Review

T Cells in the Pathogenesis of ANCA-Associated Vasculitis: Current Knowledge

(T cells / ANCA / vasculitis / effector memory cells / regulatory cells)

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Abstract. AAV are a group of systemic immune-mediated diseases with a strong and highly specific association with ANCA. In recent years, there has been increasing evidence that ANCA might play a direct pathogenic role in triggering AAV. Nevertheless, effectors of cell-mediated immunity prevail in the inflammation sites in patients with AAV. Numerous studies found increased markers of T-cell activation in AAV. Moreover, this activation persisted even in remission and despite treatment. Finally, successful therapeutic attempts using T cell-directed treatment were also reported. There has therefore been substantial evidence that T cells are involved in the pathogenesis of AAV, even though the exact mechanisms are yet to be elucidated. In this review, recent findings on the contribution of T cells to the pathogenic processes in AAV will be briefly summarized. Special emphasis will be placed on the Th1/Th2 concept, the role of T-regulatory cells, and the role of effector memory T cells in the pathogenesis of AAV.

Introduction

Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS) are a group of systemic immune-mediated diseases with a strong and highly specific association with anti-neutrophil cytoplasmic autoantibodies (ANCA). The disorders are characterized by necrotizing inflammation of predominantly small- to medium-sized vessels. Together with renal-limited vasculitis (RLV, also referred to as idiopathic rapidly progressive glomerulonephritis), they are therefore all ranked among ANCA-associated vasculitides (AAV).

ANCA are circulating autoantibodies directed against different target antigens located in azurophilic granules of polymorphonuclear leucocytes and the peroxidase-positive lysosomes of monocytes. In WG, ANCA are usually directed against proteinase 3 (anti-PR3) and have a cytoplasmic type of immunofluorescence (c-ANCA). In MPA the target antigen is mostly myeloperoxidase (anti-MPO) and the type of immunofluorescence is perinuclear (p-ANCA). Both anti-MPO and, less frequently, anti-PR3 can also be found in RLV (Jennette and Falk, 1997; Savage et al., 1997). In CSS, only about 40 % of patients are ANCA-positive (mostly anti-MPO) (Sinico et al., 2005).

ANCA are known to be an important diagnostic tool, and usually correlate with disease activity. In recent years, there has been increasing evidence that ANCA might play a direct pathogenic role in triggering AAV (reviewed in: Sarraf and Sneller, 2005; Bosch et al., 2006; Morgan et al., 2006; van Paassen et al., 2007). However, as ANCA are class-switched IgG antibodies (mainly IgG1 and IgG4), T cells are most likely involved in their production by B cells and plasma cells (Brouwer et al., 1991). Many other findings also support the role of T cells in the pathogenesis of AAV and will be reviewed in this paper. Since most studies involve Wegener's granulomatosis only, the emphasis in this review was also placed on WG. However, some of the pathogenic mechanisms mentioned refer to AAV in general.

T Cells in Crescentic "Pauci-Immune" Glomerulonephritis and Vasculitic Lesions

Rapidly progressive, pauci-immune, crescentic necrotizing glomerulonephritis is the hallmark of ANCA-associated renal vasculitis. The term "pauci-immune" reflects the relative lack of immunoglobulin and com-

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Abbreviations: AAV – ANCA-associated vasculitides, ANCA – anti-neutrophil cytoplasmic autoantibodies, DTH – delayed-type hypersensitivity, IFN – interferon, IL – interleukin, MPA – microscopic polyangiitis, MPO – myeloperoxidase, PR3 – proteinase 3, RLV – renal-limited vasculitis, TCR – T-cell receptor, TEM – effector memory T cells, WG – Wegener's granulomatosis.

plement depositions in affected glomeruli (Jennette and Falk, 1997). Given that the effectors of humoral immunity are absent, the role of cellular immunity in the process of glomerular injury seems likely. In experimental models, delayed-type hypersensitivity (DTH), a manifestation of cell-mediated immunity induced by sensitized T cells, was shown important for crescent formation (Huang et al., 1994). In patients with pauci-immune glomerulonephritis, activated T cells, macrophages, fibrin, and tissue factor, i.e. mediators of DTH, were prominent at the site of glomerular injury (Cunningham et al., 1999). Furthermore, monocytes/macrophages and T cells predominate not only in renal, but also in pulmonary (Gephardt et al., 1983) and nasal infiltrates (Rasmussen and Petersen, 1993) in AAV patients.

MPO- and PR3-Specific T Lymphocytes

T-lymphocyte proliferation was observed after *in vitro* stimulation with PR3 and, to a lesser extent, with MPO in patients with AAV. In these patients, T cells proliferate in response to crude granular extract of neutrophils, inactivated purified PR3 or MPO, and PR3- and MPO-derived peptides. However, similarly as in other autoimmune diseases, proliferation was also observed in PR3- or MPO-stimulated T cells from healthy controls (Brouwer et al., 1994; Griffith et al., 1996; King et al., 1998; Popa et al., 2002). Whilst an increased frequency of MPO-specific T lymphocytes was reported in patients with active MPO-ANCA-associated vasculitis (Griffith et al., 1996), other authors found no correlation between ANCA titres/activity of the disease, and PR3- or MPO-stimulated T-cell proliferation (Brouwer et al., 1994; King et al., 1998). Even though some PR3-derived peptide sequences were suggested as potential targets of T-cell responses in AAV, this field clearly requires further studies, and the exact role of PR3- and MPO-specific T cells in the pathogenesis of AAV remains unclear (Ballieux et al., 1995; King et al., 1998; van der Geld et al., 2000).

T-cell activation in AAV

Several studies found increased serum markers of T-cell activation in AAV, including soluble IL-2 receptor or soluble CD30. In patients with WG, the levels of soluble IL-2 receptor (sIL-2R) correlate well with disease activity (Schmitt et al., 1992, Stegeman et al., 1993) and might even indicate imminent relapse according to some authors (Schmitt et al., 1992), even though this was not confirmed by others (Sanders et al., 2006). Soluble CD30 (sCD30) was also shown to correlate with disease activity (Wang et al., 1997; Sanders et al., 2006). Although immunosuppressive treatment leads to a decrease in levels of both sIL-2R and CD30, their levels remain increased in comparison with healthy controls. Interestingly, increased levels of these soluble markers were associated with persistent or renewed ANCA positivity in a recent study, but did not predict risk for relapse (Sanders et al., 2006). On the basis of these observations, the authors concluded that ANCA positivity might relate to a complex, potentially T-cell driven, immune activation in AAV patients.

Naïve T cells are mature T cells that have not yet encountered antigen in the periphery (for the associated surface markers see Table 1). After recognition of the antigen and subsequent immune response, T cells acquire an activated phenotype ($CD25^+$, $CD62L^{low}$) and might further differentiate into a memory T cell. In contrast to soluble T-cell markers and to activation markers on B lymphocytes, activation markers on T cells (e.g. CD25 and HLA-DR) seem not to correlate with disease activity; they are up-regulated even in remission and despite treatment (Popa et al., 1999; Marinaki et al., 2005). In a recent study, this phenomenon called persistent T-cell activation (defined as either increase in $CD25^+CD4^+$ lymphocytes and/or decrease in the total number of $CD4^+CD45RO^-$ naïve cells) was shown to be associated with disease severity (Marinaki et al., 2006).

Several mechanisms might contribute to T-cell activation in AAV, such as aberrant co-stimulation or expression of co-stimulatory molecules, or homeostatic expansion (Kälsch et al., 2005). Nevertheless, special emphasis was placed on the role of regulatory T cells (Tregs) in AAV in the last few years. Tregs are a subset of $CD4^+$ T cells, characterized by high-level expression of surface CD25 and intracellular transcription factor forkhead box P3 (FoxP3) (Sakaguchi, 2005). Decreased frequency and/or impaired function of Tregs have been described in several autoimmune diseases, e.g. multiple sclerosis (Viglietta et al., 2004), rheumatoid arthritis (Ehrenstein et al., 2004), and systemic lupus erythematosus (Crispin et al., 2003). Until recently, no difference in numbers or function of Tregs had been described in AAV (Kälsch et al., 2005; Marinaki et al., 2005). Contrary to previous findings, Abdulahad et al. (2007b) reported on expanded proportion of Tregs that poorly suppressed $CD4^+CD25^-$ effector T-cell proliferation in patients with WG in remission. Such a functional defect of Tregs might support the development of inflammation and autoimmunity in WG, but data from animal and further *in vitro* studies are still needed (Lamprecht et al., 2007).

Table 1. Naïve and memory T cell-associated markers

Naïve T cells	Memory T cells	
	Effector memory T cells	Central memory T cells
$CD45RA^+$	$CD45RA^-$	$CD45RA^-$
$CCR7^+$	$CCR7^-$	$CCR7^+$
$CD62L^+$	$CD62L^-$	$CD62L^+$

CD62L = L selectin (adhesion molecule)

Th1/Th2 Paradigm in AAV

Based on their cytokine profile and related ability to generate different types of immune effector responses, $CD4^+$ T-helper lymphocytes can be classified into two distinct subsets: type 1 (Th1) and type 2 (Th2). In general, Th1 cells characteristically produce interferon gamma (IFN-γ) and are involved in cell-mediated inflammatory reactions. They promote macrophage activation and DTH. Th2 cells, producing interleukin (IL)-4, IL-5, IL-10 and IL-13, are associated with phagocyte-independent host responses, encourage non-complement-fixing IgG and IgE production, and enhance eosinophil proliferation and function (Mosmann and Sad, 1996; Del Prete, 1998).

Both Th1 and Th2 cells differentiate from naïve precursors (Th0) following specific antigen stimulation via their α/β T-cell receptor (TCR). Antigen dose and antigen affinity can influence Th subset development. The key factor in directing Th cell polarization is the cytokine milieu, but co-stimulatory signals are also important. The main inducers of the Th1 profile are IFN-γ and IL-12. In recent years, IL-18 and IL-27 have been recognized as other promoters of Th1 polarization. IFN-γ but not IL-12 may participate in inhibiting Th2 responses (Szabo et al., 2003). Of the Th2-related cytokines, IL-4 promotes differentiation of naïve $CD4^+$ cells into Th2 cells, whereas IL-10 inhibits Th1 cytokine synthesis. As for the co-stimulatory molecules, CD80 and CD86 signal via CD28, constitutively expressed on T cells. Inhibition of CD28 blocks Th2 responses without blocking Th1 responses (Rulifson et al., 1997).

Differences in surface membrane markers on Th1 and Th2 cells subsequently result in different intracellular signalling events. In addition, Th1 and Th2 cells seem to express different patterns of chemokine receptors. CXCR3, the receptor for IFN-γ-inducible chemokines (e.g. IP-10, Mig and I-TAC), and CCR5 are predominantly expressed at high levels on Th1 cells. On the other hand, CCR3 and CCR4 are associated with Th2 cells (Syrbe et al., 1999).

The Th1/Th2 paradigm in AAV, in particular in WG, has been discussed recently in many studies (Wang et al., 1997; Ludviksson et al., 1998; Csernok et al., 1999; Müller et al, 2000; Balding et al., 2001; Kiene et al., 2001; Zhou et al., 2002; Sanders et al., 2003; Lamprecht et al., 2003a, b; Masutani et al., 2003; Lamprecht, 2005; Tipping and Kitching, 2005). In localized WG, i.e. early WG restricted to the respiratory tract, T cells in nasal inflammatory infiltrates were shown to abundantly express CD26, an optional Th1 marker. In the same study, higher numbers of IFN-γ-positive cells in localized WG than in generalized disease were found both in nasal infiltrates and in peripheral blood. On the contrary, IL-4 mRNA was detected in higher amounts in nasal biopsies in generalized WG (Müller et al., 2000). Th2 environment in nasal granuloma in generalized WG was also detected in another study. In this study, increased IL-4 expression but no IFN-γ were observed in nasal biopsies in generalized active WG (Balding et al., 2001).

Both localized and generalized WG displayed upregulated expression of both Th1-type-associated CCR5 and Th2-type-associated CCR3 chemokine receptors in flow-cytometric analysis of circulating T cells. Nevertheless, predominant CCR5 expression on T cells as well as in granulomatous lesions was noted in localized WG, which may favour stronger recruitment of Th1-type cytokine-secreting cells into inflammatory lesions (Lamprecht et al., 2003a, b).

As mentioned above, plasma levels of sCD30, a member of the tumour necrosis factor receptor family and a Th2 marker, have been shown to be significantly increased and to correlate with disease activity in generalized WG. These findings further support the hypothesis that generalized WG may be associated with Th2-type immune response (Wang et al., 1997).

In summary, there seems to be an aberrant Th1-type response that might play a role during initiation of WG in patients with localized WG, when PR3 is often not yet detected. On the contrary, there seems to be significant appearance of Th2 cells and less prominent Th1 phenotype and Th1 cytokine production in granulomatous lesions of the upper respiratory tract (and eventually also in peripheral circulation) in patients with generalized vasculitis. This increasing complexity and "shift" of immune response have been hypothesized to be a consequence of B-cell expansion and T cell-dependent PR3-ANCA production during disease progression (Lamprecht, 2005), potentially triggered by interaction between neutrophils and autoreactive T and B cells within granulomatous lesions. Persistent antigenic stimulation, e.g. by *Staphylococccus aureus* (a known risk factor for relapse in WG (Stegeman et al., 1994)), might contribute to the shift in the immune response (Sanders et al., 2003).

Nevertheless, data regarding the type of Th response in WG are not entirely consistent. Predominant IFN-γ production by T cells from peripheral blood, bronchoalveolar lavage, and granulomatous nasal lesions was reported in patients with generalized WG by other authors (Csernok et al., 1999). Furthermore, in renal lesion of the patients with generalized AAV, the polarization towards Th1-type response was found, but the lesions contained Th2 cells as well (Balding et al., 2001; Masutani et al., 2003; Tipping and Kitching, 2005).

Increased IFN-γ production in WG patients was noted in another study, possibly induced by increased amounts of IL-12, produced by monocytes in both active and inactive patients with WG. Moreover, the authors demonstrated that *in vitro* IFN-γ production might be inhibited by exogenous IL-10 (Ludviksson et al., 1998). In addition to its association with Th2-type immune response, IL-10 is a cytokine with immunosuppressive and anti-inflammatory potential. Levels of IL-10 were high at diagnosis in patients with WG and decreased subsequently. Intriguingly, low levels of IL-10 were associated with increased relapse rate in

a recent study (Sanders et al., 2006). Polymorphisms in genes for IL-10 associated with WG have already been described (Zhou et al., 2002).

Even though less thoroughly studied, the Th2-type immune response was reported in CSS, where T cells producing IL-4 and IL-13 may drive the eosinophilic inflammation (Kiene et al., 2001). However, less information is available on the Th1/Th2 polarization in MPA.

Collectively, the Th1/Th2 concept provides important clues for understanding the complex pathogenesis of AAV. However, the role of specific Th subsets in various disease stages in AAV has yet to be established.

The Role of Effector Memory T Cells in AAV

Numerous studies have given attention to the altered phenotype of T cells in AAV, especially in WG. The expansion of a subset of circulating T cells lacking co-stimulatory molecule CD28 has been reported repeatedly (Giscombe et al., 1998; Moosig et al., 1998; Komocsi et al., 2002; Giscombe et al., 2006). However, surprisingly very little is still known about this immunological phenomenon.

The expansion of $CD28^-$ T cells starts early in the disease process and correlates with organ involvement and disease progression from localized to generalized WG (Moosig et al., 1998; Komocsi et al., 2002). Most studies show that circulating peripheral blood $CD4^+CD28^-$ T cells as well as those within granulomatous lesions are a major source of Th1-type cytokine secretion (Komocsi et al., 2002). Moreover, expanded $CD28^-$ T cells express the differentiation marker CD57, the activation marker and adhesion molecule CD18, Th1-type chemokine receptor CCR5, and upregulate HLA-DR and CD152 (CTLA-4), which indicates that these cells belong to the so-called late differentiated or effector memory T cells (TEMs) (Giscombe et al., 1998; Moosig et al., 1998; Steiner et al., 2001; Komocsi et al., 2002; Giscombe et al., 2006; Lamprecht et al., 2006; Abdulahad et al., 2007a). Shortened telomeres and oligoclonality indicate cytokine- and/or antigen-driven expansion and replicative senescence of TEMs (Grunewald et al., 1998; Vogt et al., 2003).

In recent years, great progress has been made in understanding T-cell differentiation processes. As proliferation of T cells toward TEMs requires a strong and persistent immune trigger, the presence of such an antigenic stimulus in WG patients seems likely. Contrary to central memory T cells, TEMs do not express CCR7, the lymph node homing receptor, and thus fail to migrate to lymphoid organs (Table 1). However, they are capable of migrating to the sites of inflammation and producing pro-inflammatory cytokines (Abdulahad et al., 2007a).

The apoptosis process was proved impaired in $CD28^-$ TEMs by increased expression of anti-apoptotic protein Bcl-2 (Vallejo et al., 2000). Although not yet proved, CTLA-4 could contribute to this Bcl-2-increased expression. In addition, the aforementioned defective function of Tregs in WG may also account for TEM expansion (Abdulahad et al., 2007b). Last but not least, two recently identified T-cell function-associated genetic factors predisposing to granulomatous inflammation (HLA-DPB1*0401) and PR3-ANCA positivity (PTPN22*620W) might be related to both persistent T-cell activation and TEM expansion in WG (Jagiello et al., 2004, 2005).

Taken together, Th1-type TEMs might contribute to the chronic granulomatous inflammation and development of autoimmunity in WG. In accordance with previous findings regarding the Th1/Th2 concept, exaggerated Th1-type response in respiratory tract with clonal expansion of TEMs could sustain the chronic granulomatous inflammation and promote formation of ectopic "lymphoid-like" tissue within granulomatous lesions. Subsequently, this might induce PR3-ANCA formation resulting in ANCA-induced vasculitis (Lamprecht et al., 2006). Therefore, suppression of TEM cells might become an important goal in therapeutic strategies in AAV in the future.

Dendritic Cells

Dendritic cells (DC) play an important role in regulating immune responses to both foreign and self-antigens through antigen presentation, co-stimulatory signals and soluble factors. DC also influence the Th1/Th2 balance. Involvement of specific receptors on DC in response to particular self-antigens may contribute to the development of an autoimmune disease. Interestingly, PR3 (the autoantigen in WG) was shown to be able to induce maturation of DC by an interaction with the protease-activated-receptor-2 (PAR-2) and to evoke a stronger Th1-type response than in healthy and diseased controls, thus favouring granuloma formation in WG (Csernok et al., 2006). In a recent study, a novel suppressant of DC function (NK026680) prevented the development of rapidly progressive glomerulonephritis and perinuclear antineutrophil cytoplasmic antibody in mice, which further supports the role of DC in the pathogenesis of AAV (Saiga et al., 2006). More studies with DC are therefore awaited in the future.

Conclusion - Therapy Targeted to T Cells

Conventional immunosuppressive treatment in AAV is still connected with high potential toxicity. In oncoming years, the emphasis will be placed on finding more selective therapeutic approaches. As summarized above, T cells play an important role in the pathogenesis of AAV. In patients with refractory WG, T-cell depleting therapy with anti-CD52 antibodies (alemtuzumab) and anti-thymocyte globulin successfully induced remission, but its use was also limited by serious side effects, in particular infectious complications (Lamprecht et al., 2006, Schmitt et al., 2004).

As CTLA-4 is up-regulated on TEMs in WG, blocking of CTLA4-mediated co-stimulation (CD28/CD80

and CD86 pathway) and up-regulation of anti-apoptotic Bcl-2 expression by CTLA-4-immunoglobulin (Abatacept) might help modulate the pathologic immune response in AAV. Another therapeutic approach might, for instance, target Kv1.3 K^+ channels, which are specific functional markers of TEMs. In rat models of rheumatoid arthritis, specific Kv1.3 blockers have already been successfully used (Beeton et al., 2006).

In conclusion, improving our insights into pathogenic mechanisms in AAV is of particular importance for both defining prognostic factors and development of novel therapeutic possibilities with presumably lower toxicity.

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