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Pathogenesis of Wegener's granulomatosis: current concepts

Pasha Sarraf and Michael C. Sneller

Wegener's granulomatosis (WG) is a complex autoimmune syndrome that is characterised by upper/lower respiratory necrotising granulomatosis, glomerulonephritis and small-vessel vasculitis. Since Wegener's 1936 description, considerable advances in recognition and treatment have changed this disease from a rapidly and uniformly fatal illness to a chronic disease characterised by remissions and relapses. The serendipitous discovery of anti-neutrophil cytoplasmic antibodies (ANCAs) as a marker associated with WG focused attention on the potential pathogenic role of these antibodies and has recently led to the development of novel animal models that might facilitate our understanding of the disease pathogenesis. Future animal models of this disease will have to account for the role of both ANCA-mediated pathology and granulomatous inflammation to enable us to understand the chronic and persistent features of WG in humans.

Vasculitis is a clinicopathological process characterised by inflammation and necrosis of blood vessels leading to vessel occlusion and ischaemia of tissues supplied by the affected vessel(s). The primary vasculitis syndromes represent a heterogeneous group of disorders in which vasculitis occurs in the absence of any underlying infectious, neoplastic or autoimmune disease. Wegener's granulomatosis (WG) is a distinct form of systemic vasculitis characterised by necrotising granulomatous inflammation involving the respiratory tract, together with glomerulonephritis and variable degrees of disseminated small-vessel vasculitis. The first part of this review summarises the important clinical and pathological features of WG. The second section focuses on the immunopathological mechanisms underlying antibody-mediated pathology and granulomatous inflammation that might initiate and perpetuate tissue damage in WG.

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Institute URL: http://www.niaid.nih.gov

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

Features of WG

Clinical features

WG can develop at any age. Approximately 15% of patients are less than 19 years of age, but only rarely does the disease occur before adolescence; the mean age of onset is approximately 40 years (Refs 1, 2). WG affects men and women equally, and Caucasians are generally over-represented in studies and surveys (Ref. 1). The incidence of WG is regionally varied, from 4 per million in Olmsted County, MN, USA (Ref. 3) to as many as 15 per million in northern Norway (Ref. 4). Variations in seasonal incidence have been noted, with highest occurrence in the winter months (Ref. 5). Preliminary studies show a weak association between the disease and certain environmental exposures, such as heavy metals and farming, but these require further verification (Refs 6, 7).

Most patients seek medical attention because of upper and lower respiratory tract symptoms or both (90%); these are often accompanied by generalised symptoms of fatigue, fever or joint pain (arthralgias) (Refs 1, 2, 8). Patients often present with severe upper respiratory tract abnormalities, such as paranasal sinus pain and drainage, and purulent or bloody nasal discharge with nasal mucosal ulceration. Nasal septal perforation may follow, as can erosion of the nasal cartilage leading to saddle nose deformity. Pulmonary involvement is seen in 85–90% of patients and can be manifested as asymptomatic infiltrates or clinically expressed as coughing, expectoration of blood (haemoptysis), shortness of breath (dyspnea) and chest discomfort (Ref. 1).

On initial presentaion, only 12–18% of patients have glomerulonephritis; however, this eventually develops in about 80% of cases. The glomerulonephritis is often rapidly progressive and, if left untreated, will result in irreversible renal failure in the majority of cases (Ref. 1). In addition to the respiratory tract and kidney, virtually any organ system can be affected by WG.

Pathological features

The distinctive triad of granulomatous inflammation, necrosis and vasculitis of the respiratory tract distinguishes WG from other forms of systemic vasculitis (Refs 9, 10). In the lung, granulomatous inflammation usually produces solitary or multiple discrete parenchymal nodules, which may be bronchocentric, angiocentric or interstitial (Refs 1, 11). Within these nodules, an outer rim of granulomatous inflammation surrounds randomly scattered areas of necrosis and/or vasculitis (Fig. 1). The two most common patterns of necrosis seen in WG are neutrophilic microabscesses and geographic necrosis (Ref. 11).

Outside the respiratory tract, although granulomatous lesions may be found, vasculitis is more common (Ref. 12). The histopathology of vasculitis in WG is varied and includes acute leukocytoclastic capillaritis, necrotising arteritis, granulomatous vasculitis and/or venulitis. In the lung, although vasculitis is generally found in areas involving the pulmonary nodules, in a small minority of cases it may occur in isolation. The vasculitis may or may not be granulomatous in nature, and larger vessels often have only focal involvement (Fig. 1).

The renal lesion in WG is characterised by a focal, segmental necrotising glomerulonephritis (Ref. 13). Unlike other forms of glomerulonephritis, glomerular pathology in WG typically lacks evidence of immune complex deposition and is often referred to as 'pauci-immune'. Granulomatous or vasculitic lesions are rarely found in kidney biopsy specimens.

Treatment and prognosis

WG can follow a varied clinical course, which is strongly influenced by treatment. Untreated, generalised WG is usually lethal. In one series of untreated patients, the mean survival time was five months, and greater than 90% of patients died within two years of diagnosis (Ref. 9). Current therapy for WG involves the initial use of intensive immunosuppressive regimens consisting of high-dose glucocorticoids and a cytotoxic agent (usually cyclophosphamide) (Ref. 14). With this therapy, complete disease remission can be achieved in the majority of patients. Following remission, immunosuppressive therapy is tapered and eventually discontinued (Ref. 14). Although current regimens can induce remission in 80–100% of patients, relapse of disease occurs in at least 50% of patients, usually after discontinuation of immunosuppressive therapy (Ref. 15).

Pathogenesis

The involvement of upper airways and lungs with granulomatous inflammation suggests that WG may be initiated by an aberrant cell-mediated immune response to an exogenous or even endogenous antigen that enters through, or resides in, the respiratory tract. In addition, WG is highly associated with autoantibodies that

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

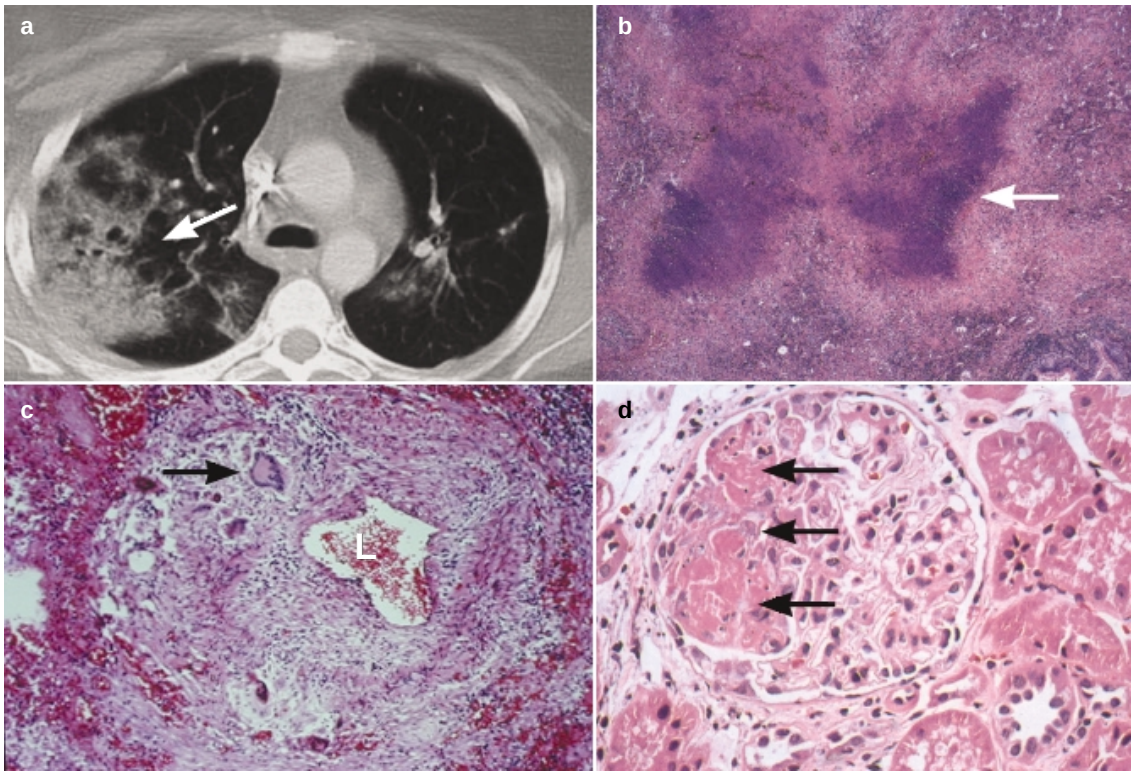

Figure 1. Features of Wegener's granulomatosis. (a) Computed tomography (CT) chest scan of a patient with Wegener's granulomatosis (WG) showing typical nodular lung infiltrate with cavitation. (b) Low-power view of granulomatous inflammation and geographic necrosis in a lung biopsy from a patient with WG. (c) Granulomatous vasculitis of a small pulmonary artery in the lung of a patient with WG (L, vessel lumen). The vessel wall is markedly thickened with an inflammatory infiltrate that includes multinucleated giant cells (black arrow). (d) Glomeruli showing segmental necrosis with early crescent formation (black arrows).

recognise specific neutrophil cytoplasmic proteins, suggesting that abnormalities of humoral immunity also play a role in pathogenesis. Although discussed individually, these two potential pathological mechanisms probably operate together to produce vessel and tissue damage in WG.

Anti-neutrophil cytoplasmic antibodies

A major advance in the study of WG came in 1982 when Davies and colleagues identified a serum factor shown to be IgG that stained the cytoplasm of neutrophils in eight patients with nephritis, five of whom probably had WG (Ref. 16). This observation went largely unnoticed for two years until another group, who were studying antinuclear factors, showed similar neutrophil staining in four patients characterised as having 'arteritis' (Ref. 17). Subsequently, several groups demonstrated that anti-neutrophil cytoplasmic antibodies (ANCAs) had a remarkable specificity for WG, and focused attention on the potential pathogenic role of these autoantibodies (Refs 18, 19, 20). Although ANCAs have been described that recognise a variety of myeloid antigens, only antibodies that react with proteinase 3 (Pr3) and myeloperoxidase (MPO) have consistently been linked to vasculitis syndromes (Ref. 21). Pr3-ANCA is the predominant autoantibody found in patients with WG, being present in more than 90% of patients with active disease (Ref. 22).

The ANCA antigens

Pr3, a 29 kDa protein, is one of four neutral serine protease homologues localised in the azurophilic

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

granules, secretory vesicles and specific granules of neutrophils and monocytes (Ref. 23). It is regulated at the transcriptional level during granulocyte development. Pr3 is expressed as a preproenzyme that is processed intracellularly; after removal of the preregion, the pro-form undergoes maturation and folding via glycosylation and disulphide-bond formation, and the mature form is stored in granules upon terminal differentiation of neutrophils. Although cells excrete pro-Pr3 in small amounts, the majority of the Pr3 found extracellularly is in its mature enzymatically active form. The resting neutrophil expresses some Pr3 on its membrane and this is increased by neutrophil activation. This finding has been clearly reproduced in samples from healthy human subjects with acute inflammatory conditions (Refs 24, 25). The nature of the Pr3 interaction with the cell membrane has not been fully defined but seems to be a covalent non-charge-dependent association (Ref. 26). Nonmyeloid cells, such as certain endothelial cell lines, can be induced to express Pr3 in vitro (Ref. 27). Whether nonmyeloid cells express Pr3 in vivo has not been systematically addressed.

Enzymatically active Pr3 cleaves and degrades extracellular matrix components, allowing neutrophil migration (Ref. 28). Other functions associated with Pr3 include endothelial cell binding and activation in vitro (Ref. 29), cleavage of tumour necrosis factor α (TNF-α) to a biologically more active form (Ref. 30), and degradation of C1 inhibitor (Ref. 31). The function of Pr3 is regulated by the serine protease inhibitor α_1-antitrypsin (Ref. 32), which is encoded by a group of highly polymorphic genes producing proteins with a variable Pr3-binding and -inhibitory capacity (Ref. 23).

MPO is a tetrameric, granule-associated haeme protein of 144 kDa. It is localised in the azurophilic granules of neutrophils and monocytes, and forms 5% of the dry weight of neutrophils and roughly a third of that proportion in monocytes (Ref. 33). MPO is strongly cationic and can generate oxidants from hydrogen peroxide (H_2O_2) and other substrates, including chlorine and nitrite, which are bactericidal; MPO therefore plays an important role in microbial killing (Ref. 33). MPO secreted from myeloid cells binds to endothelial cells through heparan/heparin-containing glycosaminoglycans and in a charge-dependent manner, and is subsequently rapidly transcytosed to the basolateral membrane, where it binds to molecules such as fibronectin (Ref. 34). Mice deficient in MPO have a mild compromise of the innate immune system, including increased susceptibility to fungal infections (Ref. 35). In the absence of MPO, stimulated neutrophils usually exhibit a prolonged respiratory burst, suggesting a role for MPO by-products in self-limiting the inflammatory response (Ref. 33).

Several in vitro observations suggest potential mechanisms by which ANCAs could contribute to the pathogenesis of WG. As indicated above, in unstimulated neutrophils, MPO and Pr-3 reside predominantly in cytoplasmic granules and are presumably not accessible to extracellular antibodies. However, when neutrophils are activated by pro-inflammatory cytokines such as TNF, granules containing MPO and Pr3 move to the cell membrane, where they can interact with extracellular ANCAs. As first demonstrated by Falk et al., in vitro exposure of cytokine-primed neutrophils to ANCAs leads to neutrophil degranulation and release of reactive oxygen species (Ref. 36). Further observations by Porges et al. suggested that ANCA-induced neutrophil activation is mediated via the FcγRIIa signal transduction system (Ref. 37). These investigators found that ANCA-induced neutrophil activation was dependent on engagement of the FcγRIIa receptor on neutrophils by the Fc region of ANCAs. Thus, ANCA-induced neutrophil activation appears to require both binding of the $F(ab)_2$ region of ANCA to Pr3 (or MPO) and subsequent engagement of the FcγRIIa receptor by the Fc region. ANCA-activated neutrophils can adhere to and kill endothelial cells in vitro (Refs 29, 38). Activation of neutrophils and monocytes by ANCAs also induces the release of pro-inflammatory mediators including cytokines such as interleukin 1 (IL-1) (Ref. 39) and IL-8 (Ref. 40) and bioactive lipids such as leukotriene B4 (Ref. 41). These in vitro observations have led several investigators to postulate a scenario in which the abnormal production of ANCAs could lead to neutrophil-mediated vascular injury (Fig. 2).

Experimental models of ANCA vasculitis

Active immunisation models

Direct attempts to generate an animal model of ANCA vasculitis have focused mostly on an active immunisation strategy. In one study, the autoimmune-prone Norway brown rats (NBRs) were immunised with human MPO, and then five weeks later the renal circulation was isolated and

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Published by Cambridge University Press

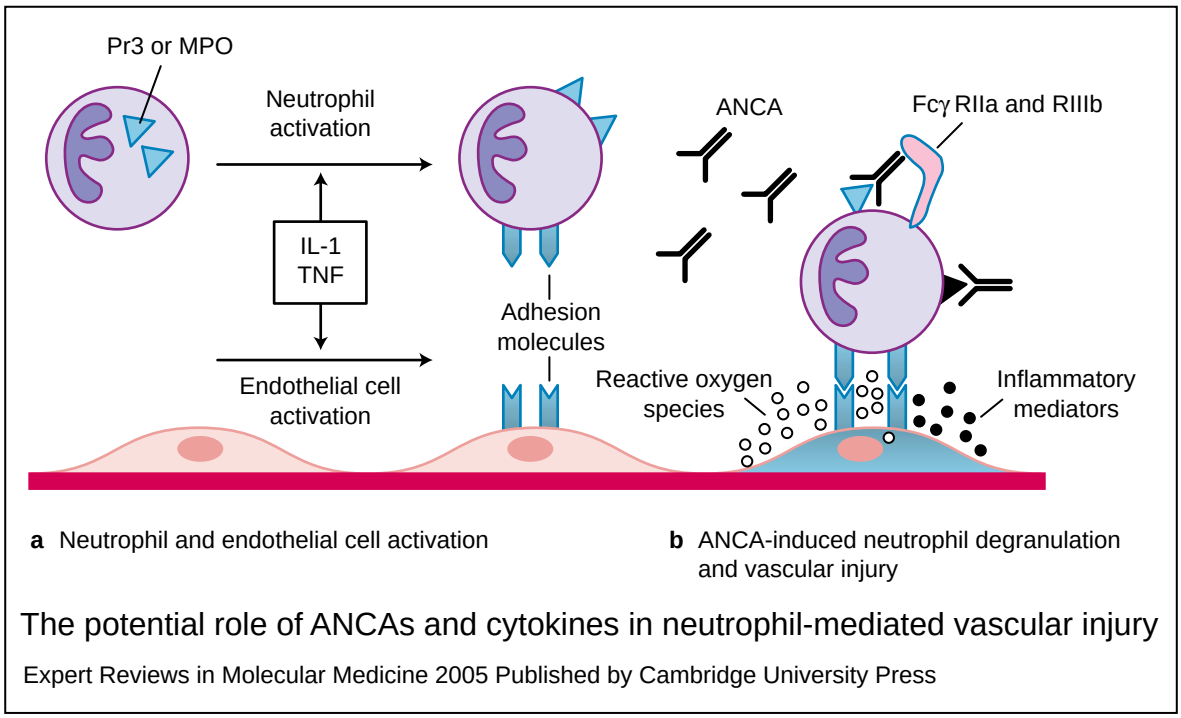

Figure 2. The potential role of ANCAs and cytokines in neutrophil-mediated vascular injury. (a) Neutrophil and endothelial cell activation. Infection or other inflammatory stimuli lead to the production of pro-inflammatory cytokines [e.g. interleukin 1 (IL-1), tumour necrosis factor (TNF)], which induce resting neutrophils to express proteinase 3 (Pr3) and myeloperoxidase (MPO) on their cell surface. IL-1 and TNF also induce neutrophils and endothelial cells to increase their expression of adhesion molecules. (b) ANCA-induced neutrophil degranulation and vascular injury. The increased expression of adhesion molecules leads to binding of activated neutrophils to the vascular endothelium. Circulating ANCAs (anti-neutrophil cytoplasmic antibodies) bind to membrane-associated Pr3 or MPO and the FcγRIIa receptor, inducing neutrophil degranulation and generation of oxygen radicals, which results in endothelial cell injury and inflammation. Activation of neutrophils by ANCAs also results in release of inflammatory mediators such as IL-1 and IL-8, and bioactive lipids such as leukotriene B4. Figure based on illustration published in Ref. 94. ANCA, anti-neutrophil cytoplasmic antibody.

human MPO, human neutrophil lysosomal extracts or H_2O_2 in various combinations was injected (Ref. 42). With this strategy, animals with circulating anti-MPO were exposed to a direct inflammatory stimulus in their kidneys. All immunised rats mounted an antibody response that recognised the injected human MPO, but also crossreacted with endogenous rat MPO. However, none of these rats developed de novo glomerular proliferative lesions until their kidneys were perfused with H_2O_2 together with MPO or neutrophilic cell lysate, suggesting that crossreacting endogenous ANCA could recognise and exacerbate an acute insult.

In another set of experiments, NBRs were injected with subnephritogenic doses of anti-glomerular basement membrane (GBM) antibody two weeks after immunisation with human MPO (Ref. 43). Soon after injection of anti-GBM, glomeruli exhibited strong staining for MPO both on infiltrating neutrophils and in the extracellular space. Notably, this staining was not specific to the immunised rats. However, whereas control rats developed a mild nephritis without crescent formation (a histological marker of severe inflammatory injury to the glomerulus; Fig. 1d), immunised rats uniformly developed severe haematuria and proteinuria, together with massive glomerular deposition of fibrin, which is a marker of necrosis. These experiments have been interpreted as showing an in vivo role for ANCAs in exacerbating inflammation initiated by a primary stimulus.

MPO-knockout mice

Although mouse or rat MPO proteins crossreact with human anti-MPO ANCAs, it has not been possible to break tolerance against endogenous

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

MPO in order to produce autologous antisera. Therefore, MPO-knockout mice were used in a strategy to produce and transfer mouse anti-MPO sera to healthy mice in order to examine the pathological effects of this antibody in vivo (Ref. 44).

MPO-knockout mice were immunised with mouse MPO or bovine serum albumin (BSA) and the production of anti-MPO antibodies was detected by immunofluorescence. Subsequently, either splenocytes (adoptive transfer) or purified immunoglobulins (passive transfer) were transferred, in a dose-dependent manner, into *Rag2*$^{-/-}$ mice (which lack functioning B and T cells) or wild-type mice. MPO-immunised splenocytes produced detectable anti-MPO antibodies in *Rag2*$^{-/-}$ mice as early as three days post-transfer. At the time of sacrifice, 13 days post-transfer, all mice had developed renal failure, and had rising blood urea nitrogen and creatinine, as well as urinary blood, protein and leukocytes. On pathology, all *Rag2*$^{-/-}$ mice that had received anti-MPO splenocytes developed crescentic (80%) and necrotic (60%) lesions in their glomeruli. Unlike the glomerular lesion in WG, immune complex and complement deposition was found in these lesions (i.e. they were not 'pauci-immune'). None of the control mice developed necrotising glomerular lesions, but deposition of immune complexes and complement was demonstrated. In the passive-transfer experiments, a single intravenous (iv) dose of immunoglobulins, leading to high circulating levels of anti-MPO, caused haematuria, proteinuria and leukocytouria by three days post-injection. Consistent with findings in the active-transfer experiments, all *Rag2*$^{-/-}$ and wild-type mice that received anti-MPO had glomerular histology demonstrating crescents and necrosis but no immune complexes were detected. The degree of pathology was more pronounced in the *Rag2*$^{-/-}$ mice (mean 10.8% crescents, 13.2% necrosis) as compared with wild-type mice (mean 3.3% crescents, 4.7% necrosis), and no pathology was seen in mice receiving anti-BSA regardless of their genotype. Creatinine was not altered in either group.

In both adoptive- and passive-transfer experiments, only incidental nonglomerular pathology was noted. In the adoptive model, there was one case of necrotising arteritis in the spleen and lymph nodes, a similar case in the lungs, one necrotising granulomatous lesion in the spleen, and five out of 16 animals had pulmonary capillaritis. In the passive-transfer experiments, two out of six mice had pulmonary alveolar capillaritis and three out of six mice demonstrated cutaneous lesions with leukocytoclasia and fibrinoid necrosis on pathology.

The authors interpreted the results of these experiments as providing direct evidence that ANCAs are sufficient to induce disease in mice. However, the authors did not determine whether the murine anti-MPO antibodies were capable of activating neutrophils. Also unclear was whether MPO expression on neutrophils per se was a requirement for the observed pathology or if released circulating MPO behaved as a planted antigen in the glomerular lesions. Further experiments in this model are needed to elucidate whether the observed pathology is secondary to immune-complex formation or direct activation of MPO on the surface of neutrophils by ANCAs.

An additional observation emphasised by the authors is that these animals can develop granulomatous lesions on active transfer of antibody and cells, arguing that ANCAs alone can create the host of disease pathology seen in WG and microscopic polyangiitis (inflammation of multiple blood vessels, of different types) (Refs 44, 45). However, it should be pointed out that granulomatous inflammation in a lymph node was detected only in a single mouse; granulomatous inflammation in the respiratory tract (the hallmark of WG) was not reported in any animal (Ref. 44). Whether incidental granulomatous inflammation in a single experimental animal is proof that anti-MPO antibody recreates the pathophysiology of WG is open to speculation.

Pr3-knockout mice

Although human MPO can elicit an antibody response in the mouse, human Pr3 does not trigger a crossreactive antibody response against mouse Pr3 (mPr3); nor do human anti-Pr3 antibodies crossreact with mPr3. Since mPr3 does not trigger a detectable antibody response in wild-type mice, a strategy similar to the one employed for the production of anti-MPO antibodies was utilised for Pr3 (Ref. 46). mPr3 and neutrophil elastase exhibit high sequence homology, and so double-knockout mice were created to ensure specificity of immunisation and the ability to examine a wide range of epitopes on Pr3. Mice were immunised with two antigens: full-length mPr3 as well as pro-mPr3. Both pools of antisera from these mice bound to activated but not

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Published by Cambridge University Press

resting wild-type murine neutrophils in vitro. Continuous antisera administration resulted in a serum anti-Pr3 level with a half-life of around 11 days (close to the normal half-life of murine IgG), indicating that the antibody was not being rapidly cleared via immune-complex formation. After antisera administration, the mice were sacrificed to evaluate any ANCA-mediated pathology. By contrast to the findings described above with anti-MPO-treated mice, none of the anti-Pr3-treated mice exhibited any signs of pathology in their lungs or kidneys.

To evaluate the role of ANCAs after neutrophil priming, the investigators administered serial subcutaneous injections of TNF to activate the inflammatory response in a localised manner. Prior to TNF injections, the mice were systemically injected with a single dose of antiserum from mock-immunised or Pr3-immunised mice. After four daily injections of TNF, the mice were sacrificed and the skin examined. Tissue staining with the anti-neutrophil marker Ly-6G revealed a substantial influx of neutrophils into the TNF-injected foci. TNF alone caused a mild inflammatory infiltrate in mice treated with mock-immunised antisera, but foci from mice treated with Pr3-ANCA-containing sera were twice as large. These results were interpreted as evidence for a distinct pro-inflammatory effect of systemic Pr3-ANCA on local inflammatory responses. Furthermore, to evaluate the role of systemic neutrophil priming on ANCA-mediated effects, mice were primed with both low- and high-dose bacterial lipopolysaccharide (LPS), and then injected with mock or immune antisera. Assessment of the lungs and kidneys from these animals for evidence of pathology indicated no significant difference between animals receiving mock versus anti-Pr3 sera, despite clear signs of LPS-mediated pulmonary capillaritis and inflammatory infiltration.

These thorough experiments do not provide clear evidence of a supportive role for Pr3-ANCA in mediating or amplifying an activated inflammatory response. The reason for the discordance between the ANCA effects following systemic versus local immune priming is unclear, but the authors suggest this might be due to rapid neutralisation of Pr3 within the circulation. With regards to the enhanced localised TNF-induced inflammatory foci, the observed results could be due to a localised Arthus reaction (the local inflammatory response resulting from deposition of immune complexes in tissues) (Ref. 47). In this case, TNF injected locally recruits and activates a neutrophilic infiltrate to degranulate, thereby releasing endogenous mPr3. Freshly released mPr3 forms immune complexes with passively administered anti-Pr3 sera, producing a reaction that propagates inflammation through complement activation and further leukocyte recruitment.

IgG preparations from patient sera

None of the experimental models described above addresses whether ANCAs can actually bind to and activate neutrophils under the conditions found in vivo. A corollary to the ANCA hypothesis is that neutrophils expressing Pr3 on their surface can be expected to have surface-bound ANCAs. The only study to date to address this issue found no evidence for surface-bound IgG on neutrophils ex vivo (Ref. 48). In this study, neutrophils isolated from WG patients with active disease and high-titre ANCAs were examined for surface-bound IgG. To circumvent technical issues with degradation and receptor internalisation, experiments were performed both on ice and at room temperature. Using anti-Ig to detect surface-bound antibody, robust expression of surface Pr3 on isolated neutrophils was demonstrated (mean 70% of neutrophils positive in WG versus 22% in healthy controls), but no difference in surface-bound IgG between healthy controls and WG patients was found (mean 7% in WG versus 14.9% in healthy controls). Furthermore, no statistical difference in binding of high-ANCA, low-ANCA and healthy donor sera to primed neutrophils from healthy controls (induced to express high levels of surface Pr3; mean 73% of neutrophils positive) was found. These effects were unaltered by temperature, arguing against degradation or internalisation of the complex as a reason for the negative findings. The use of a purified IgG fraction from ANCA-positive samples in further binding experiments revealed a more robust binding of IgG from ANCA-positive sera as compared with IgG from healthy donors. This binding appeared to be Pr3 specific since preincubation with anti-Pr3 monoclonal antibodies (mAbs) diminished the binding of ANCA-containing IgG to a greater extent than the binding of IgG from healthy donors. Given this disparity in binding between ANCA-containing plasma versus ANCA-containing IgG preparations, the investigators indirectly explored the affinity of the polyclonal antibodies within the IgG preparations. Low quantities of

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

heat-inactivated normal serum (1–5%) was sufficient to displace binding by 50%, while 100% inhibition was seen at higher concentrations of serum. The addition of irrelevant antibody or albumin, as well as increasing the reaction volume, all also inhibited ANCA binding. Interestingly, when rabbit polyclonal anti-human Pr3 antibody was used there was no binding inhibition by plasma or volume increase.

These results suggest that the binding of ANCA in IgG preparations from patient sera occurs with very low affinity. This observation, combined with the inability to detect surface IgG on Pr-3-expressing neutrophils from WG patients, would appear to contradict the central tenet of the ANCA hypothesis; that ANCA can bind and activate neutrophils in vivo. However, it is possible that most of neutrophil priming and activation in vivo occurs locally and in association with activated endothelial cells (Ref. 49). Activated neutrophils that have bound ANCA after adhering to endothelial cells would not be detected by examining peripheral blood samples.

Do ANCAs play a primary role in WG pathogenesis?

Although ANCAs are highly associated with WG, and the experimental observations described above suggest mechanisms whereby these autoantibodies can produce vasculitis, several clinical observations argue against a primary role of ANCAs in the pathogenesis of WG: first, patients can have active WG in the absence of ANCAs (Refs 22, 50); second, the levels of the antibody titres do not correlate well with disease activity (Ref. 50); and third, patients with WG in remission can continue to have high ANCA titres for years without experiencing a recurrence of disease (Ref. 50). These clinical observations suggest that the presence of ANCAs is neither necessary nor sufficient to produce the clinical manifestations of WG. In addition, it is not clear how ANCAs could give rise to granulomatous inflammation, which is the hallmark of WG. The precise role of ANCAs in the pathogenesis of WG will not be defined until a therapeutic agent can be found that selectively inhibits ANCA production and the effects of this inhibition are observed.

Granulomatous inflammation

The granulomatous response

The granuloma, a term coined by Virchow over a century ago to describe the tumour-like granulation tissue in patients with tuberculosis, can be defined as a focal, chronic, predominantly mononuclear, tissue reaction to poorly degradable ingested antigens. Granulomatous inflammation is a normal host response to infection with various intracellular pathogens such as mycobacteria and fungi (Refs 51, 52). It can also occur in hypersensitivity reactions to foreign antigens, whether biological or chemical (e.g. sarcoidosis or chronic berryliosis) (Ref. 51). The common factor for the initiation and maintenance of a granulomatous response is the presence and persistence of an antigen. By localising and containing the various cellular constituents of the innate and adaptive immune system in close proximity, the granulomatous response can also be seen as an attempt to contain the inflammatory focus and minimise damage to surrounding tissue.

Both functional T cells and mature macrophages are necessary for generation of an efficient granulomatous response to pathogens (Ref. 52). On first encounter with an organism, tissue-resident γδ T cells (Ref. 53), natural killer (NK) cells (Ref. 54), mast cells and local histiocytes (Ref. 55) compose the first wave of a response (Fig. 3a). The activation of these cells subsequently amplifies the immune response by recruiting neutrophils (Ref. 56) and $CD4^+$ T cells, with recruitment of cytotoxic T cells occurring later in the course of the disease (Ref. 57). Activated dendritic cells migrate to various lymph nodes to activate antigen-specific T cells (Ref. 54) (Fig. 3b). T cells are subsequently enriched in sites of active inflammation to coordinate a response, by signalling to various constituents of the innate and adaptive immune system. Over the course of days to weeks, a loose amalgam of cells (consisting mostly of monocytes) forms a structure around the foreign invader (Ref. 58). With the arrival of T cells, monocytes accelerate their differentiation, becoming either mature macrophages or epithelioid giant cells, and form a tight granuloma surrounded by a rim of T cells (Fig. 3c). B cells are also seen in the periphery of the granuloma and, although their numbers are variable, they can form up to 20% of the cellular component of a granuloma (Ref. 59).

Much of what is known about the immunopathogenesis of granulomatous inflammation comes from detailed studies in animal models of infection with mycobacteria (Ref. 60). Studies in gene-disrupted mice and evidence from human diseases support the primacy of $CD4^+$ T cells in initiating and maintaining

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

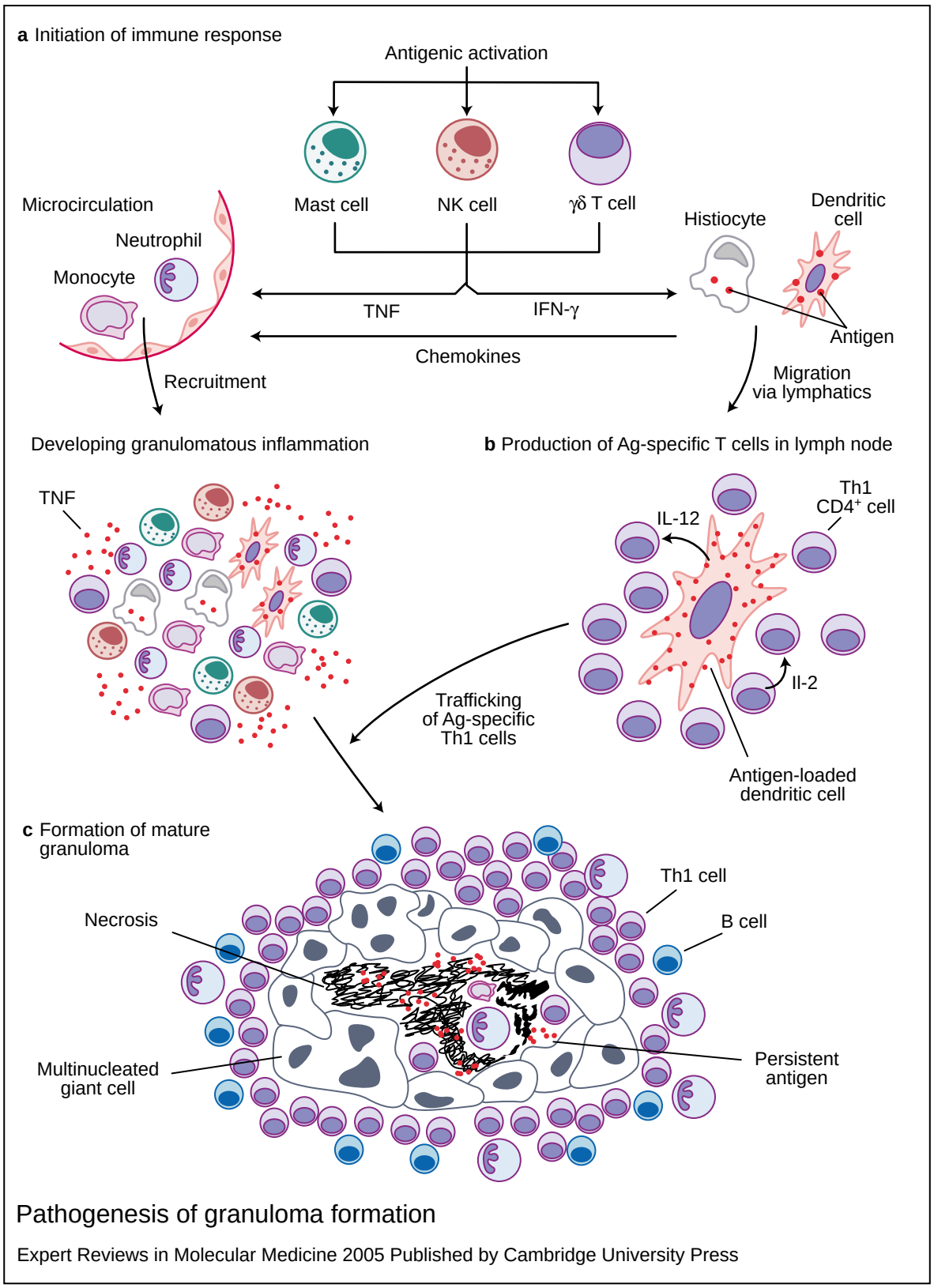

Figure 3. Pathogenesis of granuloma formation. *(See next page for legend.)*

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

Figure 3. Pathogenesis of granuloma formation. *(Legend; see previous page for figure.)* (a) Initiation of immune response. Within seconds to minutes after exposure to antigen, resident cells initiate cellular recruitment. Pre-stored tumour necrosis factor (TNF) released by mast cells recruits neutrophils, which in turn signal to and activate tissue and circulating monocytes. Interferon γ (IFN-γ) produced by local natural killer (NK) and γδ T cells further activates resident tissue histiocytes and dendritic cells. These latter cells release a host of chemokines and TNF, which alter the local microcirculatory environment and facilitate cellular trafficking into the tissue. Within minutes to hours, activated antigen-loaded dendritic cells migrate to peripheral lymph nodes via the lymphatic channels. (b) Production of antigen-specific T cells. Antigen-loaded dendritic cells travel to local lymph nodes and initiate a lymphocytic response. Dendritic cells produce interleukin 12 (IL-12) and present antigen to naive $CD4^+$ T cells. Under the influence of IL-12, naive $CD4^+$ cells differentiate into T helper 1 (Th1) cells. Activated Th1 $CD4^+$ T cells secrete IL-2, which promotes T-cell survival and proliferation, leading to expansion of the population of antigen-specific Th1 cells. (c) Formation of mature granuloma. Within hours to days after antigen exposure, activated Th1 $CD4^+$ cells preferentially traffic to sites where the microcirculation has been altered by TNF and chemokines produced by resident cells. If the source of antigen is not eradicated, inflammation persists. The interaction between Th1 $CD4^+$ T cells and activated macrophages leads to production of IFN-γ and TNF, which results in further maturation of macrophages. Over the course of several days to weeks a mature granuloma is formed. Other cells, including but not restricted to neutrophils and B cells, are found in various proportions in the mature granuloma (see text and references for further details).

granuloma formation. Mice rendered CD4 deficient by gene knockout of major histocompatibility complex (MHC) class II (Ref. 61) or CD4 (Ref. 62) exhibit delayed, poorly organised, granuloma formation and increased mortality in response to infection with *Mycobacterium tuberculosis*. In human immunodeficiency virus (HIV) infection, selective depletion of $CD4^+$ T cells by this virus is associated with increased susceptibility both to tuberculosis and to the disseminated infection resulting from *Mycobacterium avium* (Ref. 63). In HIV-infected patients with mycobacterial infections, the extent of granuloma formation is correlated with peripheral $CD4^+$ T-cell counts in that patients with low $CD4^+$ T-cell counts exhibit defective granuloma formation (Ref. 64).

Role of chemokines and cytokines

The coordinate regulation of granulomatous inflammation is orchestrated by the production of chemokines (reviewed in Ref. 65) and cytokines. Although upregulation of a number of cytokines is seen in animal models of granulomatous inflammation, the available evidence suggests that the T helper type 1 (Th1) cytokines interferon γ (IFN-γ), IL-12 and TNF are absolutely necessary for normal granuloma formation and maintenance (Ref. 52). In animal models of infectious granulomatous inflammation, IFN-γ is produced early in the infection by NK cells and later by Th1 cells. IFN-γ has several effector functions relevant to granuloma formation, including activation of macrophage bactericidal mechanisms, induction of TNF secretion by macrophages, activation of the endothelium to promote adhesion of $CD4^+$ T cells, and promotion of Th1 differentiation (Ref. 52). Studies in knockout mice have clearly shown that IFN-γ is required for normal granuloma formation in response to experimental infection with mycobacteria (Refs 66, 67). By contrast to wild-type mice, knockout mice deficient in IFN-γ or IFN-γ receptor (IFN-γR) do not develop mature granulomas or protective immunity following experimental infection with mycobacteria (Ref. 68). The granulomas in these knockout mice are poorly formed, with increased neutrophilic infiltration and necrosis.

IL-12 is a heterodimeric cytokine produced primarily by antigen-presenting cells (Ref. 69). In addition to enhancing proliferation and cytotoxicity of NK cells and cytolytic T cells, IL-12 is a potent inducer of Th1 differentiation and hence IFN-γ production. When mice with disruption of IL-12p40 are experimentally infected with mycobacteria they show increased mortality and abnormal granulomas (Ref. 70). As was the case with IFN-γ-knockout mice, the granulomatous lesions in IL-12-knockout mice are poorly formed, with increased neutrophilic infiltration and necrosis. Taken together, these studies indicate that IL-12-induced IFN-γ production is essential for normal granuloma formation in these animal models.

Analysis of humans with rare genetic mutations in the genes encoding IFN-γR, IL-12 receptor β1 or IL-12p40 supports a central role for IL-12 and IFN-γ in granulomatous inflammation and immunity to intracellular pathogens. Individuals with these mutations exhibit impaired granuloma formation and increased susceptibility to infection with intracellular pathogens (Refs 71, 72, 73).

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
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TNF is another Th1 cytokine that appears to be crucial for normal granuloma formation. The major source of TNF is mononuclear phagocytes, although T cells are also capable of producing substantial amounts of this cytokine (Ref. 74). TNF is a potent cytokine with a broad range of activities including upregulation of adhesion molecules on endothelium, activation of macrophages to kill intracellular bacteria, and induction of cellular apoptosis. Mice with targeted disruption of the gene encoding TNF exhibit increased mortality when experimentally infected with mycobacteria (Refs 75, 76). $TNF^{-/-}$ mice infected with *M. tuberculosis* exhibit levels of antigen-specific T-cell proliferation, IFN-γ production and macrophage activation that are comparable with wild-type mice. However, granuloma formation in these mice is retarded and markedly abnormal; granulomas form poorly, contain large numbers of organisms, and exhibit extensive necrosis and pronounced neutrophilic infiltration. By contrast to wild-type mice, T cells in $TNF^{-/-}$ mice are confined to the perivascular and peribronchial areas and are not seen within the inflammatory lesions. This latter finding, as well as the extensive neutrophilic influx and necrosis seen in $TNF^{-/-}$ mice, suggests that TNF plays an important role in controlling local cellular traffic in granulomatous lesions. TNF may function to limit the influx of neutrophils that cause tissue damage, yet promoting the recruitment and migration of T cells into granulomas where they can interact with macrophages.

Granuloma formation in WG

As indicated above, pathogenic granulomatous inflammation is the defining feature of WG. This pattern of inflammation is identical to that produced in response to infection with mycobacteria, fungi and other intracellular pathogens; however, in WG, the granulomatous histopathology occurs in the absence of any identifiable infecting organisms. This fact has led to the concept that a tissue autoantigen or environmental stimulus initiates an aberrant immune response that is persistent and polarised to a Th1 phenotype. The substantial presence of $CD4^+$ T cells in pathological specimens in WG, and ex vivo studies of these cells, support this idea. Peripheral blood $CD4^+$ T cells from patients with active WG produce 10–20 times the levels of IFN-γ produced by cells from healthy volunteers (Ref. 77). In addition, TNF production by $CD4^+$ T cells is enhanced, as is the production of IL-12 by purified monocytes. The levels of IL-4, IL-5 or IL-10, considered to be cytokines characterising a Th2 phenotype, are not increased. This immunoregulatory imbalance is correctable: the addition of IL-10 suppresses the abnormal IFN-γ secretion in a dose-dependent manner.

In other studies, isolated polyclonal or cloned T cells derived from broncheoalveolar lavage fluid or from nasal biopsy specimens show an increased predominance of Th1 cytokines, with normal IL-4 production (Ref. 78). Furthermore, in situ histochemical analysis of IFN-γ mRNA expression in nasal biopsy specimens reveals a predominance of this cytokine. These findings suggest that patients with WG have an immunoregulatory defect that leads to excessive production of Th1 cytokines (TNF and IFN-γ) by activated $CD4^+$ T cells in response to environmental insults (such as infections) and/or autoantigens. The excessive production of TNF and IFN-γ by $CD4^+$ T cells could serve to initiate and perpetuate the granulomatous inflammatory vascular lesion that is characteristic of WG. The nature of the immunoregulatory defect is unclear but it could involve either excessive production of IL-12 or underproduction of IL-10.

Given the close association between ANCAs and WG, an obvious possibility is that autoreactive $CD4^+$ T cells specific for Pr3 are also responsible for the pathogenic Th1 responses described above. Various investigators have tried to demonstrate expanded MPO- or Pr3-specific T-cell populations in patients with WG (Refs 79, 80). In these experiments, peripheral blood from WG patients was isolated and subsequently exposed to MPO or Pr3, in either active or heat-inactivated form, and the subsequent T-cell proliferative response was quantified. To date, the results of these experiments have not demonstrated expansion of MPO- or Pr3-specific T cells in WG. Although MPO and Pr3 have modest effects on lymphocytic proliferation, these effects are seen in both patient and control samples. This suggests that MPO and Pr3 act as nonspecific mitogens rather than as specific stimulatory antigens.

Although an autoantigen(s) responsible for initiating the pathogenic Th1 responses is yet to be identified, one group has found indirect evidence for antigen-driven $CD4^+$ T-cell expansion in WG (Ref. 81). Grunewald et al. have reported clonal expansion of $CD4^+$ cells in the peripheral blood from selected WG patients. These researchers

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
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screened a cohort of WG patients and identified four individuals with dramatic expansions of $CD4^+V\beta8^+$ T cells. Sequence analysis of the Vβ8 complementarity-determining region 3 region revealed that the expansions were clonal and shared a common sequence motif. The four patients were unrelated but they all expressed the HLA-DRB1*0401 allele. This study concluded that the $CD4^+V\beta8^+$ T-cell expansions observed in these four patients were the result of exposure to a common antigen. However, it remains unclear whether the expanded clones were responding to a disease-producing autoantigen or a common microbial antigen (such as cytomegalovirus) that is unrelated to disease pathogenesis. Another study failed to find evidence of clonal T-cell expansion in the lungs of patients with active WG (Ref. 82). Failure to find a clonal expansion in this study might be a result of inadequate sampling, as bronchoalveolar lavage fluid rather than tissue biopsies were examined. Alternatively, clonal expansions might have been present at a frequency that was below the detection of the assay. In certain animal models of antigen-driven Th1 inflammation (e.g. experimental autoimmune encephalomyelitis and mycobacterial infection), antigen-specific T cells dominate the inflammatory infiltrate early in the course of the disease (Ref. 83). However, in later stages, a large proportion of cells at inflammatory sites are nonspecifically activated T cells that contribute to ongoing inflammation by producing low levels of cytokines. Thus, in later stages, the predominance of nonspecifically activated T cells at sites of disease could 'dilute' the ability to detect an antigen-specific population of T cells that initiated the process. Depending on when and where the T-cell compartment of patients is sampled, such clones can be easily missed. Whether such a phenomenon occurs in WG is speculative and further studies would need to be designed to address this issue.

In summary, current evidence supports the hypothesis that the granulomatous inflammation in WG is driven by activated $CD4^+$ T cells producing Th1 cytokines. However, the nature of the antigen(s) that initiates this process remains unclear, as does the exact reason why immunoregulatory pathways fail to control the inflammation once initiated. The inconsistent demonstration of a specific antigen-driven T-cell process and the inability to identify an antigen make creation of new animal models particularly difficult. The relationship between this Th1 reaction and ANCAs also remains obscure. An intriguing, but unproven, hypothesis suggests that, under the influence of an inciting agent, epithelial cells upregulate expression of surface Pr3, which reacts with circulating ANCAs and thereby initiaties a cascade of inflammation and cell death. In response, and in an attempt to restrict tissue damage, activated T cells establish a granulomatous response that persists. The degree and propagation of this response would relate to the extent of tissue damage and might ultimately lead to breaking of tolerance and a chronic inflammatory response. Although attractive in theory, the findings of granulomatous inflammation in ANCA-negative patients and the fact that patients with high-titre ANCA can remain in remission despite experiencing bouts of immune activation (for instance following exposure to everyday pathogens), are contradictory to this hypothesis.

Implications for future therapy

With a better understanding of the pathogenesis of WG it has become possible to develop and test therapeutic regimens that utilise biological agents to target specific elements of the inflammatory response. The hope is that such agents will prove effective at modulating pathogenic inflammation without causing systemic immunosuppression or other deleterious effects that are typically associated with conventional immunosuppressive drugs.

One such targeted approach has been directed towards blocking cytokines thought to be involved in Th1-mediated inflammation. The availability and success of anti-TNF agents for treatment of rheumatoid arthritis, together with the importance of this cytokine in the pathogenesis of granulomatous inflammation, prompted the study of an anti-TNF agent in WG. In this trial, etanercept (a recombinant fusion protein containing the extracellular portion of the p75 TNF receptor linked to the Fc portion of human IgG1) was used in combination with standard therapy in patients with active WG (Ref. 84). The addition of etanercept to standard therapy did not prove beneficial in reducing the frequency of early disease relapses. Nevertheless, these negative findings do not rule out the possibility that anti-TNF agents could be beneficial in the treatment of WG. The rate of sustained remission in the non-etanercept arm of this study was significantly higher than predicted in the pre-study sample size calculations (Ref. 85).

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Thus, a beneficial effect of etanercept may have been missed as a result of insufficient sample size (Type II error). Alternatively, the dose of etanercept used in this study might have been inadequate to block fully the actions of TNF. The complexity of anti-TNF therapy in the treatment of inflammatory diseases is illustrated by the effects of two different anti-TNF agents in Crohn's disease (an autoimmune granulomatous disease of the intestinal tract). As was the case in WG, etanercept was found to be ineffective in the treatment of Crohn's disease (Ref. 86). However, infliximab (a chimeric monoclonal anti-TNF antibody) is effective in the treatment of Crohn's disease (Ref. 87). The reason for the difference in efficacy between these two anti-TNF agents is unknown, but might be related to the in vitro observation that infliximab (but not etanercept) can bind to transmembrane TNF and induce killing of TNF-expressing cells (Refs 88, 89). Other possible therapeutic approaches that could prove beneficial include the use of either anti-IL-12 mAbs or recombinant IL-10 to dampen pathogenic Th1 responses.

The evidence for the role of ANCAs in amplification of inflammatory signals in vitro has led to attempts to inhibit production of these autoantibodies specifically. Rituximab, a chimaeric mAb against CD20, has been used increasingly in patients with B-cell lymphomas (Ref. 90). An important property of rituximab is its ability to induce death of both malignant and normal $CD20^+$ B cells. The death of B cells appears to result from induction of complement-mediated events and through antibody-dependent cellular cytotoxicity (Ref. 91). In a small case series of WG patients, rituximab has been reported to be effective both in lowering ANCA titres and inducing disease remission (Ref. 92). However, patients in this case series were also treated with high-dose corticosteroids and plasma exchange, making it difficult to determine what role concomitantly administered immunosuppressive treatments might have played in the observed responses. Whether B-cell depletion with rituximab can inhibit ANCA production and produce clinical benefit requires further study (Ref. 93).

Conclusion

The pathogenesis of WG is complex and probably involves a variety of mechanisms acting in concert to bring about necrotising granulomatous inflammation and vasculitis. In recent years, there has been considerable progress in dissecting the immunological abnormalities present in WG. However, the primary immunopathogenic events that initiate the process of granulomatous inflammation and blood vessel damage are still largely unknown. Although the cause of WG remains a mystery, recent advances in molecular and cellular immunology have defined many of the effector mechanisms that mediate granulomatous inflammation and vascular damage. In this regard, modulation of the inflammatory response by specific cytokine and cytokine antagonists is now possible and might prove beneficial in the treatment of WG.

Acknowledgements and funding

The authors thank E. Mueller for her insightful comments, and the anonymous peer reviewers for their suggestions. This manuscript was written as part of the authors' duties as employees of the Federal Government.

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Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press

Further reading, resources and contacts

Web resources

Website listing ongoing clinical trials in the USA:

http://www.clinicaltrials.gov/

Website for the European Vasculitis Study Group (EUVAS):

http://www.vasculitis.org/

Website for Wegener's granulomatosis patients:

http://www.wgassociation.org/

Textbooks

Ball, G.V. and Bridges, S.L., eds (2002) Vasculitis, Oxford University Press, Oxford, UK

Hoffman, G.S. and Weyand, C.M., eds (2002) Inflammatory Diseases of Blood Vessels, Marcel Dekker Inc., New York, USA

Monograph

Matteson, E.L. (1999) A History of Idiopathic Vasculitis, Mayo Foundation for Medical Education and Research, Rochester, MN, USA

Articles

Original descriptions of Wegener's granulomatosis by Heinz Klinger and Friedrich Wegener:

Klinger, H. (1931) Grenzformen der Periaretritis nodosa [Borderline forms of Periaretritis nodosa]. Frankfurt Ztschr Pathol 42, 455-480

Wegener, F. (1936) Uber generalisierte, septische Gefasserkrankungen [On generalized, septic vascular diseases]. Verhandl deutsch Gesellsch Pathol 29, 202-210

Wegener, F. (1939) Uber eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystems und der Nieren [A unique rhinogenic granulomatosis with especial involvement of the arterial system and the kidneys]. Beitr Pathol Anat allg Pathol 102, 36-68

Original study establishing the efficacy of cytotoxic therapy in treatment of Wegener's granulomatosis:

Fauci, A.S. and Wolff, S.M. (1973) Wegener's granulomatosis: studies in eighteen patients and a review of the literature. Medicine (Baltimore) 52, 535-561, PubMed: 4748591

Two articles reviewing the clinical utility of ANCAs (anti-neutrophil cytoplasmic antibodies):

Schmitt, W.H. and van der Woude, F.J. (2004) Clinical applications of antineutrophil cytoplasmic antibody testing. Curr Opin Rheumatol 16, 9-17, PubMed: 14673383

Langford, C.A. (2004) Antineutrophil cytoplasmic antibodies should not be used to guide treatment in Wegener's granulomatosis. Clin Exp Rheumatol 22, S3-6, PubMed: 15675126

An article examining the role of antibody-idiotype networks in the origin of Pr-3 ANCA. The findings suggest that autoimmunity can be initiated through an immune response against a peptide that is antisense or complementary to the autoantigen, which then induces anti-idiotypic antibodies (autoantibodies) that crossreact with the autoantigen:

Pendergraft, W.F., 3rd et al. (2004) Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. Nat Med 10, 72-79, PubMed: 14661018

A commentary outlining current knowledge of the genetics of Wegener's granulomatosis:

Jagiello, P., Gross, W.L. and Epplen, J.T. (2005) Complex genetics of Wegener granulomatosis. Autoimmun Rev 4, 42-47, PubMed: 15652778

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Published by Cambridge University Press

Features associated with this article

Figures

Figure 1. Features of Wegener's granulomatosis.
Figure 2. The potential role of ANCAs and cytokines in neutrophil-mediated vascular injury.
Figure 3. Pathogenesis of granuloma formation.

Citation details for this article

Pasha Sarraf and Michael C. Sneller (2005) Pathogenesis of Wegener's granulomatosis: current concepts. Expert Rev. Mol. Med. Vol. 7, Issue 8, 13 May, DOI: 10.1017/S146239940500921X

Accession information: DOI: 10.1017/S146239940500921X; Vol. 7; Issue 8; 13 May 2005
Published by Cambridge University Press