern of necrotizing granulomatous inflammation observed in GPA and EGPA. Granulomatosis that is pathologically identical to that observed in systemic GPA and EGPA may occur as an isolated process, usually in the respiratory tract, which is considered a localized expression of GPA or EGPA. Interestingly, granulomatosis occurring in the absence of identifiable vasculitis or glomerulonephritis is less often associated with ANCA (10).

Eosinophilic Granulomatosis with Polyangiitis

EGPA (also known as Churg–Strauss syndrome) is characterized by a nonvasculitic prodrome of asthma and eosinophilic inflammation, such as eosinophilic pneumonia or eosinophilic gastroenteritis (8, 9). The vasculitis and glomerulonephritis of the vasculitic phase of EGPA may be indistinguishable from those of MPA and GPA, although they usually have much more intense infiltration of eosinophils as a component of the inflammation. The necrotizing granulomatous inflammation of EGPA also resembles that of GPA, although in the former there are almost always more numerous eosinophils than in the latter. However, pathologic identification of numerous eosinophils in granulomatous inflammation or vasculitis is not adequate for a diagnosis of EGPA; a history of asthma and blood eosinophilia, along with granulomatosis, is required.

CLINICAL EVIDENCE FOR ANCA PATHOGENICITY

The high frequency of ANCA in patients with very distinctive pathologic lesions suggests the possibility, but does not prove, that the production of ANCA is involved in the pathogenesis of these lesions. However, a contradictory finding, as revealed by current clinical analytical methods, is that a minority of patients with clinical and pathologic features of ANCA-associated vasculitis do not have ANCA. More incriminating is the

correlation of ANCA titers with response to treatment and with recurrence of disease (17), but this correlation is not uniform (18).

The efficacy of anti-B cell therapy and of plasma exchange in treating ANCA-associated vasculitis is consistent with an important role for antibodies in pathogenesis. For example, treatment with anti-CD20 humanized antibodies, which dramatically depletes B cells and lowers circulating antibody levels, is an effective therapy for AAV (19, 20). Plasma exchange, which reduces circulating ANCA titers, also is beneficial (21).

The pathogenicity of ANCA was suggested by the finding of clinical evidence for pulmonary and renal disease in a neonate who acquired circulating MPO-ANCA by transplacental passage from a mother with MPA (22); however, no additional cases have been reported. A case of a neonate who had transplacental transfer of ANCA with no symptoms of vasculitis has been reported (23).

Specific drugs induce ANCA formation; these include propylthiouracil, allopurinol, D-penicillamine, hydralazine, and levamisole (which may be a contaminant of cocaine) (24). Patients with drug-induced ANCA may develop lesions that are indistinguishable from those of MPA, GPA, or EGPA (24, 25).

IN VITRO EVIDENCE FOR ANCA PATHOGENICITY

ANCA target antigens in both neutrophils and monocytes. For ANCA to be pathogenic, one logical theory is that they must interact with neutrophils and monocytes and cause them to attack vessels, resulting in vasculitis. Numerous experiments have confirmed that ANCA activate both neutrophils and monocytes in vitro.

Incubation of normal human neutrophils with MPO ANCA immunoglobulin G (IgG) or PR3-ANCA IgG results in activation, causing a respiratory burst that generates toxic oxygen radicals and degranulation that releases numerous destructive enzymes (25–27). Not all ANCA are equally effective in activating neutrophils in vitro. ANCA IgG from AAV patients with active disease cause more in vitro activation than do ANCA from patients in remission (27). This finding suggests that there may be certain ANCA antibody classes or epitope specificities that are more pathogenic than others.

Activation of neutrophils by ANCA requires the availability of low numbers of antigens at the neutrophil surface to interact with antibodies. Some antigens, especially PR3, may be present constitutively on normal neutrophils (28); however, neutrophils must be stimulated (primed) by inflammatory stimuli (e.g., cytokines) to release ANCA antigens at the surface or in the nearby microenvironment before the neutrophils can be fully activated by these antibodies. The surface display of ANCA antigens may involve binding to specific surface receptors (29). The requirement for increased surface availability for neutrophil activation by ANCA is demonstrated in vitro by markedly enhanced activation of neutrophils by ANCA IgG after priming with low doses of tumor necrosis factor α (TNF- α), which induces surface release and binding of MPO and PR3 from neutrophils (25, 26). Interaction of ANCA with antigens may also be facilitated by an increase in the expression of MPO and PR3 genes in the circulating neutrophils of AAV patients (30). Normally, expression of MPO

and PR3 genes is suppressed before mature neutrophils leave the bone marrow (BM). However, AAV patients have abnormal epigenetic regulation of this suppression, which causes the continued expression of MPO and PR3 genes in circulating neutrophils. This continued expression, in turn, may enhance interaction with ANCA (30).

Once ANCA binds to antigen(s), neutrophil activation is induced both by Fc receptor engagement (31) and by cross-linking of Fab'2 (32). Fc receptor engagement is probably the most important step (33). Fc receptor engagement occurs (*a*) between ANCA bound to the surface of neutrophils and adjacent Fc receptors on the undulating surface of the same neutrophil, (*b*) between neutrophil Fc receptors and ANCA bound to antigens on the surface of adjacent neutrophils and endothelial cells, and (*c*) with free floating complexes of ANCA and ANCA antigens in the microenvironment of the inflammation. MPO- and PR3-ANCA antigens are released from neutrophils at sites of inflammation and bind to endothelial cells (34, 35). This process results in localized in situ formation of complexes that augment inflammation, including complement-mediated inflammation (34). The finding that most AAV patients with NCGN have at least small amounts of immune complexes supports a role for local immune-complex formation in ANCA vasculitis and glomerulonephritis (36). Neutrophil extracellular traps (NETs) that contain chromatin fibers and neutrophil proteins, including MPO and PR3, are released by ANCA-activated neutrophils and can be identified at the sites of injury in ANCA NCGN kidney biopsy specimens (37). ANCA antigens in NETs can serve as targets for localized in situ immune-complex formation. Although AAV has a paucity of immunoglobulin deposits compared with those found in immune-complex vasculitis and anti-GBM vasculitis, most patients have deposits at the sites of inflammation and necrosis. In contrast, immune-complex vasculitis and anti-GBM vasculitis patients have deposits of immunoglobulin throughout the involved microvasculature, not preferentially at the sites of inflammation.

Endothelial injury by ANCA-activated neutrophils has been demonstrated in multiple in vitro systems (38–40). Incubation of neutrophils and ANCA IgG with endothelial monolayers causes the death of endothelial cells. This process is facilitated by cytokine priming of both neutrophils and endothelial cells. Flow-based adhesion assays have demonstrated that ANCA can stimulate neutrophils to adhere to and penetrate through endothelial monolayers, mediated by integrins and chemokines, which simulates events that occur in AAV (41).

Although most in vitro studies of leukocyte activation by ANCA have focused on neutrophils, in vitro studies have also shown that ANCA IgG can activate monocytes (26, 42–46). These studies have revealed that ANCA antigens (PR3 and MPO) are downregulated during the transformation of monocytes into macrophages. This downregulation indicates that ANCA can interact directly only with monocytes and early exudative macrophages, not with mature macrophages. As we review in the next section, animal model studies indicate that neutrophils and not monocytes are sufficient and necessary for ANCA-mediated vascular inflammation.

ANIMAL MODEL EVIDENCE FOR ANCA PATHOGENICITY

As reviewed above, beginning soon after the discovery of ANCA, numerous in vitro experiments readily documented that ANCA IgG can activate neutrophils and monocytes to mediate inflammatory processes that would be required to induce vasculitis in vivo. However, a convincing animal model for AAV was not developed until 2002, when a mouse model of MPO-ANCA disease was described (47). A clear-cut animal model of PR3-ANCA disease is still lacking, although several putative models have been proposed (48).

Antimyeloperoxidase Immunoglobulin G Induces Pauci-Immune Necrotizing and Crescentic Glomerulonephritis and Small-Vessel Vasculitis in Mice

The first mouse model of ANCA disease was produced through the induction of an immune response to MPO in mice that had a knockout of the *MPO* gene (MPO KO mice) (47). MPO KO mice immunized with MPO developed a robust MPO immune response with high titers of circulating anti-MPO antibodies. After adequate doses of these anti-MPO antibodies were injected intravenously into wild-type (WT) C57BL/6J (B6) mice, within 6 days all the mice developed hematuria and proteinuria and, at postmortem examination, had NCGN that was identical by light microscopy and immunohistology to pauci-immune NCGN in AAV patients (Figure 3*a,b*) (47). Although all the B6 mice developed NCGN, on average only 10% of glomeruli were affected. Other strains of mice (for example, 129S6/SvEv) developed more severe NCGN in which more than 60% of glomeruli were affected (49). In addition to pauci-immune NCGN, a minority of mice injected with anti-MPO IgG developed systemic SVV with vasculitis in multiple organs, for example, leukocytoclastic angiitis in the skin (Figure 3*c*), hemorrhagic pulmonary alveolar capillaritis (Figure 3*d*), and arteritis affecting small arteries in multiple organs (47). These mice with systemic SVV resembled AAV patients with MPA, whereas the mice with NCGN but no evidence of systemic SVV resembled AAV patients with renal-limited disease. A few mice that had received anti-MPO IgG developed granulomatous inflammation resembling GPA.

NCGN and systemic SVV were also induced by intravenous injection of anti-MPO IgG into recombinase-activating gene 2 knockout (Rag2 KO) B6 mice that lacked both functioning B lymphocytes and T lymphocytes (47). There was no difference between the NCGN in these mice and that in WT B6 mice, indicating that functioning T cells are not required for the pathogenesis of the NCGN or SVV in this animal model.

In the same study, NCGN and systemic SVV were induced by the transfer of splenocytes from MPO KO mice that had been immunized with MPO but not by transfer of splenocytes from either MPO KO mice immunized with bovine serum albumin or nonimmunized control mice (47). The splenocytes contained both B cells and T cells that populated the Rag2 KO mice. After 13 days, all the mice that had received anti-MPO or control splenocytes developed mild to moderate glomerular immune-complex deposits, but only mice that had received anti-MPO splenocytes developed severe NCGN; granulomatous inflammation; and systemic necrotizing vasculitis, including necrotizing arteritis and hemorrhagic pulmonary capillaritis. The glomerular immune-complex deposits apparently arise from the introduction of a competent immune system into previously immune-deficient mice and may be a result of the clearance of circulating antigens that had not been cleared in the immune-deficient

mice. This background of glomerular immune-complex deposits may have acted synergistically with the anti-MPO IgG to cause more severe disease in the Rag2 KO mice that received anti-MPO splenocytes (approximately 80% glomerular crescents), compared with Rag2 KO mice that received anti-MPO IgG (approximately 10% crescents), even though both had similar titers of circulating anti-MPO.

Neutrophils Are Required and Sufficient to Mediate Antimyeloperoxidase Immunoglobulin G–Induced Pauci-Immune Necrotizing and Crescentic Glomerulonephritis and Small-Vessel Vasculitis in Mice

To evaluate the role of neutrophils in anti-MPO-induced murine NCGN and SVV, WT B6 mice were injected intravenously with a single dose of neutrophil-specific NIMP-R14 rat monoclonal antibody prior to injection of anti-MPO IgG (50). Within 16 h, NIMP-R14 markedly depleted circulating neutrophils but not monocytes. The mice received intravenous murine anti-MPO IgG 16 h after injection of either NIMP-R14 or control rat IgG. After 5 days, the mice injected with anti-MPO IgG without neutrophil depletion developed hematuria and proteinuria, whereas the mice that were depleted of circulating neutrophils by NIMP-R14 had no urine abnormalities. Levels of circulating anti-MPO IgG in the neutrophil-depleted mice were similar to those in the control mice. All the mice that received normal rat IgG before receiving anti-MPO developed NCGN, whereas none of the mice that had neutrophil depletion with NIMP-R14 developed NCGN. These observations indicate that neutrophils are required to mediate anti-MPO-induced NCGN and that monocytes are not sufficient to mediate the injury. This finding does not exclude the possibility that monocytes contribute to lesion induction; it shows only that they are not sufficient to cause injury in this model system.

Glomerular leukocyte immunohistologic phenotyping revealed that mice that received anti-MPO without neutrophil depletion had increased glomerular infiltration by neutrophils (Figure 3*b*) and by monocytes and macrophages, but not by lymphocytes. Neutrophils were most numerous in glomeruli with inflammation and necrosis, and macrophages clustered within crescents (50).

Another mouse model was used to determine whether or not BM-derived cells are sufficient to cause anti-MPO-induced NCGN and SVV in the absence of MPO in other cell types (51). At the time, there was controversy over whether or not endothelial cells produced ANCA antigens and whether they were an important target for ANCA. Anti-MPO IgG was injected intravenously into chimeric mice created by transplanting WT BM into irradiated MPO KO mice or MPO KO BM into irradiated WT mice. Chimeric MPO KO mice with circulating MPO-positive neutrophils developed NCGN, whereas chimeric WT mice with circulating MPO-negative neutrophils did not. This observation indicating that BM-derived cells are not only sufficient but also required for induction of NCGN by anti-MPO IgG (51).

Above, we describe the synergistic effect of neutrophil priming (see the section titled *In Vitro Evidence for ANCA Pathogenicity*). For example, TNF primes neutrophils for surface display of ANCA antigens, which facilitates interaction with ANCA and resultant neutrophil activation (26, 27). This synergistic effect has been confirmed by use of the mouse model of anti-MPO-induced NCGN. Intravenous injection of bacterial lipopolysaccharide (LPS)

increased anti-MPO-induced glomerular neutrophil accumulation and the severity of NCGN (52). LPS increased levels of circulating TNF- α and subsequently increased circulating MPO. In vitro, anti-MPO IgG induced activation of murine neutrophils only after priming with TNF- α . Treatment with anti-TNF- α attenuated the LPS-mediated worsening of anti-MPO IgG-induced NCGN. These observations confirmed that proinflammatory stimuli, such as increased circulating cytokines, act synergistically to induce ANCA-mediated inflammation, apparently by priming neutrophils to interact with ANCA.

Alternative Complement Pathway Activation and C5A Receptor Engagement Are Required for Antimyeloperoxidase Immunoglobulin G–Induced Pauci-Immune Myeloperoxidase in Mice

Although AAV is characterized by a paucity of immunoglobulin and complement in inflamed vessels and glomeruli, most patients have at least focal complement deposition at the sites of injury (53). Complement activation, especially alternative pathway activation, is an important mediator of injury, even in lesions that do not have conspicuous deposition of complement detectable by immunohistology (54). Observations in the murine model of AAV demonstrate that complement plays an important role (55–58); this evidence is supported by immunohistologic studies on NCGN in AAV patients (59).

Induction of NCGN and SVV by injection of either anti-MPO IgG or anti-MPO splenocytes can be completely blocked by complement depletion with cobra venom factor (55). The requirement for complement activation was confirmed by the failure of C5 KO mice to develop NCGN after injection of a nephritogenic dose of anti-MPO IgG. The role of specific complement activation pathways was investigated using both C4 KO mice that cannot activate the classic or lectin-binding pathways and factor B KO mice that cannot activate the alternative pathway. After injection of anti-MPO IgG, the C4 KO mice developed NCGN comparable to WT disease, whereas the factor B KO mice developed no NCGN. These observations indicate that the alternative pathway plays a critical pathogenic role (55).

To investigate a possible mechanism for complement involvement in pathogenesis, and to support a role for complement in human AAV, investigators incubated IgG isolated from patients with MPO-ANCA or PR3-ANCA, or from healthy controls, with normal human neutrophils primed with TNF- α . The supernatant from the reaction with MPO-ANCA or PR3-ANCA IgG (but not control IgG) caused activation of complement in normal plasma. Activation of complement by ANCA-activated neutrophils generates C3a (55) and C5a (56), which are chemotactic for neutrophils and activate neutrophils. This finding is consistent with other observations that activated neutrophils can activate complement (60). C5a also primes neutrophils for ANCA-induced activation (56).

Activation of C5 and engagement of the C5a receptor are critical events in the mediation of AAV in this animal model (56, 57). Pretreatment of mice with a C5-inhibiting monoclonal antibody (BB5.1) 8 h before injection of anti-MPO IgG and LPS prevented development of NCGN and markedly reduced glomerular neutrophil influx (56). BB5.1 injection 1 day after injection of anti-MPO IgG resulted in a marked reduction in NCGN severity.

NCGN and SVV are induced when WT BM is transplanted into irradiated MPO KO mice that have been immunized with MPO. However, transplantation of BM from C5a receptor KO mice results in markedly reduced induction of NCGN (57).

The relevance of these findings in a mouse model to AAV patients is supported by immunohistologic studies on ANCA-associated NCGN that have demonstrated deposition of C3d, factor B, and factor P in glomeruli and small blood vessels at sites of active inflammation (58). Mannose-binding lectin (a marker of the lectin-binding pathway) and C4d (a marker for the classic and lectin-binding pathways) were not detected in AAV patients (58). Patients who showed immunohistologic evidence for complement activation in glomeruli (i.e., C3c deposition) had a higher level of proteinuria and poorer initial renal function than did patients without C3c deposits (59). These observations indicate that ANCA activation of neutrophils initiates an amplification loop wherein activation of neutrophils activates complement, which in turn recruits and primes more neutrophils for activation by ANCA and further activation of complement (Figure 4).

Activation of neutrophils by complement, as well as Fc receptor engagement, utilizes signaling pathways that are potential targets for therapy in AAV. Studies in the mouse model support this strategy. Phosphatidylinositol 3-kinase γ isoform (PI3K γ) is required for many signaling pathways involved in neutrophil activation. PI3K γ -deficient mice are protected from disease induction by anti-MPO IgG, and a PI3K γ inhibitor prevents disease induction in vivo and blocks neutrophil activation in vitro (61). Bacterial endoglycosidase treatment of IgG destroys IgG's ability to bind to Fc receptors or to activate complement but does not interfere with antigen binding. Anti-MPO IgG treated with bacterial endoglycosidase could not activate neutrophils in vitro and did not induce NCGN when injected into mice (62). These studies provide additional support for the role of complement activation and Fc receptor engagement in the pathogenesis of AAV, and they suggest novel therapeutic strategies.

Rat Model of Myeloperoxidase-ANCA ANCA-Associated Vasculitis

A rat model of anti-MPO-induced NCGN and SVV has confirmed the observations made in murine models (63, 64). WKY/NCrIBR rats immunized intramuscularly with human recombinant MPO developed not only antibodies against human MPO but also antibodies that reacted with rat MPO (63). These rats developed NCGN and pulmonary capillaritis with hemorrhage. This disease induction was prevented by administering an anti-rat TNF- α monoclonal antibody (CNTO 1081), which is the same effect observed in the mouse model (52). Anti-TNF also ameliorated pulmonary hemorrhage in mice immunized with MPO.

The importance of synergistic proinflammatory stimuli has been demonstrated by adjuvant modification and intravital microscopy of rat mesenteric vessels (63, 64). The addition of pertussis toxin and killed *Mycobacterium tuberculosis* to the adjuvant used for immunization of WKY rats with human MPO increased the incidence of NCGN and pulmonary hemorrhage (64). Lewis, Wistar-Furth, and brown Norway rats similarly immunized with human MPO did not develop NCGN or SVV despite the presence of anti-MPO antibodies, which indicates a genetically determined susceptibility similar to that found in mouse strains (64). Nonimmunized WKY rats were pretreated with anti-TNF or vehicle 1 h before TNF- α .

or saline was infused. Leukocyte rolling and firm adhesion were measured by intravital microscopy both before and after TNF- α administration (63). In saline-treated rats, rolling remained stable or increased slightly, whereas after intravenous TNF- α , rolling was reduced dramatically. TNF- α -induced reduction in rolling and adhesion was blocked in rats pretreated with anti-TNF. The studies in mouse and rat models of AAV that have revealed a role for TNF- α in pathogenesis (52, 63) are in accord with clinical trials in AAV patients that have shown a beneficial therapeutic effect from use of the anti-TNF inhibitors infliximab (65) and adalimumab (66).

Animal Models of PR3-ANCA ANCA-Associated Vasculitis

No well-validated animal models of PR3-ANCA AAV have been developed to date. However, several promising models have been reported recently and await confirmation.

The induction of circulating anti-PR3 antibodies alone is not sufficient to induce NCGN or SVV. For example, even if rats and mice immunized with recombinant human PR3, recombinant mouse PR3, or chimeric human/mouse PR3 develop circulating anti-PR3 antibodies, they do not develop NCGN or SVV (67).

Using a strategy similar to the first MPO-ANCA model that employed MPO KO mice as a source for pathogenic anti-MPO antibodies, investigators immunized PR3/neutrophil elastase KO mice with recombinant murine PR3 (68). The mice developed anti-PR3 that reacted with murine PR3 and bound to PR3 on the surface of murine neutrophils. However, injection of this anti-PR3 into mice did not induce NCGN or SVV, even in mice pretreated with LPS. The circulating anti-PR3 enhanced cutaneous inflammation at sites of intradermal injection of TNF, which suggests that slight in vivo augmentation of neutrophil activation occurred.

A promising model involving immunization of autoimmunity-prone nonobese diabetic (NOD) mice with recombinant mouse PR3 resulted in high titers of anti-PR (69). These mice do not develop evidence for vasculitis; however, transfer of splenocytes from these mice to immunodeficient NOD-severe combined immunodeficiency (SCID) mice caused the development of NCGN and SVV. No disease developed in NOD-SCID mice that received splenocytes from control mice. Also, no disease developed when splenocytes from B6 mice immunized with recombinant PR3 were transferred into Rag2 KO B6 mice, which suggests that autoimmune-prone NOD mice may have greater susceptibility to PR3-ANCA disease.

An ex vivo model of PR3-ANCA disease used rat lungs perfused with TNF-primed human neutrophils and monoclonal anti-PR3 antibodies to model vascular injury induced by PR3-ANCA (70). Marked edema but no overt vasculitis was observed; therefore, this is not a convincing AAV model.

A novel approach has studied the induction of vasculitis by human PR3-ANCA in mice with a human immune system (71). Chimeric mice were generated through the injection of human hematopoietic stem cells into irradiated NOD-SCID interleukin (IL)-2R γ KO mice. The chimeric mice were injected with PR3-ANCA IgG or control IgG. After 6 days, 39% of

the PR3-ANCA-injected mice had hematuria, compared with none of the controls. Pathologic postmortem examination of the mice that had received PR3-ANCA revealed focal pulmonary hemorrhage, pulmonary capillaritis, and usually mild glomerulonephritis. These results are encouraging, but the observed lesions do not closely resemble those in human AAV.

Animal Models of LAMP2-ANCA ANCA-Associated Vasculitis

As discussed above, antibodies specific for LAMP2 have been reported in patients with active AAV (4), although another study did not confirm this finding (5). Also, whether LAMP2 antibodies induce NCGN in rats is controversial (4, 5). One group observed NCGN in rats injected with anti-LAMP2 (4), whereas another did not (5). Thus, the pathogenicity of LAMP2 antibodies remains unsettled.

IMMUNOGENESIS OF THE ANCA AUTOIMMUNE RESPONSE

The discussion so far has focused on the pathogenesis of lesions once a patient has developed an ANCA autoimmune response. Equally relevant is the genesis of the autoimmune response that causes circulating ANCA. The precise cause of ANCA autoimmunity is not known, but it is likely to be multifactorial and varied among individuals and may involve a complex interplay between innate and acquired characteristics of the immune system, as well as environmental and genetic influences. T cells and B cells are involved in the generation of IgG autoantibodies. Thus, T cells may provide too much positive regulation or not enough negative regulation. B cells may be more receptive to positive regulation or less receptive to negative regulation. The possibilities are myriad, and hard evidence for the most important mechanisms for the genesis of the ANCA response is lacking. Two proposed mechanisms are molecular mimicry between bacterial and self-antigens (4) and initiation of the response by peptides that are complementary to the autoantigens (73).

The molecular mimicry theory pertains primarily to LAMP2-ANCA, which may (4) or may not (5) be important in the pathogenesis of AAV. A LAMP2 peptide has 100% homology to a bacterial adhesin, FimH. Immunization with FimH induces circulating anti-FimH antibodies that cross-react with LAMP2 (4). Theoretically, an infection by fimbriated bacteria bearing FimH could induce an immune response that would cross-react with LAMP2, resulting in the induction of AAV. In one study, rats immunized with FimH produced antibodies to rat and human LAMP2 and developed pauci-immune NCGN (4), but in another study, the same method did not produce NCGN (5). This issue warrants further investigation.

Induction of Autoimmunity by Autoantigen Complementary Peptides

Another theory for the genesis of the ANCA autoimmune response proposes that the initial immune response is not to the autoantigen but rather to a peptide that is complementary to the autoantigen epitope (73, 74). Complementary pairs of peptides have molecular structures that align with and bind to each other. An example of complementary peptides is the sense and antisense products for a genetic locus. A role for antigenic complementarity in the

induction of autoimmunity was postulated before the evidence for a role in AAV was proposed (75, 76). In AAV, the postulate is that an initial immune response is directed against an epitope on a peptide that is the antisense product or a mimic of the antisense peptide, rather than against the sense autoantigen peptide (Figure 5) (73). This theory derived from the finding that patients with PR3-ANCA AAV have not only circulating antibodies against PR3 peptides (anti-PR3) but also a separate set of antibodies against antisense peptides that are complementary to the autoantigen epitopes on PR3 (anti-cPR3 peptides) and are anti-idiotypic antibodies that bind to anti-PR3 antipodes. PR3-ANCA AAV patients also have circulating anti-cPR3 CD4⁺ Th1 memory T cells (77). T cells that respond to the cPR3 peptide are not detected in MPO-ANCA patients. The *HLA-DRB1**15 allele, which is predicted to bind to the cPR3 peptide with high affinity, is significantly over-represented in PR3-ANCA patients, which may predispose them to generation of a pathogenic PR3-ANCA autoimmune response (77).

Theoretically, an immune response to an antisense cPR3 peptide (or an exogenously derived mimic of antisense cPR3) produces anti-cPR3 antibodies. The anti-idiotypic response to anti-cPR3 antibodies cross-reacts not only with the idiotope on anti-cPR3 antibodies but also with the portion of the PR3 molecule (the autoantigen) to which the peptide is complementary (Figure 5). The cPR3 peptide could arise endogenously from transcription of PR3 antisense or could be exogenous. For example, it could be derived from an infectious pathogen that has a peptide that mimics cPR3. Teleologically, peptides that are complementary to antimicrobial proteins such as PR3 and MPO could be beneficial to pathogens by binding to and neutralizing the antimicrobial function of PR3 and MPO. Several pathogens that are known to be associated with PR3-ANCA have peptides that mimic cPR3; they include Ross River virus, *Staphylococcus aureus*, and *Entamoeba histolytica* (73). Infection by these microorganisms may initiate an immune response to the peptide mimic of antisense PR3, which in turn would result in anti-idiotypic antibodies that react with the PR3 sense peptide (i.e., PR3-ANCA).

T Cell Dysregulation and ANCA Autoimmunity

If an ANCA autoimmune response arises, it should be downregulated to maintain immunologic tolerance of self. Autoimmune responses are normally held in check by B cell and regulatory T cell (Treg) systems. Even healthy individuals have low levels of circulating “natural” autoantibodies to PR3 and MPO (78). Pathogenic levels of autoantibodies appear to arise not from the emergence of a previously forbidden clone but rather through a loss of effective downregulation (suppression) of autoimmune reactivity (79).

Adjuvant effects, such as those from environmental exposures, may augment autoimmune responses that otherwise would have been held in check. This finding may explain the association between AAV and exposure silica, which is an immune response adjuvant (80, 81).

T cells are important regulators of B cell functions, including antibody production. Patients with PR3-ANCA and GPA have abnormalities in the number and function of Tregs. CD25 is expressed on recently activated effector T cells. Tregs in peripheral-blood mononuclear cells can be identified through assessment of Foxp3 expression on CD4⁺CD25⁺ T cells. GPA

patients have an increased fraction of CD4⁺CD25⁺ T cells, although the percentage of Foxp3-positive cells is decreased (82). The percentage of Tregs is inversely related to the rate of disease relapse. In addition, CD4⁺CD25^{hi} T cells are able to suppress T cell proliferation in response to PR3 in healthy controls but not in ANCA-positive patients, suggesting a defect in regulatory function. In patients with active GPA, an increased proportion of CD4⁺Foxp3⁺ cells is associated with a more rapid induction of remission. Thus, GPA patients have abnormalities in the number and function of Tregs; these abnormalities are most pronounced in patients with the most active disease.

MPO-specific interferon- γ -producing T cells are increased in MPO-ANCA AAV patients compared with healthy controls and MPO-ANCA patients in remission (83). CD4⁺CD25⁺ Tregs do not seem to play a role in maintaining low numbers of MPO-specific T cells, because increased MPO-specific responses are not accompanied by reduced Tregs and the FoxP3 levels are diminished.

The IL-17 axis appears to play a role in ANCA disease, which is similar to other autoimmune diseases (84). IL-23 induces the differentiation of CD4⁺ T cells into potentially pathogenic T helper cells (Th17 cells) that produce IL-17, IL-6, and TNF- α . Serum IL-17A and IL-23 are elevated in acute AAV patients compared with healthy controls, and these elevated levels are not consistently suppressed by immunosuppressive treatment (84). Patients with higher levels of IL-23 have more active disease and higher ANCA titers compared with those with lower levels of IL-23. Autoantigen-specific IL-17-producing T cells appear to play a role in the induction and maintenance of the pathogenic ANCA.

In addition to circulating T cells, T cells in tissue, especially tissue affected by AAV granulomatous inflammation, may augment the ANCA autoimmune response and may be involved in the maintenance and progression of the ANCA autoimmune state (85). Moreover, granulomatous lesions contain numerous antigen-presenting dendritic cells that may facilitate the ANCA autoimmune response (86).

Mouse models have been used to investigate the role of T cells in the genesis of the ANCA response as well as in the pathogenesis of inflammatory lesions. For example, when B6 mice were immunized with human MPO, they developed anti-MPO antibodies and anti-MPO T cells (87). No disease developed in these mice, but injection with anti-GBM antibodies resulted in more severe glomerulonephritis than did injection of anti-GBM into mice not immunized with MPO. MPO KO mice immunized with MPO and given anti-GBM antibodies developed immune responses to MPO that were similar to the responses of their WT counterparts, but they failed to develop glomerulonephritis. CD4⁺ T cell depletion in this model attenuated crescentic glomerulonephritis without altering anti-MPO titers, and B cell-deficient mice, with no anti-MPO, developed severe crescentic glomerulonephritis. In this model, anti-MPO CD4⁺ T cells appeared to act with macrophages to amplify glomerular injury caused by anti-GBM antibodies. In the same model, mice deficient in the Th17 effector cytokine IL-17A were protected from developing glomerulonephritis (88), and Toll-like receptors and Th17 CD4 cells appeared to be involved in causing glomerulonephritis (89). Thus, in this model, T cells play a role not only in the genesis of the autoimmune

response but also in the induction of glomerulonephritis. However, the relevance of this model to human AAV is unclear because of the requirement for anti-GBM antibodies.

PATHOGENIC MECHANISM OF ANCA-ASSOCIATED VASCULITIS

The clinical observations, in vitro experiments, and animal model studies of AAV are consistent with the theoretical pathogenic events illustrated in Figure 6. The initial event is development of a pathogenic ANCA immune response. This event is probably a multifactorial process that may include genetic predisposition (e.g., genetically determined specific T cell receptors and abnormal neutrophil expression of ANCA antigens), environmental adjuvant factors (e.g., silica exposure), an initiating antigen (e.g., ANCA antigen complementary peptide), and failure to suppress the autoimmune response (e.g., ineffective T cell regulation) (Figure 5).

Once pathogenic ANCA are in the circulation, they activate neutrophils by reacting with ANCA antigens. Monocytes also are activated, but they appear to be less important in producing acute vascular injury. ANCA-induced neutrophil activation is facilitated by neutrophil priming, for example, with cytokines such as TNF- α . Priming causes the release and display of ANCA antigens at the surface of neutrophils, where they are available to interact with ANCA. Binding of ANCA to ANCA antigens on the surface of neutrophils and in the microenvironment of the inflammation (e.g., on the surface of endothelial cells) activates neutrophils through Fab' binding to ANCA on neutrophils and, more importantly, through Fc receptor engagement. Activated neutrophils release factors (e.g., properdin) that activate the alternative complement pathway leading to the generation of C5a, which not only activates neutrophils but also recruits more neutrophils to the site of inflammation. Complement activation established an inflammatory amplification loop that causes very destructive, localized necrotizing inflammation. This severe acute injury elicits an innate inflammatory response that recruits monocyte and T lymphocytes, which replace the neutrophils that have undergone leukocytoclasia during the acute inflammation. Mild injury may resolve with remodeling of the vessel to normal structure. More severe injury persists; more monocytes mature into macrophages, and fibroblasts and myofibroblasts are activated to lay down interstitial collagen, resulting in fibrosis/sclerosis of injured vessels and adjacent tissue.

The pathogenic mechanisms that cause extravascular granulomatosis in AAV have not been elucidated by specific experimental observations. However, the pathology of the lesions and our understanding of the interaction between neutrophils and ANCA are consistent with the theoretical mechanism illustrated in Figure 7. Patients who develop GPA may have an inflammatory condition that precedes the onset of AAV, for example, a staphylococcal upper respiratory tract infection. This infection may not only initiate an ANCA autoimmune response (e.g., by exposure to staphylococcal peptides that are complementary mimics of PR3 antisense peptides) but may also cause the accumulation of numerous activated neutrophils in the mucosal tissues of the upper respiratory tract. Once an ANCA autoimmune response occurs, ANCA appears not only in the circulating plasma but also in the extravascular interstitial fluid. On the basis of in vitro experimental observations, reactions between interstitial ANCA and extravascular neutrophils would lead to the same

sequence of events that occur in vessel walls as a result of neutrophil activation. Extravascular neutrophils would be activated to produce intense, localized, acute inflammation that would cause tissue necrosis and fibrin formation from the exudation and spillage of plasma from injured microvasculature in the lesion. The acute injury would elicit a mononuclear leukocyte response, including the influx of monocytes that would transform into macrophages and multinucleated giant cells. ANCA interactions with monocyte ANCA antigens might facilitate giant cell formation. The early necrotizing granulomatous lesions would have the characteristics described as pathergic granulomatosis. Over time, the neutrophil-rich acute necrotizing lesions would accrue large numbers of mononuclear leukocytes (e.g., monocytes, macrophages, and lymphocytes) and take on a more typical granulomatous appearance with numerous macrophages and multinucleated giant cells, which initially surround a zone of amorphous necrotic debris and eventually associate with varying degrees of fibrosis.

The prodromes that precede the vasculitis phase of EGPA (e.g., asthma and eosinophilic pneumonia) have numerous extravascular eosinophils. Although eosinophils are conspicuous, this acute inflammation also includes neutrophils. Theoretically, ANCA in the interstitial fluid of EGPA patients with numerous extravascular eosinophils and admixed neutrophils could activate the neutrophils, as has been proposed for GPA. Eosinophils do not contain MPO or PR3, but they could be activated by ANCA-activated neutrophils. For example, ANCA bound to respective antigens derived from neutrophils engage Fc receptors on eosinophils and cause activation. Activated neutrophils and eosinophils in EGPA would then mediate extravascular granulomatosis through a mechanism similar to that postulated for neutrophils in GPA (Figure 7), but with numerous eosinophils involved as well. These hypothetical pathogenic scenarios have not been proven but are feasible, according to current experimental evidence, and are setting the stage for future investigations.

Glossary

ANCA	antineutrophil cytoplasmic autoantibody/autoantibodies
MPO	myeloperoxidase
PR3	proteinase 3
LAMP2	lysosomal-associated membrane protein 2
AAV	ANCA-associated vasculitis
MPA	microscopic polyangiitis
GPA	granulomatosis with polyangiitis, also known as Wegener's granulomatosis
EGPA	eosinophilic granulomatosis with polyangiitis, also known as Churg–Strauss syndrome
NCGN	necrotizing and crescentic glomerulonephritis

SVV	small-vessel vasculitis
TNF	tumor necrosis factor
BM	bone marrow
NETs	neutrophil extracellular traps
WT mice	wild-type mice
Rag2 KO B6 mice	recombinase-activating gene 2 knockout immune-deficient mice
NOD mice	nonobese diabetic mice
SCID mice	severe combined immunodeficiency mice
FimH	antigen on fimbriated gram-negative bacteria
cPR3 peptide	complementary PR3 peptide
Treg	regulatory T Cell

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Figure 1. Segmental acute necrotizing ANCA-associated vasculitis lesions with (a–c) fibrinoid necrosis (hematoxylin and eosin stain) (*large arrow*) and (a,c) leukocytoclasia (*small arrow*). (a,b) The inflammatory infiltrate includes a mixture of neutrophils and mononuclear leukocytes. (c,d) A Masson trichrome stain is useful in distinguishing between (b) acute segmental fibrinoid necrosis and (d) chronic segmental sclerosis.

Figure 2.

Acute pulmonary lesions in (a,b) microscopic polyangiitis and (c,d) granulomatosis with polyangiitis (GPA). (a) Alveolar capillaritis with extensive neutrophilic infiltration and hemorrhage [hematoxylin and eosin (H&E) stain]. (b) Extensive disruption of the silver-positive alveolar capillary basement membranes in the center of the photomicrograph (Jones silver stain). (c) An acute GPA granulomatous lesion with a central zone of intense neutrophilic infiltration (microabscess) with adjacent multinucleated giant cells (H&E stain). (d) A more chronic GPA granulomatous lesion with necrotic debris and adjacent palisading macrophages (H&E stain).

Figure 3. Glomerulonephritis and vasculitis induced in a B6 mouse 6 days after intravenous injection of mouse anti-MPO (myeloperoxidase) immunoglobulin G. (a) Segmental necrotizing glomerulonephritis with fibrinoid necrosis [hematoxylin and eosin (H&E stain)]. (b) Immunohistochemical staining of neutrophils showing segmental accumulation at sites of necrosis. (c) Leukocytoclastic angiitis in the skin (H&E stain). (d) Hemorrhagic pulmonary alveolar capillaritis (Masson trichrome stain).

Figure 4.

The putative pathogenic event in acute ANCA-associated vasculitis. (*Left to right*) Neutrophils that have been primed (e.g., with cytokines) release ANCA antigens at the cell surface and into the adjacent microenvironment, where they bind ANCA. Activated neutrophils release factors that activate the alternative complement pathway, which in turn recruits and primes more neutrophils for activation by ANCA and further activation of complement, resulting in an amplification loop. ANCA-activated neutrophils adhere to and penetrate vessel walls and release destructive mediators that cause vascular necrosis.

Figure 5.

Multiple events contributing to the pathogenesis of ANCA-associated vasculitis, including (a) genesis of the autoimmune response by an inciting antigen, (b) loss of tolerance that allows the autoimmune response to persist, (c) abnormally increased expression of ANCA target antigens in neutrophils, and (d) cytokine-induced increased release to ANCA antigens at the surface of neutrophils and into the inflammatory microenvironment. The autoimmune response is initiated by a peptide antigen (Ag1) that is complementary to the autoantigen (Ag2). The responding B cells (B1) produce antibodies (A1) directed against the complementary peptide. The A1 antibodies stimulate an anti-idiotypic response (B2, A2) that cross-reacts with the autoantigen (Ag2). Perpetuation of the pathogenic anti-Ag2 response requires loss of tolerance due to dysfunction of regulatory T cells (Tregs).

Figure 6.

A putative sequence of events in the pathogenesis of ANCA-associated vasculitis. (*Top to bottom*) Loss of tolerance allows for production of pathogenic levels of ANCA. ANCA activate primed neutrophils by binding to ANCA antigens at the surface of neutrophils and in the microenvironment of the inflammation. ANCA-activated neutrophils mediate acute necrotizing injury with fibrinoid necrosis and leukocytoclasia (compare with Figure 1*a*). The acute injury elicits an innate inflammatory response that recruits monocytes and T lymphocytes, which replace the neutrophils and lead to either resolution of the injury or development of localized fibrosis/sclerosis.

Figure 7.

Putative events in the pathogenesis of extravascular granulomatosis. (*Upper left*) Extravascular neutrophils are activated to produce (*upper middle*) intense localized acute inflammation, which causes (*upper right*) tissue necrosis and fibrin formation. The acute injury elicits a mononuclear leukocyte response, including (*bottom*) the influx of monocytes that transform into macrophages and multinucleated giant cells.