Pathogenesis of Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitis and Potential Targets for Biologic Treatment

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Key Words

Antineutrophil cytoplasmic autoantibody-associated vasculitis · Granulomatosis with polyangiitis · Microscopic polyangiitis · Glomerular disease · Biological therapy

Abstract

Antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAV) are autoimmune diseases in which the small vessels are inflamed. Clinical observations suggest a pathogenic role for ANCA. Such a role is supported by in vitro experimental data and animal models, particularly for myeloperoxidase-ANCA. An in vivo pathogenic role of ANCA directed to proteinase 3 has, however, not been fully substantiated. Additionally, the pathogenic role of B cells, T cells, and the alternative pathway of complement in AAV have been elucidated. Insight into these pathogenic pathways involved in AAV has opened and will further open new ways for targeted biologic treatment. In this review the pathogenesis of AAV and potential targets for biologic treatment are discussed.

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Introduction

Since the Chapel Hill Consensus Conference (CHCC), vasculitides have been defined according to the size of the involved vessels, the histopathology of the lesions,

and clinical findings. Granulomatosis with polyangiitis (GPA, formerly known as Wegener's granulomatosis), microscopic polyangiitis (MPA), and eosinophilic GPA (EGPA, formerly known as Churg-Strauss syndrome) are primary vasculitides primarily affecting small vessels, i.e. small arteries, arterioles, capillaries, and venules [1]. GPA is hallmarked by the classic triad of small-vessel vasculitis, extravascular necrotizing granulomatous inflammation, and pauci-immune necrotizing crescentic glomerulonephritis. MPA is defined as necrotizing vasculitis, with few or no immune deposits, that predominantly affects small vessels, but without the typical granulomatous inflammation present in GPA. Finally, EGPA is characterized by asthma, eosinophilia, and small-vessel vasculitis. Collectively, they are designated as antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAV). As EGPA is clinically different from the other AAV, it is beyond the scope of this review.

The AAV are characterized by the presence of autoantibodies against either proteinase 3 (PR3) or myeloperoxidase (MPO), which are enzymes contained in the primary granules of neutrophils and peroxidase-

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positive lysosomes of monocytes [1]. A recent genome-wide association study has shown that PR3-ANCA versus MPO-ANCA-positive AAV patients display distinct genetic associations – even more than their associated diseases, GPA and MPA, respectively [2]. PR3- and MPO-AAV are also characterized by clinical and histopathological differences irrespective of the associated diseases, i.e. GPA and MPA. Renal-limited disease occurs mainly in MPO-AAV. In contrast, PR3-AAV patients show more widespread extrarenal organ involvement [3]. Additionally, PR3-ANCA is strongly associated with upper and lower respiratory tract granulomatous inflammation. Finally, patients with PR3-AAV are at increased risk to experience relapses of disease [4–6].

Pathogenesis of AAV and Conventional Therapy

A central event in the effector phase of the disease is ANCA-mediated neutrophil activation, which leads to degranulation of proteolytic enzymes, oxygen radical production, and amplification of the inflammatory response via activation of the alternative pathway of the complement system [7]. Eventually, these effects result in vasculitis and lead to organ dysfunction with renal failure as a prominent manifestation. Conventional therapy of AAV was introduced in the 1970s and consists of administration of cyclophosphamide together with steroids, which induces remission in the majority of patients [8, 9]. Subsequently, cumulative cyclophosphamide dosages have been reduced by switching therapy after induction of remission to maintenance with azathioprine or methotrexate [10, 11]. However, conventional therapy is associated with severe side effects and often fails to restore renal function. In patients who needed renal replacement therapy at diagnosis at our center, 29% did not regain renal function and 23% died within 6 months [12]. Also, in this cohort approximately 50% of patients with PR3-AAV experienced a relapse within 5 years after diagnosis accompanied by increasing renal damage [12]. In individual patients it is difficult to predict if and when a relapse will occur. Thus, the main challenges in the field of AAV are to develop more specific and less toxic therapies, and to identify markers that will improve monitoring of disease activity and predict impending relapses. To meet these challenges, a better understanding of the key pathogenic pathways involved in AAV is essential. This might subsequently result in new targets for therapy.

Arguments for a Pathogenic Role of ANCA

In the great majority of patients with AAV, ANCA can be detected. After successful induction of remission, ANCA titers decrease or disappear in many patients. However, the relation between ANCA levels and disease activity is not straightforward, limiting its clinical use. In a previous retrospective study we found that patients with PR3-AAV who remain ANCA positive after induction of remission have increased risk of experiencing a relapse [13]. Also, after successful induction of remission, rises in ANCA may precede a relapse. However, this relation is far from perfect and the time to event relation may be over 6 months [14]. Tomasson et al. [15] recently performed a meta-analysis of nine studies and found that a rise in or persistence of ANCA during remission was only modestly predictive for disease relapse. Finally, a strong argument for a pathogenic role of MPO-ANCA comes from the observation of glomerulonephritis and pulmonary hemorrhage in a neonate born from a mother with MPO-AAV. Transient occurrence of vasculitis in the neonate was thought to be caused by transplacental transfer of MPO-ANCA [16]. Thus, data from clinical observations suggest, but do not prove, that ANCAs are pathogenic.

A pathogenic role for MPO-ANCA is suggested by *in vivo* animal studies. Xiao et al. [17] immunized MPO-deficient mice with mouse MPO. As a result, these mice developed an immune response to mouse MPO. Next, splenocytes from these mice were transferred to immunodeficient and normal mice. These recipient mice developed pauci-immune necrotizing crescentic glomerulonephritis and systemic necrotizing small-vessel vasculitis. Transfer of IgG alone from these MPO-deficient mice immunized with MPO into normal mice resulted in pauci-immune focal necrotizing crescentic glomerulonephritis. Little et al. [18] induced anti-MPO necrotizing vasculitis in Wistar-Kyoto rats by immunizing rats with human MPO. The development of a model for PR3-ANCA disease has not been as successful, although promising models are being developed. Splenocyte transfer from autoimmunity-prone nonobese diabetic (NOD) mice immunized with murine PR3 to nonobese diabetic-severe combined immunodeficiency mice resulted in the development of vasculitis and severe glomerulonephritis, but not in granuloma formation [19]. Recently, a model was described in which mice received human hematopoietic stem cells, which resulted in a human-mouse chimeric immune system. After passive transfer of PR3-ANCA, the mice developed mild glo-

Fig. 1. A proposed model of disease mechanisms and therapeutic applications for autoimmune modulation in ANCA-associated systemic vasculitis. (1) Superantigens and peptidoglycans from *Staphylococcus aureus* stimulate antigen-presenting cells in the respiratory tract to produce IL-23 (2), which then induces skewing in the T cell response towards Th17 cells and release of IL-17 (3). IL-17 promotes the release of the proinflammatory cytokines IL-1 β and TNF- α from macrophages, which are essential for the priming of neutrophils (membrane expression of PR3) and upregulation of adhesion molecules on their surface as well as on the vascular endothelium (4). IL-17 also induces chemokine secretion in bronchial epithelial cells that attract primed neutrophils to the infected tissue, which subsequently adhere to the endothelial cells (5). Released PR3 from neutrophils can be processed and presented by antigen-presenting cells/B cells to T cells (6). As T regulatory cells (T_{reg}) fail to inhibit this autoimmune response in GPA, an autoreactive immune response can proceed, in which B cells can differentiate to ANCA-producing plasma cells (7), whereas T cells might undergo repeated stimulation by PR3-pulsed antigen-presenting cells resulting in a pool of T_{EM} cells including IL-17-pro-

ducing Th cells and T-follicular helper (T_{fh}) cells that produce IL-21 (8). ANCA can also induce the release of BAFF from activated neutrophils. Both BAFF and IL-21 (released from T_{fh} cells) synergize in stimulating plasma cell differentiation and promote the survival of autoreactive B cells (7). Deficiency or dysfunction of regulatory B cells results in failure to inhibit both autoreactive B and T cells. ANCAs activate neutrophils that adhere to endothelial cells, resulting in local production of reactive oxygen species (ROS) and release of proteolytic enzymes that damage vascular endothelial cells (5). Moreover, the expanded population of CD4+ T_{EM} cells in peripheral blood, resulting from persistent activation by PR3, upregulate their NKG2D cytotoxic protein and remain in the circulation during remission. When the disease becomes active, the MICA protein is upregulated on several vascular endothelial cells (especially in the kidney), which attract T_{EM} cells to the inflammatory areas (9). The MICA protein on the target cells can bind to NKG2D on the T_{EM} cells, which in turn enhances their cytotoxic function to kill the target cell in a perforin- and granzyme-dependent way, ending in vasculitis (from Lepse et al. [57] with permission).

merulonephritis and lung hemorrhage, but no granuloma formation was detected [20].

In conclusion, data from animal studies clearly suggest, if not prove, a pathogenic role for MPO-ANCA. Subsequent studies in the MPO-ANCA animal models have elucidated the various pathways involved, enabling testing of targeted treatment. A limitation is the absence of an animal model for granulomatous inflammation in PR3-AAV.

Anti-TNF- α Therapy

Owing to the importance of TNF- α in the mechanisms of inflammation, TNF- α blockers have been widely used for the treatment of autoimmune diseases. Also in GPA, after small studies in refractory disease showed promising results, a randomized trial was initiated [21]. However, the randomized placebo-controlled trial evaluating the efficacy of etanercept, one of the TNF- α inhibitors, in addition to standard of care, failed to show higher efficacy in the achievement of sustained remission in GPA patients compared to placebo [22]. Additionally, in this study more solid malignancies were initially observed in the intervention group, although during long-term follow-up this could not be solely attributed to etanercept use [23]. A steroid-sparing role of the TNF- α inhibitors infliximab and adalimumab in remission induction has been suggested in small uncontrolled studies [21]. Currently, however, TNF- α inhibitors are not recommended in AAV.

B cells

The major breakthrough of the last decade in AAV has been the introduction of B cell-targeted therapy [5, 24]. The RAVE trial showed equivalence of rituximab B cell-targeted therapy to standard immunosuppressive therapy [5, 6]. These clinical studies have led to great interest in the role of B cells in AAV, but their exact role is not completely clear. B cells are present in lesions in patients with AAV and they are the precursors of ANCA-producing plasma cells; however, B cells are also critical players in the regulation of immune responses [25]. With the success of B cell-targeted therapy by rituximab, other B cell-depleting agents could have clear therapeutic potential in AAV. Ofatumumab is a novel, potentially more potent anti-CD20 monoclonal antibody [26]. It binds CD20 with greater avidity leading to greater complement-dependent cytotoxicity. Other B cell targets could also be of potential

interest. Epratuzumab is an anti-CD22 monoclonal antibody. CD22 is expressed on the surface of mature B cells and acts as a negative regulator of B cell receptor signal transduction. In contrast to B cell depletion by rituximab, it acts as an immunomodulatory agent inducing B cell anergy. Studies in systemic lupus erythematosus have shown potential efficacy and safety [27, 28].

Several studies have found elevated levels of B cell-activating factor belonging to the TNF superfamily (BAFF) [29, 30]. BAFF is a known positive regulator of B cell survival, differentiation, and proliferation. The BAFF receptor is expressed in many stages of the B cell lineage, including plasma cells. In AAV, controversial data exist regarding the correlation between circulating BAFF levels and ANCA titers. Nagai et al. [31] reported increased BAFF levels in active MPA patients which correlated with MPO-ANCA titers. Bader et al. [32] found increased levels in GPA which correlated inversely with ANCA titers. However, we have not found a significant difference in BAFF levels between ANCA-positive and ANCA-negative patients [29]. BAFF might prove to be a potential target for therapy. Belimumab, an anti-BAFF monoclonal antibody, is approved for systemic lupus erythematosus, and is being investigated in a clinical trial as additional relapse prevention therapy for AAV (ClinicalTrials.gov No. NCT01663623). Therefore, a novel therapeutic approach could be maintenance treatment by the anti-BAFF agent belimumab after induction of remission by B cell-targeted therapy.

An increasing body of evidence indicates that in autoimmunity the interplay between pathogenic and protective B cell functions is dysregulated. Several studies have found a deficiency in the regulatory B cell compartment. Decreased CD5+ B cells, a B cell population enriched for regulatory cells, were found to be associated with the occurrence of relapses [33]. Additionally, two recent studies found numerical, but not functional, deficiencies in regulatory B cells [34, 35]. These studies imply a role for the balance between regulatory and effector B cell functions which might have therapeutic implications. Therefore, the future direction for therapy in autoimmunity might not be B cell depletion, but instead restoring the balance between B cell regulators and effectors.

T cells

Several lines of evidence support a role for T cells in the pathogenesis of AAV [36]. Lymphocyte-depleting therapy, mainly T cells, with anti-CD52 antibodies

(alemtuzumab) has been shown to induce remission in AAV patients, suggesting a role of T cells in disease pathogenesis [37]. We previously demonstrated an imbalance in CD4 T cell subsets in peripheral blood of patients with AAV [38]. In particular, circulating CD4 T effector memory (CD4+ T_{EM}) cells were reduced during active disease, and these cells were detected in the urine of patients and correlated with renal disease activity [39]. Therefore, CD4+ T_{EM} cells are supposed to act as a key trigger of kidney damage and disease expression/relapse in AAV. Importantly, T cell-mediated lesions in AAV patients treated with rituximab (RITUXVAS trial) were shown to be important predictors of renal outcome, whereas the presence of B cells in the renal tissue of those patients did not provide any independent predictor variables related to renal outcome [40]. These results suggest that in addition to anti-B cell therapy, therapy directed at T cells, specifically CD4+ T_{EM} cells, may improve renal outcomes in AAV.

In this setting, alemtuzumab would not be a suitable therapy since this treatment regimen is associated with severe adverse events in the elderly and those with renal failure [37]. As CD4+ T_{EM} cells are major players in AAV, a promising approach for treating AAV could be to specifically target these effector cells and leave the other T cells untouched. Concerning this issue, Chandy and colleagues [41] have shown that CD4+ T_{EM} cells are characterized by high expression of surface Kv1.3 channels (approx. 1,500 channels per cell), whereas naïve and central memory T cells express lower levels of these channels (approx. 250 channels per cell). Therefore, Kv1.3 channels may serve as an attractive target for specific immunomodulation in T_{EM} cell-mediated inflammatory autoimmune diseases. Importantly, selective blockade of Kv1.3 channels ameliorates disease in several animal models without compromising the protective immune responses to acute infection [42, 43]. Gocke et al. [44] found that Kv1.3 is required for expression of proinflammatory cytokines, whereas its absence leads to increased expression of the anti-inflammatory cytokine IL-10. Thus, it is tempting to speculate that pharmacological blockade of Kv1.3 channels contribute to skewing of CD4+ T cell differentiation towards a regulatory phenotype which might be beneficial for autoimmune-mediated vascular diseases. Human phase 1A and 1B trials of a Kv1.3-blocking agent were recently completed in healthy volunteers, and the efficacy of this agent in patients needs to be assessed.

In AAV patients, persistent T cell activation has been reported during active disease as well as in remission [38, 45]. Therefore, targeting T cell activation in AAV by abatacept may represent a potential therapeutic approach. Abatacept is a fully human fusion molecule of cytotoxic T lymphocyte antigen 4 (CTLA4-Ig) and the immunoglobulin Fc region that modulates the CD28-mediated T cell costimulatory pathway which is required for T cell activation [46]. It is currently approved for rheumatoid arthritis. An open-label pilot study involving relapsing patients with nonsevere GPA demonstrated sustained disease control with abatacept [47].

Besides targeting T cell activation, one can also consider targeting their effector cytokines, such as IL-17 and IL-21, which are in large part responsible for their pathogenic action. Elevated serum levels of IL-17A and increased autoantigen-specific IL-17-producing cells (Th17) have been demonstrated in AAV patients during disease convalescence compared with healthy controls [48, 49]. IL-17A cytokines seem operative in lesion development [48]. Therefore, therapeutic approaches targeting IL-17A could be of interest. Several IL-17A blockers, including the anti-IL-17A monoclonal antibodies secukinumab and ixekizumab and the anti-IL-17 receptor subunit A monoclonal antibody brodalumab have been evaluated in clinical trials [50, 51]. Secukinumab seems to be the most advanced with respect to clinical evaluation in rheumatoid arthritis [50].

As Th17 cells are pathogenically important in AAV, blocking IL-23, a cytokine essential for the proliferation of Th17 cells, might also have therapeutic potential in AAV. Ustekinumab is an anti-IL-12/23p40 monoclonal antibody, and might be effective in autoimmune disease related to Th17 cells.

Besides IL-17, IL-21-producing cells have also been shown to be increased in AAV patients, which also seems to be an interesting target in the treatment of autoimmune-mediated vascular inflammation [52]. Manipulation of IL-21 may have desirable therapeutic consequences as it must reduce the recruitment of inflammatory Th1 and Th17 cells to inflammatory lesions to prevent tissue damage. Furthermore, it may inhibit expansion of autoreactive B cells. Recently, phase I clinical trials using an IL-21-specific monoclonal antibody (NNC0114-0006) have been completed for rheumatoid arthritis (NCT01208506 and EudraCT-2011-005376-42), but were terminated for systemic lupus erythematosus (NCT01689025) [53]. Therefore, neutralization of IL-17 or IL-21 could also represent novel therapeutic approaches for patients with AAV.

Complement

Recently, several studies have shown the importance of the alternative pathway of complement in the pathogenesis of AAV [54]. Xing et al. [55] detected MAC, C3d, factor B, and factor P in glomeruli of patients with AAV, indicating involvement of the alternative pathway of complement. Also, elevated levels of plasma and urinary C5a levels were found indicating complement activation in human AAV [56]. Therefore, inhibition of C5a, a cleavage product of complement C5 with strong chemotactic and anaphylatoxic features, is a potential therapeutic approach. Currently an ongoing phase 2 trial is investigat-

ing CCX168, an orally administered small molecule inhibitor of C5a, in patients with AAV (ClinicalTrials.gov No. NCT01363388).

Conclusion

In AAV, unraveling the pathogenesis has led to various biological targets for therapy. Biological therapies have been successfully introduced or are under evaluation in both clinical and experimental studies. Future clinical studies preferably in international collaboration are warranted to find an optimal place for these new drugs.

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