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REVIEW

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# Primary vasculitides and vasculitis confined to skin: clinical features and new pathogenic aspects

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*Abstract* **Cutaneous vasculitis is a heterogeneous group of disorders, and may occur with virtually all syndromes of vasculitis. It can occur as an isolated dermatologic disorder or as a manifestation of a potentially life-threatening systemic vasculitis. Cutaneous manifestations vary depending on the underlying cause, the size of the vessel involved and the severity and type of inflammation. In this short review, the classification, the characteristic skin manifestations of the primary vasculitides and new pathogenic aspects are discussed.**

**Key words** Primary vasculitides · Cutaneous vasculitis · Leukocytoclastic vasculitis · ANCA · Immunopathogenesis

## Introduction

Vasculitis, as a clinicopathologic process, may occur as a primary process (primary or idiopathic vasculitis) or as a secondary feature of other diseases (secondary vasculitis) such as collagen vascular diseases, infectious disorders, malignancy, etc. The clinical spectrum of vasculitides is wide and varied. The vasculitic syndromes share a common histopathologic substrate: inflammation within blood vessels resulting in vascular obstruction with tissue ischemia and infarction. Most of the vasculitic syndromes are generally thought to be mediated by immunopathologic mechanisms. Irrespective of the primary immunopathogenic events leading to vasculitis, activation of the vascular endothelium by several cytokines plays a pivotal role in the localization and propagation of vascular injury. The majority of vasculitides affecting the skin result from immunologic injury. Various etiologic agents and other vasculitic skin conditions may cause similar clinical features, mainly palpable purpura. Skin biopsies usually show leukocytoclastic vasculitis. The immunopathogenesis of most forms of vasculitis is only beginning to be understood, but is probably varied and complex. Accurate, timely diagnosis of the vasculitic disorders is critical, because some are life-threatening.

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## Classification

Because there is no simple or uniform classification scheme for vasculitis, the various syndromes are still discussed from the clinical standpoint, or they may be classified primarily using histopathologic criteria or laboratory findings. Many investigators have attempted to classify the vasculitides. In 1952 Zeek was the first to incorporate a clinical pathologic assessment based on the size of the vessels involved in the inflammatory process in her classification of necrotizing vasculitis (Montgomery 1967). A number of classification systems have been proposed and the most recent breakthroughs have been the 1990 American College of Rheumatology Criteria (ACR 1990 criteria) and the explanation of terminology used for naming, defining, classifying and diagnosing vasculitic disorders elaborated at the Chapel Hill Conference 1992 (1992 CHC definitions). The 1990 ACR criteria have been reviewed more recently (Hunder 1996). The 1992 CHC definitions include markers with immunodiagnostic significance, for example anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis (WG), and/or immunohistologic findings, for example IgA-dominant immune deposits in Henoch-Schönlein's purpura, which are indicative of certain diseases, and have been published recently (Jennette et al. 1994a).

In former classification schemes the major problem is the lack of standardized diagnostic terms and definitions. Thus, different names have been used for the same disease and the same name has been used for different diseases. Therefore, a committee comprising internists, rheumatologists, nephrologists, immunologists and pathologists who

**Table 1** Names and definitions of vasculitides adopted by the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis (*normal type* essential components, *italicized type* usual, but not essential components) (Jennette et al., with permission)

| | |
|---|---|
| Large vessel vasculitis[a] | |
| Giant cell (temporal) arteritis | Granulomatous arteritis of the aorta and its major branches, with a predilection for the extracranial branches of the carotid artery. *Often involves the temporal artery. Usually occurs in patients older than 50 years and often is associated with polymyalgia rheumatica* |
| Takayasu's arteritis | Granulomatous inflammation of the aorta and its major branches. *Usually occurs in patients younger than 50 years* |
| Medium-sized vessel vasculitis[a] | |
| Polyarteritis nodosa[b] (classic polyarteritis nodosa) | Necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules |
| Kawasaki's disease | Arteritis involving large, medium-sized, and small arteries, and associated with mucocutaneous lymph node syndrome. *Coronary arteries are often involved. Aorta and veins may be involved. Usually occurs in children* |
| Small vessel vasculitis[a] | |
| Wegener's granulomatosis[c] | Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels (e.g. capillaries, venules, arterioles, and arteries). *Necrotizing glomerulonephritis is common* |
| Churg-Strauss syndrome[c] | Eosinophil-rich and granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels, and associated with asthma and eosinophilia |
| Microscopic polyangiitis[b,c] (microscopic polyarteritis) | Necrotizing vasculitis, with few or no immune deposits affecting small vessels (i.e. capillaries, venules, or arterioles). *Necrotizing arteritis involving small and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs* |
| Henoch-Schönlein purpura | Vasculitis, with IgA-dominant immune deposits, affecting small vessels (i.e. capillaries, venules, or arterioles). *Typically involves skin, gut, and glomeruli, and is associated with arthralgias or arthritis* |
| Essential cryoglobulinemic vasculitis | Vasculitis, with cryoglobulin immune deposits, affecting small vessels (i.e. capillaries, venules, or arterioles), and associated with cryoglobulins in serum. *Skin and glomeruli are often involved* |
| Cutaneous leukocytoclastic angiitis | Isolated cutaneous leukocytoclastic angiitis without systemic vasculitis or glomerulonephritis |

[a] *Large vessel* refers to the aorta and the largest branches directed toward major body regions (e.g. to the extremities and the head and neck); *medium-sized vessel* refers to the main visceral arteries (e.g. renal, hepatic, coronary, and mesenteric arteries); *small vessel* refers to venules, capillaries, arterioles, and the intraparenchymal distal arterial radicals that connect with arterioles. Some small- and large-vessel vasculitides may involve medium-sized arteries, but large- and medium-sized vessel vasculitides do not involve vessels smaller than arteries
[b] Preferred term
[c] Strongly associated with ANCA

together had extensive experience with diagnosing vasculitides have proposed the names and definitions given in Table 1. A number of problems still remain. There is still considerable overlap between the groups. However, any classification system based on clinical and laboratory features will be contentious, especially when the etiology of the disease is not fully understood.

## Skin lesions and subtypes of cutaneous vasculitis

The skin manifestations of the vasculitic syndromes which consist of purpurae, bullae, nodules, ulcerations, and pigmentary changes, highly overlap. However, each syndrome has characteristic features. Common clinical features of the systemic vasculitides are constitutional symptoms such as fever, fatigue and weight loss. In the large vessel arteritis (i.e. giant cell arteritis, Takayasu's arteritis), skin lesions (i.e. erythema, edema, tender nodules, linear ulceration, alopecia) are rather uncommon. Of the medium-sized vessel vasculitides, Kawasaki's disease is associated with the mucocutaneous lymph node syndrome.

### Cutaneous polyarteritis nodosa

Cutaneous polyarteritis nodosa (PAN) is a condition in which only the skin is involved, with an inflammatory neutrophilic vasculopathy of the medium-sized vessels. Cutaneous PAN is separated from the systemic form because it remains limited and is associated with a better prognosis. The site of involvement with cutaneous PAN is primarily the legs (95% of lesions are located in this region) and the typical primary lesion is a subcutaneous nodule. Skin lesions are also a prominent feature of systemic polyarteritis nodosa (classic polyarteritis nodosa). The lesions include livedo reticularis, painful subcutaneous nodules, and/or often ulcerated as well as digital gangrene. Both polyarteritis nodosa and microscopic polyangiitis (MPA, formerly microscopic polyarteritis) can affect arteries in the skin

causing nodules but, by definition (Table 1), only MPA can also affect vessels smaller than arteries and cause purpura. When defined in this fashion, polyarteritis nodosa is not associated with ANCA whereas MPA is part of the group now termed "ANCA-associated vasculitides" (see Table 1).

### Leukocytoclastic vasculitis (necrotizing vasculitis)

Leukocytoclastic vasculitis is synonymous with necrotizing vasculitis and is the prototype of cutaneous vasculitis (Gibson and Daniel Su 1995). The characteristic feature of all small-vessel vasculitides is *leukocytoclastic vasculitis* of the skin affecting primarily small post-capillary venules. The most common clinical manifestation is palpable purpura. The lesions usually occur initially as macular erythema or urticarial papules and evolve as purpura. Pruritus is absent. A small percentage of patients have lesions which appear as pustules, bullae, ulcers etc. The most common site of involvement is the lower legs. The lesions tend to be symmetrical and often aggravate after a period of prolonged standing (for review see: Claudy 1995; Jennette et al. 1994b). Histologically, leukocytoclastic vasculitis is characterized by fibrinoid degeneration of the blood vessel wall, with predominantly a neutrophilic infiltration into the vessel wall and the perivascular region associated with hemorrhage and fragments of inflammatory cells.

### Cutaneous or subcutaneous granulomatous inflammation

Cutaneous or subcutaneous granulomatous inflammation causing nodular lesions can occur in patients with WG and Churg-Strauss syndrome (CSS). The nodular lesions can show features of pyoderma gangrenosum or erythema nodosum.

### Henoch-Schönlein purpura

Henoch-Schönlein purpura is distinguished from other types of necrotizing vasculitis on the basis of its clinical features and the presence of IgA in involved vessels. The characteristic skin lesions manifest themselves as palpable purpura *(leukocytoclastic venulitis)*. In essential cryoglobulinemic vasculitis, which also features leukocytoclastic vasculitis, the pathogenic immune complexes may contain heterologous molecules generated by infectious pathogens (e.g. hepatitis C antigens).

### Urticarial vasculitis

In some patients, especially those with immune complex-mediated vasculitis with extensive complement activation, dermal small-vessel vasculitis causes focal edema resulting in urticaria. Urticaria associated with vasculitis persists longer than typical nonvasculitic urticaria (i.e. > 24 h) and often evolves into a purpuric lesion. Urticarial vasculitis is not a specific disease, but is rather a manifestation of a vasculitis that has caused markedly increased permeability of the dermal microvasculature.

### Erythema elevatum diutinum

Erythema elevatum diutinum is a form of chronic leukocytoclastic angiitis characterized by cutaneous plaques, usually on the extensor surfaces of the extremities which are initially red and later brown to yellow. This pattern of vasculitis occurs commonly as a syndrome secondary to other disease (e.g. in rheumatoid arthritis, systemic lupus erythematosus (SLE) or inflammatory bowel disease).

### Hypersensitivity vasculitis

Hypersensitivity vasculitis occurs characteristically secondarily to drugs or infections or both and belongs to the group of secondary vasculitides. Its main clinical manifestations are skin and joint symptoms. Palpable purpura is the most frequent clinical manifestation and the most common initial symptom. Other skin manifestations include macular papular rashes, urticaria and nodules. The skin lesions are predominantly located in the lower extremities, particularly on the lower part of the legs. Systemic involvement does not occur and the prognosis is excellent (Martinez-Taboada et al. 1997).

### Skin involvement in Wegener's granulomatosis

Cutaneous manifestations are not unusual in WG and occasionally may be the initial sign of the disease. The prevalence of skin involvement in WG has been reported to be between 35% and 50% (Daout et al. 1994). Histologically, cutaneous lesions in patients with WG cover a wide spectrum of findings, and leukocytoclastic vasculitis is by far the most common pathologic pattern. The histopathology of most skin lesions is non-specific, but in about 25% of patients cutaneous lesions demonstrate more characteristic histopathologic findings including necrotizing vasculitis of small and intermediate vessels, extravascular palisading granulomas, and granulomatous vasculitis. The cutaneous lesions characteristic of WG may correlate with the activity, distribution and course of disease (Barksdale et al. 1995).

## Immunopathogenesis

Most of the vasculitic syndromes are mediated by immunopathogenic mechanisms which have been traditionally classified into four types (I–IV) analogous to those described by Coombs and Gell for the various forms of hypersensitivity reactions. Accordingly, clinicopathologic and immunohistochemical studies have led to the terms "allergic angiitis" (type I reaction), "ANCA-associated

vasculitis" (type II reaction), "immune-complex vasculitis" (type III) and "vasculitis associated with T-cell-mediated hypersensitivity" (type IV reaction). We try to follow this simplistic line to describe the major pathogenic mechanisms involved in vasculitis syndromes.

### Type I: Vasculitides strongly associated with atopic disorders

Urticarial vasculitis and CSS can be described as "vasculitides strongly associated with atopic disorders", or type I reactions in the classification of Gell and Coombs. Urticarial vasculitis is a manifestation of inflammatory injury of capillaries and postcapillary venules in the skin. IgG autoantibody to IgE receptor or IgE itself causes urticarial lesions in 30% of patients with chronic idiopathic urticaria. Only a minority of (about 10%) of patients with chronic urticarial lesions have urticarial vasculitis (Wisnieski 2000). In urticarial vasculitis and CSS, activated Th2 lymphocytes play a central role through their production of cytokines, such as IL-4, IL-5 and IL-13, mediating the accumulation of mast cells, basophils and, particularly, eosinophils. Bridging of IgE receptors on these cells leads to secretion of inflammatory and toxic mediators. While urticarial vasculitis is predominantly induced by degranulation of mast cells, in CSS activation of eosinophils is a central feature, where eosinophil cationic protein, eosinophil-derived neurotoxin and lipid mediators (LTC4, PAF) seem to play a major role in induction of eosinophilic vasculitis. IL-5, in contrast to GM-CSF or IL-3, acts as a specific growth and differentiation factor for eosinophils and as a selective chemoattractant in the tissue. IL-5 also induces the expression of adhesion molecules CD18/11b, promoting an enhanced adhesion of eosinophils on human microvascular endothelial cells in vitro. IL-5, and also IL-3 and GM-CSF, activate eosinophils in vitro, leading to degranulation. Moreover, eotaxin is a selective and highly potent chemotaxin for eosinophils. It specifically binds to the chemokine-receptor CCR3, expressed on eosinophils, and activates these cells. In this regard, platelet activation factor (PAF) and eotaxin can act synergistically.

### Type II: Vasculitides strongly associated with autoantibodies

ANCA are useful diagnostic serologic markers for a group of systemic vasculitides (ANCA-associated vasculitides: WG, MPA). With indirect immunofluorescence, two distinct patterns are detected on ethanol-fixed neutrophil cytocentrifuge preparations: a granular, centrally accentuated cytoplasmic staining (cANCA) and a perinuclear staining (pANCA). ANCA-associated vasculitides feature prominent type II immune reactions, and are also termed as "vasculitides strongly associated with autoantibodies". ANCA are involved in the immunopathogenic mechanisms of these vasculitides, resulting in necrotizing inflammation of blood vessel walls and a pauci-immune character of immune-complex depositions. Here we highlight the new immunopathogenic aspects, the role of ANCA and the main cell populations (neutrophils, T cells) and their products (i.e. enzymes, cytokines) relevant to the pathogenesis of ANCA-associated vasculitides, especially of WG.

ANCA-associated vasculitides include related but clearly distinct disorders: WG, MPA and CSS. These entities share the characteristic of not having immune complex deposits in affected organs, and thus have been termed "pauci-immune". The clinical, immunohistochemical and serological diagnostic hallmarks of WG and MPA are the frequent finding of pulmonary renal vasculitis syndrome, pauci-immune vasculitis of the lung or glomerulonephritis (GN) and PR3-ANCA or MPO-ANCA, respectively. Biopsies – usually taken from kidneys or lung – typically show a necrotizing vasculitis of the small arteries, capillaries or small veins. In the kidney, necrotizing GN with crescent formation is the most prominent feature; it cannot be distinguished from other forms of crescentic GN (e.g. Goodpasture's syndrome) by light microscopy. However, immunohistochemistry shows no or only pauci-immune deposits in the "ANCA-associated vasculitides" (WG, CSS, MPA), in contrast to Goodpasture's syndrome (linear IgG deposits along the glomerular basement membrane) and Henoch-Schönlein's purpura (mesangial immune-complexes of IgA) (for review see: Gross et al. 1999).

Unfortunately, our knowledge of the etiology and pathogenesis of ANCA-associated vasculitides is still incomplete. We know that tissue destruction occurs because of host activity, but only a few of the triggers of this self-destructive process are actually known. The clue to the initiating event in AAV lies in environmental factors, such as infectious agents and environmental toxins, and genetic susceptibility. Evidence accumulating during the last few years suggests that ANCA play an active role in the immunopathogenesis of vasculitis. The pathogenesis of vasculitis includes different immunologic mechanisms that cause leukocytes to adhere to endothelial cells, penetrate into vessel walls, and release injurious products.

#### *ANCA and the pathogenesis of vasculitis*

The serologic hallmark of WG is the presence of circulating ANCA to either proteinase 3 (PR3-ANCA) in about 90% of patients or myeloperoxidase (MPO-ANCA) in 3% of patients. Changes in ANCA titers seem to reflect changes in disease activity in about two-thirds of patients (Cohen-Terveart et al. 1994; Kerr et al. 1993; Nölle et al. 1989). ANCA have been hypothesized to participate in the pathogenesis of necrotizing vasculitis based on their association with small-vessel vasculitides and the ability of these antibodies to activate neutrophils, monocytes and endothelial cells in vitro. The clinical, pathologic and experimental evidence regarding the role of ANCA in the pathogenesis of systemic vasculitis and glomerulonephritis are summarized in Tables 2 and 3. How these in vitro effects of ANCA finally lead to in vivo systemic inflammation, blood ves-

**Table 2** Clinical and pathologic evidence of the pathogenic role of ANCA

| |
|---|
| Necrotizing vasculitis and GN are strongly associated with PR3- or MPO-ANCA |
| Changes in titers of ANCA seem to reflect disease activity |
| WG responds to immunosuppressive therapy |
| WG are characterized by the absence of immunohistologic evidence for vascular wall localization of immune-complex or anti-GBM antibodies |
| IgM-ANCA are associated with pulmonary hemorrhage |
| Plasma exchange and intravenous immunoglobulins are effective in relieving the clinical symptoms |
| Recurrences are associated with IgG3-ANCA, which are more effective in activating neutrophils than IgG1 and IgG4 ANCA |
| The severity of renal lesions correlates with the number of accumulated neutrophils and with their degree of activation; the activation of neutrophils correlates with the ANCA titer |
| Active WG is associated with widespread neutrophil activation and the presence of neutrophilic alveolitis is the initial pulmonary sign in patients with recent onset disease |
| ANCA-target antigens (i.e. proteinase 3, elastase) can be detected in glomeruli crescents in glomerulonephritic conditions that are associated with neutrophilic infiltrates |

sel damage and glomerulonephritis is still a matter of speculation. Several mechanisms have been proposed to explain how the interaction of ANCA with their target antigens may result in necrotizing vasculitis. In inflammatory conditions, leukocytes have to migrate by means of a complex process across the endothelial wall to arrive at the site of inflammation. This process is not accompanied by vessel wall injury (necrosis). If ANCA cause the vasculitis in vasculitic conditions, however, the autoantibodies must interact with neutrophils and monocytes in the circulation, resulting in activation, microvascular adherence of leukocytes, and subsequent vascular inflammation and necrosis (ANCA-cytokine sequence theory; Fig. 1).

One main conclusion from recent studies investigating the potential pathogenic role of ANCA is that ANCA in combination with exogenous factors are able to aggravate a clinical inflammatory process and may result in systemic vasculitis and glomerulonephritis. Many of the basic features of mechanisms underlying the ANCA response, such as the factors responsible for the generation and perpetuation of these autoantibodies and the shaping of the ANCA immune response, remain unknown.

### *Apoptosis, ANCA and vasculitis*

Recently, much attention has been devoted to the role of apoptosis, or programmed cell death, in the pathogenesis of autoimmune disease. A putative role of apoptosis in the pathophysiology of WG has been suggested by Gilligan et al. who have reported the presence of ANCA target antigens on the surface of apoptotic polymorphonuclear neutrophils (PMN) (Gilligan et al. 1996). The demonstration that ANCA interact with primary granule constituents (i.e. PR3, MPO) on the surface of apoptotic neutrophils and that ingestion of opsonized apoptotic neutrophils modulates macrophage behavior, raises the question as to whether ingestion of ANCA-opsonized apoptotic neutrophils may influence the uptake process and functions of macrophages.

To investigate the above interactions, we investigated the effects of PR3-ANCA-opsonized apoptotic neutrophils on the uptake process and the production of TNF-α, IL-10 and IL-12, as well on the secretion of lipid inflammatory mediators, $TxB_2$, $LTB_4$ and $PGE_2$ by human monocyte-derived macrophages. We demonstrated that opsonization of apoptotic neutrophils by PR3-ANCA substantially enhanced recognition and binding by scavenger macrophages. Moreover, phagocytosis of ANCA-opsonized apoptotic neutrophils by macrophages triggered production of TNF-α and $TxB_2$. These findings indicate that PR3-ANCA opsonization of apoptotic neutrophils results in effective phagocytosis by human monocyte-derived macrophages

**Table 3** Experimental evidence for the pathogenic role of ANCA

| | |
|---|---|
| Effect of ANCA on neutrophils | Priming and apoptosis of neutrophils results in cell membrane expression of the target antigens, making them accessible for ANCA |
| | Cause degranulation and an oxidative burst of normal neu trophils primed with TNFα by binding simultaneously to Fcγ RII receptor and to the corresponding antigen expressed on the cell surface |
| | Activation of neutrophils by ANCA involves 5 lypoxygenase pathway inducing the production of leukotriene ($LTB_4$), a chemoattractant for neutrophils |
| | Induce expression of interleukin-1β and IL-8 in neutrophils |
| Effects of ANCA on monocytes | Activate monocytes (induce ROI release and the production of monocyte chemoattractant-1, potent for neutrophils; induce IL-8 production) |
| Effects of ANCA on endothelial cells (EC) | Induce expression of adhesion molecules and may enhance the adhesion of neutrophils and mononuclear cells to EC |
| | Lysis of EC previously incubated with PR3 or MPO and neutrophils |
| Effects of ANCA on PR3 | Prevent inactivation of PR3 by natural inhibitor α1-antitrypsin |

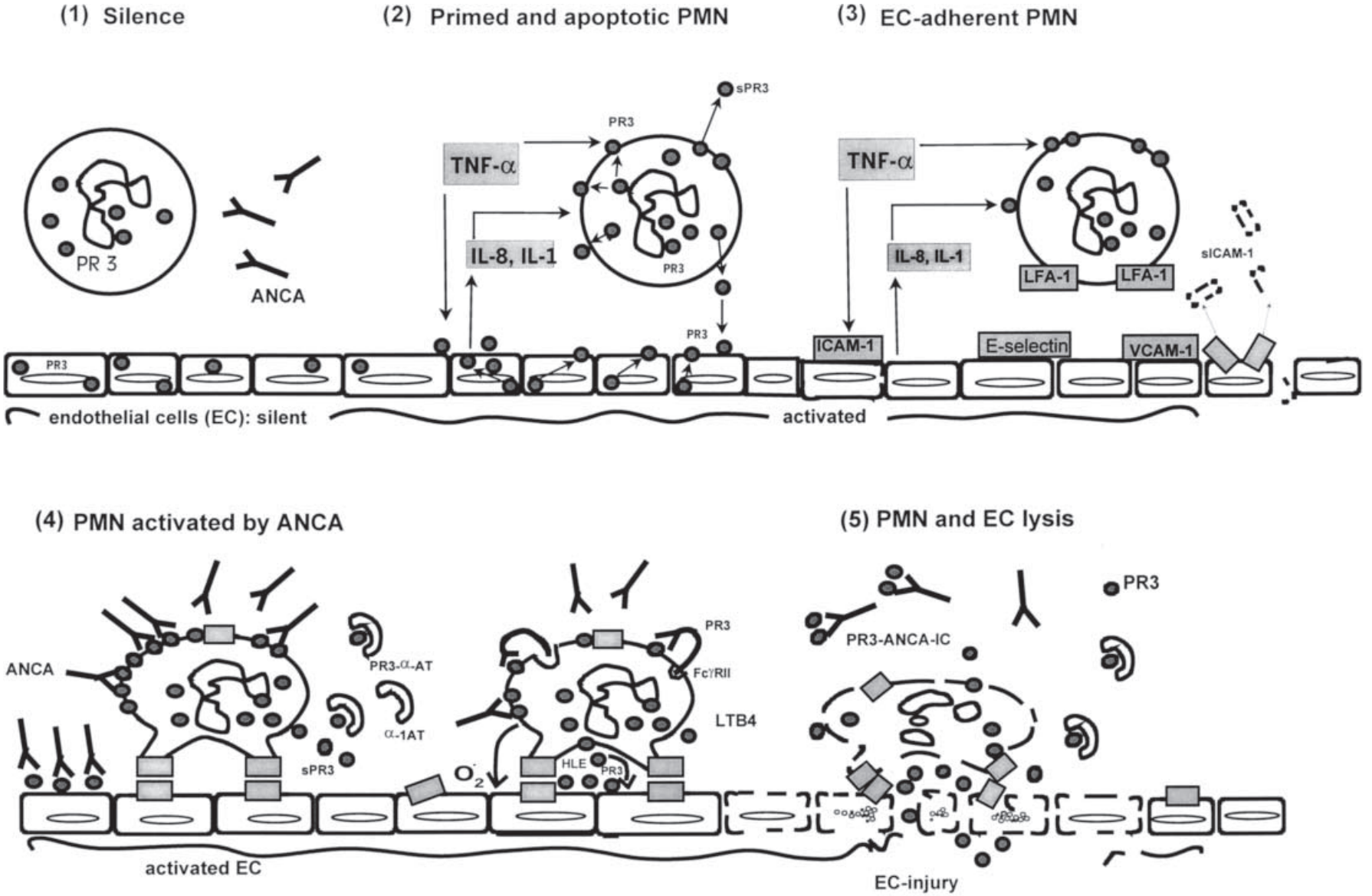


**Fig. 1** ANCA-cytokine sequence theory

and leads to upregulation of proinflammatory mediator production. Therefore, these results indicate a novel mechanism by which ANCA actively participate in apoptotic neutrophil clearance and modulate macrophage function. They may also help to explain the amplification and perpetuation of inflammation associated with severe necrotizing vascular injury. Furthermore, our observations emphasize the key role of TNF-α in the pathogenesis of vasculitides and indicate a possible avenue for therapeutic interventions (Moosig et al., submitted for publication).

Defective apoptosis regulation might lead to the persistent presence of inflammatory cells, causing damage to vessel walls. Recently, Tanigushi et al. have demonstrated that mice defective in Fas-mediated apoptotic mechanisms develop a granulomatous arteritis through macrophage activation possibly amplified by macrophage colony-stimulating factor (M-CSF). Both MRL/gld and MRL/lpr strains can spontaneously develop granulomatous arteritis, with infiltration by Mac-2 macrophages and $CD4^+$ T cells, as a result of defective apoptotic mechanisms. M-CSF has been shown to enhance Fas antigen and Mac-2 expression on spleen cells as well as granuloma formation in MRL/+ mice. This model clearly demonstrates how cytokine stimulation and interaction between T cells and macrophages can generate and potentiate permanent damage to vessel walls (Tanigushi et al. 1996).

Lorenz et al. (1997), however, have found significantly increased rates of in vitro apoptosis and upregulation of Fas/APO-1 and bcl-2 expression in a study of the regulation of apoptosis in immunocompetent cells derived from patients with autoimmune diseases (25 with SLE, 5 with WG). The authors conclude that accelerated in vitro apoptosis in SLE is nonspecific for the disease, and might be explained at least in part by the increased in vivo activation levels of peripheral blood mononuclear cells from patients with autoimmune diseases such as SLE or WG combined with in vitro incubation under "noninflammatory" conditions and growth factor withdrawal. However, these observations do not exclude the possibility that an in vivo dysregulation of apoptosis or apoptosis-related immune mechanisms is important in the pathogenesis of WG.

### *Experimental models of ANCA-associated vasculitis*

Several animal studies have been reported on ANCA and vasculitis/glomerulonephritis using various experimental approaches (for review see Heeringa et al. 1998). There are few animal models for ANCA-related vasculitis that resemble MPA. Kallenberg's group developed a model of proliferative glomerulonephritis resembling the renal involvement characteristic of MPA. The systemic autoimmune response to MPO led to severe necrotizing vasculitis with extracapillary glomerulonephritis and in some animals to extravascular granuloma formation. It is important to mention that induction of MPO-ANCA alone did not cause disease manifestations. Additional factors (infections?) inducing priming/activation of neutrophils, monocytes and endothelial cells with subsequent release of products (enzymes, oxygen species, etc.) are required. Similar models have been used to demonstrate PR3-ANCA-mediated kidney injury by our group (Mrowka et al. 1995).

Kobayashi et al. have reported that rats injected with low doses of anti-GBM antibodies along with MPO-ANCA develop more severe glomerular neutrophil infiltration and fibrin formation than rats injected with anti-GBM or anti-MPO antibodies alone (Kobayashi et al. 1995).

The MRL/lpr strain of mice has been used as an experimental model for the study of SLE and rheumatoid arthritis (Trabandt et al. 1992). These animals spontaneously develop lymphoproliferation, glomerulonephritis, arteritis and arthritis. Arteritic lesions in these mice are histologically characterized in the early stage of disease by fibrinoid necrosis and neutrophil influx, followed by a chronic infiltrate of lymphocytes and granulomatous inflammation. Recently, Harper et al. have demonstrated that 22% of female MRL-*lpr* mice develop MPO-ANCA. Anti-MPO monoclonal antibodies derived from these mice are polyreactive and react with double-stranded DNA. They bind conformational epitopes on human MPO which is also expressed by activated human neutrophils. These mice develop a clinical syndrome of vasculitis and glomerulonephritis that is distinct from immune complex disease. The results suggest that a subset of MRL-*lpr* mice develop ANCA-related vasculitis rather than SLE and may be used as a spontaneous model for human MPA.

In another model, purified human PR3-ANCA injected into Balb/c mice has been shown to cause production of mouse antibodies against human PR3-ANCA (Ab1), followed during the next month by production of anti-Ab1 antibodies (Ab2) that recognize human PR3. At the time of production of anti-Ab2 antibodies, the mice develop renal and pulmonary vasculitis similar to that in WG, suggesting a link between these two events. However, it is not clear from these experiments whether the antibodies (Ab2) also recognize murine PR3 (Blank et al. 1995). Recently, Jenne et al. have identified and characterized the murine PR3. Despite the strong similarities between human and murine PR3, PR3-ANCA from WG patients do not recognize the natively folded murine PR3 indicating that the endogenous murine homologue does not present any of those epitopes which are required to study human ANCA-induced vasculitis in mice. Consequently, it is unlikely that the disease observed in mice after immunization with PR3-ANCA is caused by pathogenic antibodies against mouse PR3 (Jenne et al. 1997).

A satisfactory animal model for ANCA-associated vasculitis has not yet been developed. None of the experimental models completely mimics the human ANCA-associated diseases. Furthermore, all of these in vivo animal models suggest, but do not definitively prove, that ANCA are pathogenic.

*The fate of polymorphonuclear neutrophils and monocytes in vasculitis*

PMN play an important role in the pathogenesis of WG: they predominate at the site of tissue injury (necrotizing vasculitis and granuloma) and they are the main target cell of the ANCA antigens. Using an $HgCl_2$-induced vasculitis model, direct evidence has recently been found for the primary role of PMN in vasculitis (Qasim et al. 1996): PMN have been shown to be essential for the induction of vasculitis and the degree of vasculitis correlates with the number of PMN. Furthermore, Hänsch et al. (1999) recently analyzed PMN from WG patients with respect to the expression of activation markers, including the high-affinity receptor for IgG, CD64, and major histocompatibility complex class II (MHC II) antigens (HLA-DP, HLA-DQ, HLA-DR). They found that PMN from the peripheral blood of WG patients expresses MHC II antigens and this expression is closely associated with active disease and declines rapidly under immunosuppressive therapy. Constitutive MHC II expression is restricted to professional antigen-presenting cells and PMN of healthy individuals normally do not express these molecules. The consequences of MHC II expression on PMN are a matter of speculation. The function of MHC II molecules is the presentation of antigenic peptides to T lymphocytes. Some data show that PMN are able to function as accessory cells for *Staphylococcus* enterotoxin-dependent T-cell proliferation (Fanger et al. 1997). This observation is particularly interesting with regard to a possible involvement of *Staphylococcus* enterotoxins in the ethiopathogenesis of WG (Cohen-Tervert et al. 1999).

In vitro experiments indicate that TNFα interacting with other cytokines plays a pivotal role in neutrophil-mediated vascular injury. Recently, Bratt and Palmblad have analyzed how cytokine stimulation of EC in vitro activates the cytotoxic capacity of PMN. Using this in vitro model of vasculitis, the authors demonstrated that IL-1β, TNF-α, and IFN-γ act as powerful promoters of cytokine-mediated neutrophil-dependent injury to endothelial cells. Many previous studies have shown that stimulated PMN can cause injury to EC. Chemotactic peptide FMLP or the physiologically occurring lipid product of arachidonic acid, $LXA_4$, directly stimulate PMN to confer a consistent cytotoxicity. The effects of these two stimuli are dependent on the release of proteases, oxygen radicals and expression of PMN adhesion molecules, and require $Ca^{2+}$ and $Mg^{2+}$ ions. However, the cytokine-mediated process described in that work is an example of endothelial injury mediated by neutrophils in the absence of neutrophil agonists. The cytotoxic process of vascular inflammation depends on expression of adhesion molecules and may be associated with stimulated nitric oxide production (Bratt and Palmblad 1997).

As well as PMN, monocytes/macrophages also play a key role in the development of vasculitis and granuloma formation in WG. Activated monocytes are detected in renal biopsies (Rastaldi et al. 1996) and in nasal biopsies from patients with WG (Müller et al. 2000). Since the ANCA antigen PR3 is also a constituent of granules from monocytes, these cells are also likely to be targets for ANCA. Monocytes can be activated to produce reactive oxygen intermediates by ANCA enhanced by priming with TNFα. Observations that ANCA stimulate monocytes to produce monocyte chemoattractant protein-1 (MCP-1) in vitro suggest that MCP-1 secretion may also participate in the formation of granulomas by amplification of local monocyte recruitment (Casselman et al. 1995). Furthermore, monocytes are potent regulators and producers of cytokines, especially when stimulated via endotoxin or Fcγ receptor crosslinking. In this context, recent findings have demonstrated that FcγR crosslinking produces IL-8 in monocytes (Ralston et al. 1997). These observations indicate that ANCA binding to PR3 expressed on the surface of monocytes may result in FcγR crosslinking and subsequent IL-8 release. Importantly, only TNFα-primed monocytes, which expressed surface PR3, released IL-8 in response to IgG-PR3-ANCA, implying that the surface interaction of PR3 with IgG-PR3-ANCA is critical in this disease process.

Recently, Bruce et al., have demonstrated that monocytes from patients with primary systemic vasculitis (i.e. WG, CSS) have an increased capacity to generate reactive oxygen species. Further study is required to determine if the reactive oxygen species generated act as a mechanism to promote vessel wall injury, or healing in this condition (Bruce et al. 1997).

*Wegener's granulomatosis: a Th1-granulomatous inflammation*

WG begins with granulomatous changes, as Fienberg was able to establish after decades of research. The primary granulomas form and develop in connective tissue, but without vascular involvement (Fienberg et al. 1981). In his last paper on this topic Friedrich Wegener wrote: "the vasculitis that accompanies the granulomatous disease is a secondary feature that represents a later stage" (Wegener 1990). Thus, insights into the leading immunopathogenic mechanisms in WG should come from studies concentrating on cells from the "pathergic granuloma" or its surroundings (e.g. bronchoalveolar lavage, BAL).

Granuloma formation is usually a host tissue response to a foreign antigen. Studies of intercellular interactions that lead to formation and maintenance of granulomas have now focused on the role of T cells (Nossal et al. 1997). In infectious diseases it has been found that in leprosy the T cells locally produce the Th1-cytokine pattern, whereas the granuloma induced by the schistosome egg is an inflammatory reaction that is tightly controlled by Th2 cytokines (Libraty et al. 1997; Wyn et al. 1995). Since the histologic lesion of WG is largely composed of cells of the monomyelocyte lineage (neutrophilic granulocytes, macrophages, epithelioid cells, and giant cells) together with lymphocytes and eosinophilic granulocytes, considered morphologically the lesion could equally be induced by Th1 or Th2 cytokines (Carter et al. 1996). Consequently,

we have turned our focus onto the cytokine profile of the lesional T cells (or the "nearby T cells" of the BAL).

In a preliminary study, we have found highly elevated soluble CD30 (sCD30) in the plasma of WG patients (Wang et al. 1997). This finding in association with the variable numbers of eosinophils in the granuloma, the moderate blood eosinophilia, and the slight elevation of IgE and the autoimmune phenomena (including ANCA) seen in many patients with WG led to the working hypothesis that WG could be a Th2-associated condition. On the other hand, studies on T cells from peripheral blood of WG patients have clearly shown that these cells exhibit increased secretion of IFN-γ but not of IL-4, IL-5 and IL-10, thus demonstrating that at least the periphery shows a Th1 response (Csernok et al. 1999; Ludviksson et al. 1998).

Recently, our group has investigated the cytokine pattern (Th1 and Th2) in WG by analyzing the profile of cytokine secretion by T cells derived from tissue with granulomatous inflammation (nasal mucosal biopsy specimens) or from an area close to the site of granulomatous inflammation (BAL) and, for comparison, from peripheral blood. In this study, we used different experimental approaches and IFN-γ (Th1 pattern) and IL-4 (Th2 pattern) were detected by ELISA and a competitive RT-PCR (Table 3). Our results demonstrate that the Th1 pattern is the main cytokine profile exhibited by TCC isolated from nasal biopsy specimens displaying granulomatous inflammation and – to a lesser extent – by TCC and TCL generated from BAL cells. In addition, both polyclonal $CD4^+$ and $CD8^+$ T cells from peripheral blood and BAL produce predominantly IFN-γ. These findings fit well with the concept that T cells play a triggering role in the pathogenesis of WG.

Our results have demonstrated the existence of a clear-cut Th1 polarization of the immune response in the granulomatous inflammation in WG. Furthermore, very recently we have analyzed the phenotype of inflammatory cells in nasal biopsies from patients with localized/initial and generalized WG. The presence of CD26, a dipeptidylpeptidase predominantly expressed by T cells (operational Th1 marker) and CD30 (Th2 marker) on T cells were detected by immunochemistry. Our findings indicate, that in nasal tissues mainly $CD4^+$/$CD26^+$ T cells as well as $CD14^+$ monocytes/macrophages may contribute to a polarized Th1-like immune response in both phases of WG (Müller et al. 2000). The mechanisms responsible for the preferential development of Th1 cells in granuloma have not yet been investigated. Th1-dominant responses are very effective in eradicating infectious agents, including those hidden within the cells. However, if the Th1 response is not effective or excessively prolonged, it may become dangerous for the host due to both the activity of cytotoxic cytokines and the strong activation of phagocytic cells. The local secretion of high levels of IFN-γ may represent an important amplification loop leading to a tissue-destructive inflammatory response in WG patients. IFN-γ activates local macrophages and granulocytes to produce proinflammatory cytokines and toxic metabolites, which cause damage to the tissue and maintain the inflammation.

As mentioned above, WG typically begins with a granulomatous inflammation in the respiratory tract without vasculitis and if untreated sometimes leads ultimately to "full-blown" WG mainly as a consequence of the subsequent vasculitic disease. Considered in this manner, WG is a granulomatous diseases evolving into Th2-dominated vasculitic disease. It remains to ask by what mechanism the putative Th1/Th2 switch occurs during the disease process. In light of ongoing advances in the development of immunotherapeutic modalities, it is imperative that these studies be performed.

### Type III: Vasculitides strongly associated with immune-complexes

Circulating immune complexes or in-situ immune complex formation are widely accepted as inducers of vasculitis. The pathogenic mechanisms leading to Schönlein-Henoch purpura, essential cryoglobulinemic vasculitis, cutaneous leukocytoclastic angiitis, and classic polyarteritis nodosa can therefore be characterized as "vasculitides strongly associated with immune complexes" (type III reaction). Immunohistochemical demonstration of humoral immune components (immunoglobulins, complement factors) in situ in blood vessel walls, often combined with polyclonal hypergammaglobulinemia and autoantibody production (antinuclear antibodies, rheumatoid factor) are hallmarks of these conditions. Th2-type cytokines, such as IL-10 and IL-6, seem to play a central role.

### Type IV: Vasculitides strongly associated with T-cell-mediated hypersensitivity

The type IV immune reaction encompasses "vasculitides strongly associated with T-cell-mediated hypersensitivity" and includes large-vessel vasculitides (giant cell arteritis and Takayasu's arteritis). These are often described as granulomatous vasculitides, but without deposition of immune complexes in situ or ANCA association.

Characteristically, accumulation of lymphocytes and monocytes can be seen in blood vessel walls, with a preponderance of IFN-γ-producing $CD4^+$ T cells (Th1-type cells). Although this scheme allows a simple classification according to central pathogenic mechanisms, more than one type of immune reaction may be involved during the course of a single disease entity, and overlap syndromes may occur.

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