REVIEW

Translating basic science into patient therapy for ANCA-associated small vessel vasculitis

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ABSTRACT

ANCA (anti-neutrophil cytoplasm antibody)-associated small vessel vasculitis is an inflammatory condition associated with the production of autoantibodies to neutrophil cytoplasmic components. The disorder results in destruction of the microvasculature, infiltration of neutrophils into tissues, which is followed later by mononuclear cells, leading to injury and the formation of granulomatous lesions. Initiators for the disease are undetermined but a pro-inflammatory environment is required. Other influencing factors may include environmental triggers, genetic propensity or infectious agents. The primary cellular event in the condition involves the neutrophils, which are likely to be responsible for the majority of tissue injury. Binding of the autoantibody to neutrophils initiates cell activation via a complex intracellular signalling cascade, culminating in the release of pro-inflammatory mediators, proteolytic enzymes and reactive oxygen species. Adhesion of neutrophils to endothelial cells is observed in vitro and more investigations in this area may explain the focussing of the disease to certain vessels/tissues. Current treatment regimens have substantial toxicity. Although newer developments are an improvement there is still a pressing need for more targeted therapies, which could be provided by extrapolating information emerging from basic scientific research.

INTRODUCTION

Characteristics

SVV (small vessel vasculitis) is an inflammatory disorder affecting arterioles, capillaries and post-capillary venules and is characterized by focal necrosis of the blood vessels with few or no immune deposits. The disease has a variable presentation, affecting many organs. The three main types of SVV are: WG (Wegener’s granulomatosis), MPA (microscopic polyangiitis) and CSS (Churg Strauss syndrome). These diseases share many common features, affecting the kidneys, lungs and the skin. However, granulomatous destruction of the respiratory tract is more common in WG, whereas eosinophilia and asthma support the diagnosis of CSS, and MPA uncommonly involves the upper airways or the eyes (for review, see [1]). Although patient survival rates have improved to over 80 % at 5 years, there is considerable morbidity involved with the current use of immunosuppressants. Over the past few years, the aetiology and pathogenesis of this disorder has been under intense scrutiny and potential therapeutic options are being developed.

Diagnosis

The majority of patients with SVV (90 %) produce autoantibodies to neutrophil components termed ANCA...
(anti-neutrophil cytoplasm antibodies) and these are a useful diagnostic tool. The two major antigens for ANCA are MPO (myeloperoxidase) and PR3 (proteinase 3), both expressed in the neutrophil granules. ANCA display two characteristic staining patterns when applied to ethanol fixed neutrophils [4]. A perinuclear localization (pANCA) denotes an anti-MPO ANCA and is predominantly associated with MPA or CSS, whereas cytoplasmic distribution (cANCA) is due to anti-PR3 and is frequently linked to WG and, less frequently, to MPA. ANCA are also detected using ELISA either via coating plates with the relevant antigen and incubating with patient sera prior to visualization or by coating plates with an antibody that captures the antigen before patient sera is included again.

These two different tests are most usually combined to provide a higher degree of confidence and, when used in conjunction with a clinical assessment and histology (see below), are a good determinant of the presence of ANCA-associated SVV. This is of enhanced importance as the treatment regimen for this disease is highly toxic.

Tissue biopsy is important to confirm a diagnosis of ANCA-associated disease prior to the commencement of potentially toxic immune-suppressing agents. In patients with haematuria, a renal biopsy may reveal a typical vasculitis glomerulonephritis. Biopsy of other involved tissues, such as nasal mucosa or lung tissue, may also be informative in selected patients.

**Progression**

Clinical disease activity is assessed using a standardized system such as the BVAS (Birmingham Vasculitis Activity Score). This utilizes a numerical scoring system, involving nine organs, which is weighted for importance. A high score denotes either critical organ involvement or multi-system effects and relates to mortality [2,3]. The damage (non-healing scars) caused by vasculitis or the use of drugs for its treatment is assessed using a complementary scoring system called the Vasculitis Damage Index.

ANCA-associated SVV can be fatal if left untreated. Adverse prognostic factors are known to be age, renal impairment and pulmonary involvement. Relapse is a frequent occurrence once treatment is discontinued and often long-term maintenance therapy is required. Serial measurements of ANCA may be useful in predicting the onset of relapse so allowing pre-emptive intervention, although the efficacy of this controversial [5].

**Vascular effects**

Predominantly in ANCA-associated SVV, damage to the endothelium is observed in the post-capillary venules [6]. Endothelial cells undergo cell death leading to detachment and there is evidence for this event, as circulating detached endothelial cells have been captured and quantified [7]. This denudation exposes the underlying basement membrane, initiating thrombosis and platelet de-position, and results in eventual occlusion of the lumen of the vessel. Clinically, endothelial perturbation has been observed by measuring vasodilatation in the brachial artery and dermal microvascular responses to acetylcholine.

In addition to this passive event, a more active process is taking place. This involves the endothelial cells responding to injury by expressing a pro-inflammatory phenotype, i.e. changes in adhesion molecule and cytokine patterns [8]. Both of these events provide an environment for the capture and activation of leucocytes with the corresponding downstream effects (see below).

**Mediators**

In addition to the presence of ANCA, other soluble components that may exacerbate disease are found in patients. Pro-inflammatory cytokines are thought to be of particular importance [9]. Plasma TNFα (tumour necrosis factor α) is elevated in active disease and shows a decline in remission. This probably plays a pivotal role in the initiation of both neutrophil and endothelial cell activation, along with having a host of other effects. The initiation and source of this potent mediator is undetermined, but may be driven by a prior infectious event. Circulating monocytes show increased expression of TNFα mRNA [10]. Other cytokines that have been identified in urine during active disease include IL (interleukin)-6 and IL-8, which may indicate a more localized renal production [9]. In immunohistochemical analysis, IL-8 and also IL-18 have been found to be localized within renal vasculitic lesions, often close to the sites of neutrophil infiltration [11]. Receptors for cytokines such as TNFα-RII are also detected in the serum of patients, which may prolong the half-life of the circulating molecule [12]. Soluble forms of adhesion molecules provide markers of pro-inflammatory events and sVCAM-1 (soluble vascular cell-adhesion molecule-1) has been shown to be present in urine. In serum, sE-selectin (soluble E-selectin), sICAM-1 (soluble intercellular cell-adhesion molecule-1) and sVCAM-1 are all increased. There was no difference in serum P-selectin levels and L-selectin was decreased [13].

**Evidence for the involvement of ANCA**

Clinically, increases in ANCA titre are a good indication of active disease or the onset of a relapse, but the antibodies are not present in 100% of cases. In vitro, a number of different groups have shown that ANCA have leucocyte-activating potential [14]. This work predominantly concentrated on the effects on neutrophils (see below) and, using various parameters, has shown activation of the cells, indicating a functional role in the disease.

There have been numerous attempts at the development of an animal model of ANCA-associated SVV with
Varying success [15–17]. Techniques have included the use of low levels of anti-glomerular basement membrane antibodies to prepare animals for the subsequent effects of ANCA, suggesting that initiation factors are required for the development of disease [16,17]. Recently, however, a more definitive model has been developed [18]. MPO knockout mice are immunized with MPO and produce MPO-recognizing ANCA. Either the splenocytes or isolated antibodies are then transferred to Rag−/− (lacking functional T- and B-cells) mice, resulting in several of the hallmark characteristics of ANCA-associated SVV. Histologically, necrotizing and crescentic glomerulonephritis is observed with several mice also having a pulmonary capillaritis and cutaneous vasculitic lesions. It was noted that more severe disease was evident when splenocytes, rather than isolated anti-MPO antibodies, were given, indicating both a cellular and a humoral role.

**Variation of ANCA**

There are reports of IgM ANCA being present in SVV but more usually IgG ANCA predominate [19]. It has been noted that variations in subclass and epitope recognition can occur, but there is no consensus of opinion on the precise nature of the polyclonal antibodies present as wide variation exists between patients. Data on the distribution of subclasses in the total IgG or ANCA population are controversial. There do appear to be differences between active disease patterns and remission, and also the potency of the antibody can be affected by subclass. ANCA are usually represented within each subclass but levels of IgG2 are very low, whereas IgG1 and IgG4 are the most abundant. It is currently thought that a high IgG3 relates to disease severity and is associated with relapse. This particular subclass has been shown in vitro to have a high neutrophil-activating potential.

Both epitope spreading and shift are documented but recognition is predominantly confined to a restricted number of sites that appear to be closely related [20]. This is evidenced by the ability of one patient’s ANCA to competitively inhibit the binding of ANCA from another patient [20]. The most antigenic site appears to be a surface area around or in the catalytic site of the enzymes [20]. These epitopes are in some cases conformational [20]. Binding of ANCA may or may not inhibit enzymic activity.

Additionally, the ANCA population exhibits a higher proportion of aglycosylated antibodies than normal IgG. This would probably favour the mannose-binding lectin pathway of complement activation.

ANCA-associated SVV is a multifactorial disease possibly triggered by environmental factors in a genetically susceptible individual. Understanding the genetic polymorphisms and environmental influences may help to identify individuals who are at risk of developing vasculitis, relapse or have worse prognosis. This will help us to administer targeted treatment instead of blanket therapy with powerful immunosuppressants.

**ENVIRONMENTAL FACTORS**

**Drug-induced vasculitis**

A number of case reports have suggested an aetiological role for PTU (propylthiouracil; a thionamide antithyroid medication) in ANCA-associated SVV [22,23]. Cross-sectional studies have confirmed the increased prevalence of ANCA in patients treated with PTU, the rates ranging from 33–64% [24,25]. Despite ANCA-positivity, clinical manifestations of SVV are rare in this group. How PTU induces ANCA formation is not clear. Studies have shown that the drug can accumulate in neutrophils and is oxidized to reactive intermediates [26] that can bind to self-peptides and cause T-cell sensitization [27]. Besides thionamides, drugs such as minocycline and hydralazine have been linked to pANCA and SVV [28,29]. As thionamides are widely used in the treatment of hyperthyroidism, it is important that physicians are aware of the increased risk of ANCA-positivity in this group. Symptoms of SVV should lead to immediate withdrawal of thionamides and aggressive disease may need immunosuppressive treatment.

**Silica exposure and other environmental factors**

Case reports and case-control studies have suggested an aetiological link between silica dust exposure and ANCA-associated SVV [30,31]. Studies have indicated that the intensity of silica exposure is more important than the duration of exposure. The odds ratio for high-occupational silica exposure, e.g. sandblasting, for development of SVV is much higher than for low-intensity jobs [32]. The mechanism by which silica induces ANCA-associated SVV is not clear. Pfau et al. [33] demonstrated that NZM (New Zealand mixed) mice (an inbred lupus-prone strain) exposed to silica developed autoantibodies that recognized caspase-cleared proteins on apoptotic cells. It is possible that the accumulation of apoptotic cells in an inflammatory environment leads to self-antigen presentation and antibody production. In addition to silica, other factors that have been implicated to play a role in causing SVV are solvent exposure and allergy. Recently, Lane et al. [32] reported for the first time an association between farming, especially livestock, and SVV. Further investigations are required to establish the nature of the association.

**Infection**

There is a long-standing view that infection may play an important role in the induction of WG. Stegeman et al.
[34] reported that 60–70% of patients with WG are chronic nasal carriers of *Staphylococcus aureus* and that this was a risk factor for the development of relapses of the disease. Furthermore, this group demonstrated that prophylactic treatment with cotrimoxazole, an antibiotic that potentially eliminates staphylococcal nasal carriage, reduced relapse frequency [35]. How *Staph. aureus* induces activation of vasculitis is not clear. *In vitro* studies have also shown that SACP (staphylococcal acid phosphatase), a staphylococcal antigen, can bind to cultured endothelial cells and this can be recognized by serum antibodies of patients with WG. SACP has been demonstrated in glomeruli of some patients with WG and antibodies to SACP are found in the sera of WG patients [36]. It has been hypothesized that focal immune complex formation could occur in the vessel wall. The presence of ANCA could exaggerate neutrophil responses and cause the immune complex to be degraded quickly, leading to pauci-immune glomerulonephritis [37]. Indeed, immune complexes have been demonstrated in early skin lesions in patients with WG [38]. This demonstrates that SACP could act as a planted antigen initiating glomerulonephritis and vasculitis in patients with WG.

**GENETIC FACTORS**

**HLA antigens**

A genetic contribution in ANCA-associated SVV is suggested by reports of occurrence of the disease in related individuals [39,40]. An inheritance pattern of SVV is difficult to map due to the low prevalence of the disease. Associations with HLA class II alleles have, so far, yielded inconsistent results. There are reports of a positive association with DR4 [41] and DR9 [42] and a negative association with DR13 [43,44]. Recent studies on a Japanese cohort reported an association of HLA-DRB1 0901 with microscopic polyangiitis [45].

**α1-Antitrypsin alleles**

α1-Antitrypsin is the main inhibitor of PR3 and elastase in neutrophils. A polymorphic gene with 75 alleles at the Pi (protease inhibitor) locus controls this enzyme. Homozygous-deficient alleles, PiZZ, lead to decreased serum levels of α1-antitrypsin. Several groups have shown that PR3-ANCA is associated with a deficient PiZZ phenotype in ANCA-associated SVV [46,47]. Studies have also indicated that the PiZ allele could be a risk factor for greater organ involvement, higher mortality and poor prognosis [48]. This protease/antiprotease imbalance could be important in the pathogenesis of ANCA-associated SVV. It has been postulated [49] that α1-antitrypsin could be inactivated by reactive oxygen species generated by an ongoing infection. This may prevent normal limitation of proteolytic activity after neutrophil activation and lead to the exposure of PR3 antigen to the immune system. However, the incidence of anti-PR3 antibodies is not increased in PiZZ-deficient patients [50], suggesting that α1-antitrypsin deficiency alone is not sufficient to induce vasculitis.

**PR3 expression**

PR3 is located in the azurophilic granules and in the secretory vesicles of neutrophils. Recent studies have shown that PR3 is also expressed on the outer cell membrane (mPR3) of resting neutrophils [51]. It is suggested that higher expression of mPR3 on the cell surface is a risk factor for ANCA-associated SVV [52] and is important for disease relapse in patients with WG [53]. mPR3 expression is probably influenced by genetic factors as suggested by studies on twins [54]. Gencik et al. [55] demonstrated that a specific polymorphism A(−564)G in the promoter region is associated with WG. This transcription-enhancing polymorphism can lead to PR3 overexpression. The specific gene loci responsible for mPR3 expression are yet to be identified. The levels of circulating PR3 are increased in patients with active disease [56,57] and those in remission [58]. This could be important in the pathophysiology of ANCA-associated SVV.

**FcyR (Fcy receptor) polymorphism**

Studies have shown that activation of neutrophils by ANCA is FcyRIIa dependent [59]. Polymorphisms of FcyRIIa receptors (FcyRIIa-Arg31 and FcyRIIa-His31) affect its ability to be activated by human and murine IgG isotypes. Neutrophils homozygous for His31 bind IgG3 more efficiently [60]. The IgG3 subclass of ANCA is known to be elevated during the acute phase of WG [61] and is particularly associated with renal involvement; however, studies of FcyRIIa allotypes in ANCA-associated SVV have not shown an increase in the His31 allotype irrespective of ANCA specificity or nephritis [62]. Despite lack of evidence for an aetiological role, there are suggestions that patients with homozygous FcyRIIa-Arg31 and FcyRIIa-Phe158 may be more susceptible to relapse of SVV [63].

**β2 Integrin**

Using a flow-based adhesion model, Radford et al. [64] showed that activated β2 integrin (CD11b/CD18) mediates neutrophil adhesion to, and enhanced migration across, vascular endothelial cells. This process may initiate tissue damage in vasculitis (see below). An RFLP (restriction-fragment-length polymorphism) in exon 11 of the CD18 gene has been reported to be associated with MPO-ANCA-associated SVV. No other relevant polymorphism was identified within, or in the vicinity
of, genes for ICAM-1, E-selectin, PLAUR (plasminogen activator, urokinase receptor) or CD11b [65].

**Complement system and cytokines**

C3 and C4 are important in the complement cascade and the encoding genes are located on chromosome 19 and 6 respectively. An increase in C4A3 allotype is reported in patients with SVV and the same study observed an increase in the C3F allele in PR3-ANCA-positive patients, but not MPO-ANCA-associated SVV [66].

Cytokines play an important role in driving inflammatory process. TNFα primes the neutrophil for further activation by ANCA. Other cytokines, such as IL-2, are also pro-inflammatory, whereas the IL-5 receptor is required for development of antibody-secreting cells. Studies have failed to show convincing disease associations with a TNF promoter polymorphism, IL-2 and IL-5RA microsatellites [44,45,67]. The cytokine IL-10 is essential for polarization of T-cells towards Th2 development and immunoglobulin production by B-cells. A CA repeat microsatellite IL-10.G is located in the promoter region of the IL-10 gene. This polymorphism is associated with high autoantibody production [68] and is linked to WG [69]. Recent studies using an extended association screen with microsatellite markers, representing apoptosis-related genes, showed a strong association of WG with HLA-DPB1 0401 allele [70].

**Genetic variations in T-cell-related genes**

CTLA-4 (cytotoxic T-lymphocyte antigen-4) is expressed on activated T-cells and inhibits further activation by binding to co-stimulatory molecules of the antigen-presenting cell. Thus CTLA-4 plays an important role in the down-regulation of T-cell activation. Polymorphisms of CTLA-4 have been linked to various autoimmune diseases, including Graves’s disease and diabetes. Huang et al. [71] described an association of CTLA-4 (AT)n microsatellite to WG. The prevalence of the shortest CTLA-4 allele was reduced in patients when compared with controls. The same group has recently reported that another polymorphism of CTLA-4 in the promoter region at position – 318 is also linked with WG [72].

It would appear from the data presented above there are no clear individual genetic markers that could easily be screened to predict onset of SVV or its progression. However, combinations of events may be important, as thought for FcγR. The development of DNA banks and cross-referencing of patient genotypes might provide a cluster of genes for which concordance of data would be a strong indicator of progression/outcome. With regard to onset, there is, in all probability, a genetic background on top of which external factors act. These supplementary events are again unconfirmed, but unifying themes may be an initiation of a break in tolerance or exposure to molecules of similar structure to the known antigens.

**ANTIBODY AND CELLULAR INVOLVEMENT**

**Neutrophils and monocytes**

ANCA-target antigens are now well described as being presented on the surface of ‘primed’, i.e. cytokine-treated, neutrophils. This allows the autoantibody to bind to the surface of the cell. This event in itself appears to be insufficient for activation of the cell. Additionally, ligation of the Fc portion of the antibody to FcγRs on the neutrophil is required [73], which then gives rise to the generation of reactive oxygen species, degranulation and the production of pro-inflammatory cytokines (Figure 1A). It is also now becoming clear that there are additional components to the system, importantly CD18 [74]. Continuous stirring of neutrophils during stimulation with ANCA inhibits the usual superoxide production [75]. It may be that the involvement of CD18 stabilizes the complex formed by ANCA or, alternatively, causes its relocation to areas within the plasma membrane that enable it to activate the NADPH complex.

Studies on the intracellular signal transduction pathways induced by ANCA binding to neutrophils have shown differences between the signal initiated by F(ab′)2 fragments of the antibody and those seen with whole antibody, indicating that multiple pathways are involved (Figure 1B). ANCA binding to antigen instigates activation of G-protein-coupled pathways [76], whereas ligation of the Fcγ R targets Syk kinase [77], PKCβ (protein kinase Cβ) [78] and calcium release [79]. Both portions of the antibody are required for PKB (protein kinase B) [76] activity and both are able to activate Src kinase(s) [77] and PI3Kγ (phosphoinositide 3-kinase γ) [80]. Also stimulated is the small GTPase p21ras, which is known to be a molecular switch providing the connection point for a number of pathways, and this may be pivotal in uniting the signals to provide a functional response [76]. It is noteworthy that the pathways triggered by ANCA in neutrophils are clearly different to those seen with cross-linking of FcγRs, which may provide opportunities for therapeutic interventions without concomitant down-regulation of the entire immune response [80] (see below).

As well as the above effects, ANCA are also capable of inducing neutrophils to undergo an accelerated rate of apoptosis [81]. This has been shown to be dependent on the production of reactive oxygen species [82]. Additionally, the process is dysregulated with delayed externalization of phosphatidylserine occurring and consequently reduced phagocytosis by macrophages. This leads to apoptotic neutrophils progressing more readily into secondary cell lysis, releasing their proteolytic cell contents which may cause bystander injury. Moreover, apoptotic neutrophils express increased amounts of PR3 and MPO on their surface [81] and potentially this could lead to opsonization of the cells by ANCA and their uptake by macrophages in a pro-inflammatory manner [82].
Figure 1  Effects of ANCA binding to neutrophils

(A) Extracellular events induced by the binding of ANCA to neutrophil plasma membrane. ANCA is capable of binding both antigen via its F(ab′)2 fragments and FcγR via its Fc region. This interaction leads to an activation of the cell with release of mediators such as pro-inflammatory cytokines, superoxide and granule components. (B) Intracellular events induced by the binding of ANCA to neutrophil plasma membrane. ANCA binding initiates a dual signalling cascade which involves activation of G-protein-coupled pathways by F(ab′)2 ligation and tyrosine-kinase-coupled pathways by Fc ligation. These pathways involve a number of other molecules and converge in order to produce a functional response. (C) ANCA instigates neutrophil adhesion to endothelial cells and transmigration. Binding of ANCA to the plasma membrane of neutrophils leads to their progression from rolling along the endothelial surface to firm adhesion. The production of chemotactic factors by the endothelial cells induces transmigration of the neutrophils into the subendothelial space and their resultant production of reactive oxygen species and release of proteolytic enzymes generates vascular damage. β2, β2 integrin.

Fewer studies have taken place to determine the role of the monocytes/macrophages in development and progression of SVV. Monocytes are capable of expressing both PR3 and MPO and are therefore a legitimate target for ANCA. The cells are seen within granulomas and glomerular crescents during active disease and so, by implication, play a part. Monocytes are able to release reactive oxygen species, cytokines/chemokines [MCP-1 (monocyte chemotactic protein-1), IL-8, TNFα, IL-1β and IL-12] and thromboxane in response to ANCA and consequently may contribute to the local pro-inflammatory environment [10]. Furthermore, ANCA-treated monocytes can up-regulate the surface adhesion molecules CD14 and CD18 [83] and down-regulate CD62L, giving rise to increased adhesion.

In vitro vascular modelling and release of PR3

Binding of ANCA to neutrophils induces their adhesion to cytokine-activated endothelial cells [64], in vivo this would trap them at vessel walls. If concurrently the neutrophil releases superoxide and then undergoes accelerated apoptosis without clearance (leading to secondary lysis and release of toxic intracellular contents), then bystander injury can occur. The release, particularly of PR3, has been shown to have wide-ranging effects on endothelial cells [84] leading ultimately to their apoptosis, but also provoking the production of IL-8 and MCP-1 and the increased surface expression of ICAM-1 and VCAM-1, all pro-inflammatory events.

The adhesion of ANCA-treated neutrophils to cytokine-treated endothelial cells has been modelled in vitro under physiological flow conditions which mimic the shear stresses seen within vessels (Figure 1C). The experiments have demonstrated both the importance of cytokines in the system plus the role of various adhesion molecules and chemokines. Endothelial cells treated with high concentrations of TNFα are capable of capturing neutrophils flowing past and transmigration occurs. If the endothelial cells receive only low doses of TNFα then cells roll but do not adhere firmly. If, however, the neutrophils are pretreated with ANCA then adhesion proceeds and the neutrophils transmigrate. For these events to ensue, β2 integrin engagement is necessary and our group has demonstrated [84a] that CD11a/CD18 and CD11b/CD18 both play a role, with CD11b probably being more important in promoting stable adhesion. There is also a role for chemokine receptors in this process with CXCR2 (CXC chemokine receptor 2) blockade being able to decrease the response.

B-cells

Evidence both from the animal model of Xiao et al. [18] and from patient studies [14] have implicated ANCA-IgG and, therefore, B-cells in ANCA-associated
SVV. The development of more severe disease in the RAG-1^{-/} mice which receive splenocytes, rather than purified antibody, indicates a cellular component. Additionally, the recent clinical evidence that therapeutic monoclonal antibodies against CD20, a B-cell antigen, can ameliorate WG is indicative of a continuous role for B-cells (see below).

**T-cells**
T-cells are widely believed to play a role in the development of ANCA-associated SVV. Evidence for this includes IgG subclass switching (which requires T-cell help) [85], the presence of activated CD4^{+} cells in patient sera and high levels of soluble IL-2 receptor [86], soluble CD4 and soluble CD8 [87]. More specifically, autoreactive T-cells have been demonstrated which are capable of proliferating in response to MPO or PR3 [88–90]. PR3-specific T-cells respond predominantly to a restricted number of peptide sequences, namely the signal sequence, the pro-peptide or the C-terminus [91]. Less clear is the polarization of the response, as determined by the production of specific subsets of T-helper cells (Th1 and Th2) with distinct functions and patterns of cytokine production. In the periphery, there would appear to be a Th1 bias [production of IFNγ (interferon γ), but in nasal lesions this may be reversed to Th2 (production of IL-4 and -10) [92–94]. CD4^{+}/CD28^{−} T-cells have been found in granulomas and in increased numbers within peripheral blood. They are postulated to be the effector cells of the response [95,96]. In conjunction, CTLA-4 is increased in patients and correlates with disease activity [97].

**Gene activation**
Real-time PCR and DNA microarray on leucocytes from patients and leucocytes treated with whole and F(ab′)^{2}; ANCA IgG has shown activation of a subset of genes [98]. Some of these genes were common to the response to whole IgG, some only to F(ab′)^{2}; and some responded to both. Activated genes included DIF-2 [differentiation-dependent gene-2 or IEX-1 (radiation-inducible immediate early gene), gly96 in mice or PRG1 (pituitary adenylate cyclase-activating polypeptide-responsive gene) in rat] and IL-8, along with genes for a number of molecules associated with inflammation and cell signalling. DIF-2 was up-regulated in patients and strongly correlated with disease activity and ANCA titre, but was not present in remission or controls. IL-8 was increased only in remission samples. Profiling patients in future could give rise to better-tailed therapy.

**TREATMENT**
**Current therapies [99]**
SVV treatment is effective but exhibits a high degree of morbidity and mortality in its own right. The variable pattern of the disease adds further complexity, including rapid to slow progression and relapsing/non-relapsing forms. First line therapy traditionally comprises steroids combined with an immunosuppressant, usually cyclophosphamide, which is then tailored as remission is achieved. A number of trials, organized by EUVAS (European Vasculitis Study Group), of differing combinations of these drugs have been undertaken over recent years (NORAM, CYCAZAREM, CYCLOPS). The results of these are giving rise to better, less aggressive, regimens being implemented with decreasing severity of symptoms. The role of side effects such as infection and leukopaenia have been increasingly recognized as negative prognostic factors, and prophylactic treatment is now offered where available. Later side effects of treatment include malignancy, haemorrhagic cystitis and infertility. Additional plasma exchange also provides additive benefits in severe disease, as shown by the MEPEX trial. Other approaches include the use of intravenous Ig during relapses or when the patient is refractory to treatment.

**Newer therapies**
Emerging treatments are attempting to focus on particular aspects of the disease to specifically modify, rather than broadly dampen down, the immune system. These approaches are used as complementary remedies in addition to traditional treatments, particularly in refractory cases. A number of them incorporate the use of modified antibodies to target precise pathways. Rituximab is a chimaeric monoclonal anti-CD20 antibody that targets B-cells. Use of this agent in ANCA-associated SVV leads to B-cell depletion and a decrease in circulating ANCA titres [100]. Another approach is blockade of the effects of TNFα [101]. TNFα has a myriad of functions, including increasing the pro-inflammatory nature of endothelium and activating leucocytes, whereas TNFα-positive cells are present in affected kidney and other tissues. Laboratory studies have shown that TNFα is necessary for priming of neutrophils in order for ANCA to generate a response. Two different molecules are currently in use for treatment of SVV: (i) infliximab, a chimaeric monoclonal anti-TNFα antibody [102], and (ii) etanercept, a soluble TNFα receptor fusion protein [99]. Infliximab inhibits both cell-associated and secreted TNFα and consequently all downstream effects, including the production of other cytokines and up-regulation of adhesion molecules, are inhibited. Potential problems with the use of this therapy are the increased risk of infections, particularly the reactivation of tuberculosis, the production of autoantibodies to the chimaeric antibody and malignancy. Etanercept is fully humanized and therefore provides less of a potential risk of immunogenicity. It is capable of binding two TNFα molecules per fusion protein and has a long circulating half-life. Some infections have been reported with its use, but there appears
to be less reactivation of latent tuberculosis. Adalimumab is a recently developed alternative fully humanized anti-TNFα antibody.

**Future agents**

There is an overwhelming need for more specific and less toxic treatment for SVV. Targets for novel therapies would probably involve either the effector cells, i.e. neutrophils, or the affected cells, i.e. endothelial cells. One potential area of intervention would be at the adhesion stage and could be directed at either effector or affected cells. Strategies to inhibit specific adhesion molecules include monoclonal antibodies, small peptides or small molecule inhibitors. Monoclonal antibodies to ICAM-1 and CD11a have been used in rheumatoid arthritis and allograft rejection. However, these have the same drawbacks as the use of infliximab described above. An advance from this is the use of peptides derived from the binding regions of adhesion molecules, e.g. ICAM-1 and LFA-1 (lymphocyte function-associated antigen-1; for review, see [103]). These are designed to recognize their binding partner selectively and either block binding of the native molecule or induce down-regulation of expression of the binding partner. Predominantly these are hexapeptides with a cyclic conformation that maintains their structure. Phage display has been a useful tool to screen large numbers of these peptides for maximum inhibition with minimum crossover between other adhesion molecules [104]. The third approach is via chemical inhibitors of adhesive interactions which are cheaper and easier to manufacture. Lovastatin is capable of blocking the conformational change of LFA-1 into its active structure via allosteric control. Future innovations, still at the animal model testing stage, include the use of biodegradable carriers with stealth systems [PEGylated, where PEG is poly(ethyleneglycol)] that incorporate monoclonal antibodies to adhesion molecules [105]. These have been shown to preferentially bind inflamed endothelium, but avoidance of the reticulo-endothelial system and immunogenicity have not been fully investigated. Also, the use of cyclic peptides to target drugs to particular cell types is an inviting possibility. Already methotrexate has been conjugated to a cyclic ICAM-1 peptide in order to target it to LFA-1-bearing cells. This approach could lead to less toxic therapies as lower doses would probably be required.

Another tactic with therapeutic potential is to target the intracellular signalling pathways in the effector cells so as to inhibit, for example, the release of toxic products or pro-inflammatory mediators. The obvious target cell for such an approach in ANCA-associated SVV is the neutrophil. As stated above, a variety of the intracellular events initiated by ANCA binding to neutrophils differ from those seen in a generalized inflammatory response, so it may be possible to utilize this to specifically inhibit acute disease processes. A variety of small molecule inhibitors to kinases are currently being developed that may be applicable. For example, PKC has been shown to be activated in a variety of inflammatory disorders. Broad-spectrum inhibition of the whole family of these kinases would lead to multiorgan failure and high toxicity. However, in neutrophils in ANCA-associated SVV, only the β isoform is activated. In diabetic retinopathy, the selective PKCβ inhibitor ruboxistaurin mesylate (LY333531) is currently on trial [106]. In animal models, this has been shown to decrease leukocyte adhesion in diabetic retina and is at phase III trials in patients. The treatment is administered orally, is a selective and reversible inhibitor and is well tolerated. Other pathways that may lend themselves to intervention are p21ras and Syk kinase. p21ras holds a fundamental position in the functional activation of neutrophils by ANCA. If this pathway is inhibited, cells are no longer capable of releasing superoxide. The selective inhibitor of p21ras, FTS (farnesylthiosalicylic acid), has been investigated in experimental autoimmune encephalitis models and shown to inhibit active cells within the immune system but to leave basal functions unperturbed [107]. Syk is able to be inhibited with a certain degree of selectivity and this may provide a good starting point for broader therapeutic targeting, as it plays a central role in a variety of cellular processes[108].

Gene therapies are an alternative attractive model of therapeutic interest. Of potential interest would be modification of the role of cytokines in ANCA-associated SVV. This could be applied in two ways: either production of regulatory forms or of natural inhibitors [109]. Gene therapy provides a number of advantages in respect of modifying cytokine pathways as the more usual route of recombinant protein is expensive, delivery is problematical, the cytokines themselves have a short half-life, are toxic and cannot be targeted to where required. The administration of vectors encoding the cytokine of choice provides a relatively constant delivery over long periods, can be made fairly tissue specific, is less toxic and is cheaper. For targeting purposes, the vectors can be injected at, or close to, the site at which they are required or potentially targeted to specific cell types. This would mean that a renal-specific delivery could be performed by injection or a lung-specific delivery by aerosol. The delivery systems are either viral or plasmid based, each with their own advantages/disadvantages, but technologies in this area are advancing rapidly. Methodologies for the improvement of uptake are also advancing. Potential targets for this therapy would be cytokines which regulate TNFα functions such as TGFβ (transforming growth factor β). Usually this cytokine is fibrinogenic but it is able to be produced in a latent form which, if targeted correctly, would only be processed to its active component at affected sites. Cytokine inhibitors
are much less likely to be toxic and clinical trials are underway (IFNγ receptor). Further developments for these forms of gene therapy will incorporate ‘off’ switches for use in periods of remission.

A recent description of the production of a PR3-conjugated toxin is likely to spark interest, as this specifically targets cells which can bind PR3 [110]. This was tested on hybridomas which produced anti-PR3 antibodies and was shown to induce apoptosis. The usefulness of this in a clinical setting is unclear and the cells that bind PR3 will need to be identified. There is some evidence for PR3 receptors on certain endothelial cells and, if this is the case in vivo, high degrees of vascular damage would be expected.

An alternative use for gene therapy as a tool could be linked to patient polymorphisms. A wide range of information is being compiled from DNA banks of patients with a number of autoimmune diseases, including ANCA-associated SVV. As analysis of this information is performed and links made to prognosis, gene-replacement therapies can be contemplated to supplement existing DNA sequences with those that give a more optimistic outcome.

Polymorphisms may also be useful for predicting sensitivities to drugs. A variable response in patients on infliximab therapy may be dictated by a polymorphism in the TNFα gene [111]. Also, data are emerging that adverse drug reactions to azathioprine could be due to a polymorphism in the enzyme inosine triphosphate pyrophosphatase [112]. This would indicate a strong need for more rigorous analysis of patients’ DNA. Detailed statistical analysis of available genetic information on patients may allow combination of genetic types to both predict severity of disease and allow individual tailoring of therapies.

CONCLUSION

Although the consensus of opinion is now that ANCA are central to the pathogenesis of some forms of SVV, much work needs to be done on unravelling the complexities of disease initiation and progression. A more detailed understanding of the cellular events that are initiated by binding of the autoantibodies to their target cells would undoubtedly give rise to numerous therapeutic opportunities (see Figure 2 for possible points of intervention). The need for more tailored therapies is self-evident and exploitation of emerging information from the laboratory is paramount to drive this field forward.

REFERENCES

21 Reference deleted


