CD4-positive effector memory T-cells participate in disease expression in ANCA-associated vasculitis

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Abstract

Although the cause of ANCA-associated vasculitis (AAV) remains undetermined, the presence of lymphocytic infiltrates in inflammatory lesions of patients suggests that vascular damage is immune mediated. Studies over the past decade have implicated a role for T-cells in the pathogenesis of AAV as altered T-cell phenotype has been observed in this disorder. The distribution of T-cell subpopulations has been analyzed most intensely in Wegener’s granulomatosis (WG), where an expanded population of circulating CD4+ effector memory T-cells (CD4+TEM) was demonstrated. CD4+TEM cells play a major role in the pathogenesis of several autoimmune diseases. Specific suppression of CD4+TEM cells inhibits delayed-type hypersensitivity (DTH) and has therapeutic potential in autoimmune disease. Thus, CD4+TEM cells may act as inducers of tissue injury and participate in the development of AAV. Therapies that target CD4+TEM, without impairing the activity of other lymphocyte subsets, may hold therapeutic promise for AAV.
Introduction

Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated systemic vasculitis (AAV) constitutes a group of disorders characterized by autoimmune inflammation affecting small- to medium-sized vessels, which leads to vessel occlusion and systemic organ damage. AAV is associated with the presence of ANCA that are directed in these diseases against either proteinase 3 (PR3) or myeloperoxidase (MPO). However, the primary immunopathogenic events that initiate the process of vasculitis are still largely unknown. AAV comprise basically three diseases: Wegener’s granulomatosis (WG), Microscopic Polyangiitis (MPA), and Churg-Strauss syndrome (CSS). PR3-ANCAs are predominantly associated with WG, whereas MPO-ANCAs are present in the majority (60-80%) of MPA-patients and in 35-50% of patients with CSS, particularly those with small-vessel vasculitis.1-3

As autoantibodies against neutrophil proteins are present and related to disease activity in these forms of vasculitis,4 the focus of research has been on the effects of these autoantibodies and their production. In addition, an animal model has shown that passive transfer of anti-MPO antibodies is sufficient to induce necrotizing glomerulonephritis.5 However, the distribution of IgG subclasses of ANCA in AAV, with predominance of IgG1 and IgG4, suggests an antigen-driven and T-cell-dependent immune response.6 This provides indirect evidence for T-cell involvement in AAV. Furthermore, the presence of T-cells and macrophages in vasculitic areas and granulomatous lesions,7-10, as well as the therapeutic effect of anti-T-cell treatment in patients,11,12 strongly support the view that T-cells play a role in disease manifestation. Finally, serum markers of T-cell activation have been found to be increased during remission and active AAV and to be related to disease activity. Based on these data, this review will discuss the role of T-cells in AAV. The main emphasis will be on the involvement of CD4+ effector memory T-cells in the pathogenesis of this autoimmune inflammatory disease.
Altered T-cell phenotype in ANCA-associated vasculitis

Although T-cells may play an important role in the pathogenesis of AAV, in vitro specific T-cell reactivity to PR3 or MPO was found not only in some patients with AAV but also in a comparable proportion of healthy controls, and investigators have failed to detect a relation between PR3- or MPO-specific T-cells and disease activity. Nevertheless, in patients with WG, percentages of activated circulating T-cells are increased in active disease and this increase persists during remission. This suggests that T-cell activation in WG is chronically present and possibly due to a persistent stimulus causing ongoing stimulation. In addition, analysis of soluble markers for T-cell subsets, that is for Th1 (interferon [IFN]-γ, sCD26) and Th2 (interleukin [IL]-4, IL-5, IL-10, IL-13, sCD23, sCD30), disclosed a shift towards humoral immunity (Th2) in patients with active generalized WG and CSS, whereas cell-mediated immunity (Th1) dominated in patients with MPA and localized WG. The relationship between Th1/Th2 skewing and its specific role in the pathophysiology of AAV awaits further studies.

Abnormalities of T-cell phenotypes have been analyzed predominantly in WG, whereas studies in MPA and CCS are scarce. A number of studies have shown that most of the expanded lymphocytes in the peripheral blood of WG-patients upregulate CD152 (CTLA-4), express the differentiation marker CD57, and contain and/or secrete Th1-type cytokines but lack CD28, which indicates that these cells belong to lately differentiated or effector memory T-cells. These expanded circulating lymphocytes express the memory marker CD45RO, and in localized and generalized WG upregulate the expression of the chemokine receptors CCR3 and CCR5, which mediate the entrance of these cells into the site of inflammation. Moreover, memory T-cells, mainly belonging to the CD4+ and to a lesser extend to the CD8+ T-cell population, were abundantly present in WG lung lesions. Furthermore, enrichment of CD28- memory T-cells has also been described in granulomatous lesions in nasal biopsies of WG-patients. Taken together, these data provide additional evidence for the involvement of especially memory T-cells exhibiting immediate effector function in the pathogenesis of WG and suggest that these cells contribute to inflammatory damage and the formation of granuloma by rapidly migrating into the tissues.
CD4 T<sub>EM</sub> cells are involved in the pathogenesis of various autoimmune diseases including ANCA-associated vasculitis

According to recent models of progressive T-cell differentiation, the strength of the signal delivered via the T-cell receptor (TCR) and the type and amount of cytokines present during priming determine the differentiation of T-cells<sup>27-29</sup>. Cells receiving insufficient stimulation remain nonfit and die by neglect, whereas cells receiving excessive stimulation die by activation-induced cell death (AICD). The fittest cells survive and some of these cells enter the memory pool, develop into effector (T<sub>EM</sub>) as well as central memory T-cells (T<sub>CM</sub>), and will persist long after antigen clearance. Naïve T-cells receiving a relatively weak stimulus will proliferate and develop preferentially into T<sub>CM</sub>, whereas priming by a strong stimulus results in differentiation towards T<sub>EM</sub> cells. T<sub>CM</sub> and T<sub>EM</sub> cells are defined based on the expression of the lymph node homing chemokine receptor CCR7, which is a key to unlock entry into lymph nodes<sup>30</sup>. T<sub>CM</sub> express CCR7 and efficiently home to lymph nodes, while T<sub>EM</sub> lacking CCR7 expression fail to migrate to lymphoid organs, but have acquired the capacity to migrate to sites of inflammation and to produce large amounts of proinflammatory cytokines.

Several studies have demonstrated that disease-specific autoreactive T-cells in various autoimmune diseases are co-stimulation-independent CCR7-negative T<sub>EM</sub> cells. It has been shown that myelin basic protein (MBP)-specific T-cells in peripheral blood of patients with multiple sclerosis (MS) are predominantly CD4<sup>+</sup>T<sub>EM</sub> cells<sup>31,32</sup>. In patients with type-1 diabetes mellitus (T1DM), T-cells specific for the autoantigen glutamic acid decarboxylase 65 (GAD65) are co-stimulation-independent activated CD4<sup>+</sup> memory T-cells<sup>33</sup>. Moreover, enrichment of T-cells exhibiting the T<sub>EM</sub> phenotype was demonstrated in synovial fluid of patients with juvenile idiopathic arthritis and in skin lesions of patients with psoriasis<sup>34,35</sup>. The pathogenic role of T<sub>EM</sub> was confirmed in an animal model for MS<sup>36</sup>. Passive transfer of myelin-specific T<sub>EM</sub> to naïve rats induces experimental autoimmune encephalomyelitis (EAE) in the recipient rat, which indicates the involvement of T<sub>EM</sub> cells in the pathology of autoimmunity.

In WG, a persistent expansion of CD4<sup>+</sup>T<sub>EM</sub> cells with a reciprocal decrease in naïve CD4<sup>+</sup>T-cells has been reported in patients during remission, whereas no differences were observed in the distribution of
CD8⁺T-cells subpopulations and the CD4⁺T<sub>CM</sub> cells between patients and healthy controls<sup>37</sup>. The majority of expanded CD4⁺T<sub>EM</sub> cells in WG have a FoxP3-negative nonregulatory phenotype<sup>37</sup>. Since proliferation towards T<sub>EM</sub> cells requires both a strong and persistent immune trigger, these data provide important evidence for the presence of an ongoing strong antigenic stimulus in WG-patients.

The obvious question arises why the disturbance in T-cell subpopulations in WG-patients is confined to the CD4⁺T-cells subset. Studies in mice have shown that granuloma formation was delayed and poorly organized in CD4-deficient mice in response to intravenous infection with M. tuberculosis<sup>38</sup>. In humans, HIV-infected patients with mycobacterial infection exhibit defective granuloma formation and the extent of granuloma formation in those patients was dependent on circulating CD4⁺T-cell counts<sup>39</sup>. These data point to an important role for CD4⁺T-cells in initiating and maintaining granuloma formation and suggest that CD8⁺T-cells are less important. Very recently, Ruth et al.<sup>40</sup> have demonstrated the contribution of effector CD4⁺T-cells to tissue injury in MPO-ANCA-associated glomerulonephritis. They induced experimental autoimmune anti-MPO crescentic glomerulonephritis in C57BL/6 wild-type (WT) mice by immunization with human MPO (hMPO) and recruiting neutrophils to glomeruli by administration of heterologous anti-GBM antibodies. They found that depletion of effector CD4⁺T-cells in MPO-immunized mice at the time of administration of the triggering anti-GBM antibodies resulted in a pronounced attenuation of crescent formation and macrophage influx in glomeruli, despite the presence of MPO-ANCA levels, comparable to that in control-treated mice. Furthermore, they showed that glomerular crescent formation was not reduced in the absence of MPO-ANCA, as hMPO immunized B-cell-deficient mice (μMT<sup>−/−</sup> mice) with this model developed comparable severity of glomerulonephritis despite the absence of an anti-MPO antibody response. This study suggests that effector CD4⁺T-cells act as key effectors of tissue injury in MPO-ANCA vasculitis. Importantly, data from our cross-sectional and follow-up study in WG demonstrate a significant decrease in CD4⁺T<sub>EM</sub> cells in active disease as compared to patients in remission<sup>37</sup>. A decrease in CD4⁺T<sub>EM</sub> cells during active disease may have occurred due to a selective migration of those cells into inflammatory areas in response to an as yet unknown initial trigger. In
agreement with this, CD4+ memory T-cells were present in active pulmonary lesions in patients with WG\textsuperscript{25}. In addition, Marinaki \textit{et al.}\textsuperscript{41} have reported on the association between persistent activation of CD4\textsuperscript{+} T-cells and disease severity in MPA- and WG-patients. Furthermore, Sakatsume \textit{et al.}\textsuperscript{42,43} have shown that T-cells in urine of patients with glomerulonephritis exhibit a TEM phenotype identical to the phenotype of infiltrating T-cells found in and around glomeruli.

Collectively, the aforementioned data strongly support the involvement of CD4\textsuperscript{+}TEM in the pathogenesis of AAV, and provide some clues to understanding the mechanism of disease development and relapse.

\textbf{What causes expansion of CD4\textsuperscript{+}TEM cells in WG?}

It is still unresolved what causes the expansion of CD4\textsuperscript{+}TEM cells in WG, but several possibilities may be suggested. It has been reported that \textit{in vitro} stimulation of peripheral blood mononuclear cells (PBMCs) from WG-patients with PR3 and MPO (autoantigens) and with \textit{Staphylococcus aureus} (a risk factor for relapse in WG\textsuperscript{44}) resulted in an exaggerated and a Th2-skewed cytokine response\textsuperscript{45,46}. In line with this \textit{in vitro} finding, we observed a skewing of CD4\textsuperscript{+}TEM in WG-patients in remission toward a Th2-phenotype\textsuperscript{37}, which may support the hypothesis that expansion of circulating CD4\textsuperscript{+}TEM in WG results from autoantigenic triggers or \textit{S.aureus} derived stimuli. In addition, this autoreactive response may be due to a failure in one or more self-tolerance mechanisms. Our recent results on regulatory T-cell (T\textsubscript{Reg}) function in WG-patients have demonstrated a defective suppressive function of circulating T\textsubscript{Reg}\textsuperscript{47}, which may contribute to TEM expansion and disease pathogenesis.

On the other hand, an impressive enrichment of Insulin-like growth factor 1 (IGF-1) immunoreactive cells has been demonstrated in vessel walls and in granuloma of WG-patients\textsuperscript{48}. This trophic factor promotes the conversion of the CD45 isoform of T-cells from CD45RA (naive cells) to CD45RO (memory cells) and prevents T-cells from spontaneous apoptosis by downregulating Fas-expression on activated T-cells\textsuperscript{49}. Therefore, it can be argued that the increase and survival in the TEM pool in WG-patients could be related to enhanced production of IGF-1. Moreover, CTLA-4
expression can prevent apoptosis in activated T-cells by upregulation of the Bcl-2 anti-apoptotic protein. Indeed, upregulation of CTLA-4 has been detected in T-cells from WG-patients which provides some clues to understanding the increased survival of T_{EM} cells in this disease.

Another plausible explanation for the expanded T_{EM} population is indicated by the fact that the variant of the protein tyrosine phosphatase 22 gene (PTPN22) has been shown to be a risk factor for WG. The PTPN22 protein acts as a powerful inhibitor of T-cell activation via inhibition of key molecules downstream of the T-cell receptor. Variation in the PTPN22 gene may be related to the potential for persistent T-cell activation and proliferation toward effector memory cells in patients with WG.

**CD4^{+} T_{EM} cells as a target for therapy**

Several studies in patients with autoimmune disorders have supported a pathogenic role of autoreactive CD4^{+}T_{EM} cells in disease expression. Therefore, strategies designed to specifically target the autoreactive CD4^{+}T_{EM} cells without impairing the function of other lymphocyte subsets might have value in the treatment of autoimmune diseases. Beeton *et al.* have demonstrated that the voltage-gated Kv1.3 K^{+} channel is a specific functional marker for T_{EM} cells. They found that the disease-associated autoreactive T-cells from patients with T1DM or RA are mainly CD4^{+}T_{EM} cells expressing a high level of the Kv1.3 K^{+} channel. Therefore, Kv1.3 channels can serve as a novel therapeutic target for immunomodulation of autoreactive T_{EM} in autoimmune disorders. Indeed, selective inhibition of Kv1.3 channel effectively prevented and restored autoimmune disease in EAE and suppressed delayed-type hypersensitivity (DTH) in rats. Importantly, specific Kv1.3 blockers suppress proliferation and cytokine production of autoantigen-specific T_{EM} clones from T1DM-patients and RA-patients. Thus, suppression of Kv1.3 would appear a good approach to modulate pathologic immune responses mediated by autoreactive T_{EM} cells. This may hold therapeutic promise for ANCA-associated autoimmune disease, but further studies are needed to assess this approach.
Conclusion

The specific role of T^{EM} cells in the pathogenesis of systemic vasculitis is still unclear, and further studies are needed to elucidate the exact contribution of CD4^{+}T^{EM} cells to tissue injury. Based on our findings and published reports of others, we propose the following scenario: due to impaired function of T^{Reg} cells, autoreactive CD4^{+}T-cells may escape immune regulatory mechanisms and undergo repeated autoantigenic stimulation. Autoantigens such as PR3 and MPO or superantigens from S. aureus act as stimulators of specific T-cells leading to accelerated differentiation of CD4^{+}T_{Naive} cells toward T^{EM} cells. Upon local upregulation of adhesion molecules and chemokine receptors, these T^{EM} cells migrate and accumulate in inflammatory areas and contribute to granuloma formation and tissue damage. Therefore, suppression of autoreactive T^{EM} cells could be an important goal in the treatment of ANCA-associated vasculitis.

REFERENCES


