



## CKJ REVIEW

# T-lymphocyte in ANCA-associated vasculitis: what do we know? A pathophysiological and therapeutic approach

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## ABSTRACT

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is an autoimmune condition that commonly causes kidney impairment and can be fatal. The key participation of B-lymphocytes as ANCA producers and neutrophils as target of these antibodies is widely described as the mechanism of endothelial damage in this disease. There has been a rising interest in the role of T-lymphocytes in AAV in recent years. Evidence is strong from animal models, and T-lymphocytes can be found infiltrating kidney tissue and other tissue sites in AAV patients. Furthermore, the different subsets of T-lymphocytes are also key players in the aberrant immune response observed in AAV. Polarization towards a predominant Th1 and Th17 response in the acute phase of the disease has been described, along with a decline in the number of T-regulatory lymphocytes, which, in turn, show functional impairment. Interactions between different T-cell subsets, and between T-cells and neutrophils and B-cells, also enhance the inflammatory response, constituting a complex network. Novel therapies targeting T-cell immunity are emerging in this scenario and may constitute an interesting alternative to conventional therapy in selected patients. This review aims to summarize the available evidence regarding T-cell imbalances and functional impairment, especially focusing on renal involvement of AAV.

**Keywords:** ANCA, crescentic glomerulonephritis, cytokine, glomerulonephritis, immunology, T-lymphocyte

## INTRODUCTION

Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) affects small and medium-sized vessels [1]. Renal involvement is frequent. Although ANCA trigger an immune reaction, glomerular damage is pauci-immune, given the scarce

deposition of immune complexes [2]. Several lines of evidence for the implication of T-lymphocytes in AAV exist. Firstly, biopsies from AAV patients show the presence of extensive T-cell infiltrates in different renal compartments. Secondly, studies in animal models show attenuation of histological lesions in the

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absence of different T-cell subsets, and a 20-amino acid region (MPO<sub>409-428</sub>) was identified as an immunodominant T-cell epitope by Ooi et al. in a murine model of myeloperoxidase (MPO)-induced glomerulonephritis [3]. Finally, amelioration of the disease after T-cell-directed therapies suggests a role for T-cells in the pathogenesis [4].

Here, we aim to review the recent evidence regarding the imbalance of T-cells, their related functional impairment and their therapeutic implication in AAV.

## T-LYMPHOCYTES: IMBALANCES AND DYSFUNCTION IN AAV

### Cytotoxic T-lymphocytes

CD8<sup>+</sup> T-lymphocytes, classically named cytotoxic, are important in the defence against intracellular pathogens and cancer. After recognition of peptides presented by major histocompatibility complex (MHC) class I, they cause cellular death by cytotoxicity via induction of apoptosis and secretion of the pro-inflammatory cytokines tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon-gamma (IFN- $\gamma$ ) [5]. Available data from preclinical models, and also in human AAV, point to CD8<sup>+</sup> T-cells as playing a direct role in tissue damage in AAV, at least in part, together with other innate immunity cells [6].

In a murine model of anti-MPO glomerulonephritis, Chang et al. showed that CD8<sup>+</sup> T-cell blockade diminished the extent of histological lesions and renal impairment. They also proved that CD8<sup>+</sup> T-cells selectively caused glomerulonephritis when transferred to mice with kidney-planted MPO antigen [7].

In human AAV, the proportion of activated CD8<sup>+</sup> T-cells is elevated, according to various authors [6, 8, 9]. McKinney et al. explored blood CD8<sup>+</sup> T-cell gene expression profile in AAV. They found that the higher expression of a particular subgroup of genes related to T-cell survival and expansion of memory CD8<sup>+</sup> T-cells was strongly associated with a shorter time to relapse after induction of remission treatment. Those upregulated genes were specifically implicated in interleukin (IL)-7 and T-cell receptor (TCR) signalling pathways. If these findings are validated in prospective studies and can be adapted into a clinically viable test, then this could help to identify those patients with a greater propensity to relapse, thus enabling therapy customization [10]. Kidder et al., in a T-cell marker validation study including 38 renal biopsies from AAV patients, found an increased number of CD8<sup>+</sup> T-cells in the periglomerular area and interstitium in kidneys with crescentic histology, and a significant correlation between the number of CD8<sup>+</sup> T-cells and the glomerular filtration rate. Despite the low number of T-lymphocytes infiltrating the glomeruli, CD8<sup>+</sup> T-cells predominated over CD4<sup>+</sup> T-cells [11].

Mechanistically, the importance of the CD8<sup>+</sup> T-cell subset may reside in their capability to activate polymorphonuclear (PMN) cells [6]. PMN cell activation is a key event in the pathogenesis of AAV that leads to exposure of proteinase 3 and MPO on their surface, allowing their recognition by ANCA [1]. IFN- $\gamma$  secreted by CD8<sup>+</sup> T-cells during the acute phase of AAV is a potent activator of PMN cells, thus explaining the contribution of the CD8<sup>+</sup> T-cell subset to disease pathogenesis.

### Helper T-lymphocytes

CD4<sup>+</sup> T-cells, classically named T-helper cells, orchestrate the adaptive immune response. They activate B-cells, macrophages and cytotoxic T-cells via a wide range of cytokines that, in turn,

constitute the signature of every T-helper cell subset [12]. As ANCA production is T-cell-dependent, B-cells require stimulation from previously activated T-helper lymphocytes to produce antibodies. After CD4<sup>+</sup> T-cells recognize antigens exposed by antigen-presenting cells and become activated, they are able to activate, in turn, B-cells that bind the same antigen on their B-cell receptor [13] (Figure 1). B-cell activation leads to proliferation, isotype switch and antibody secretion.

In the MPO-induced glomerulonephritis murine model, CD4<sup>+</sup> T-cell depletion diminished the extent of the disease [14]. *In vitro* proliferation and ANCA production [15] of peripheral blood mononuclear cells (PBMCs) from AAV patients in response to MPO stimulation disappeared after CD4<sup>+</sup> T-cell depletion. The beneficial effects of the use of anti-thymocyte globulin (ATG) in patients with AAV for induction of remission also constitute proof of concept [16].

Next we will further discuss the reported evidence of imbalances and functional disturbances in the different subsets of T-helper cells in AAV. Controversial results have been found in this setting, with a changing predominance of the different subsets, depending on the AAV phenotype and disease activity.

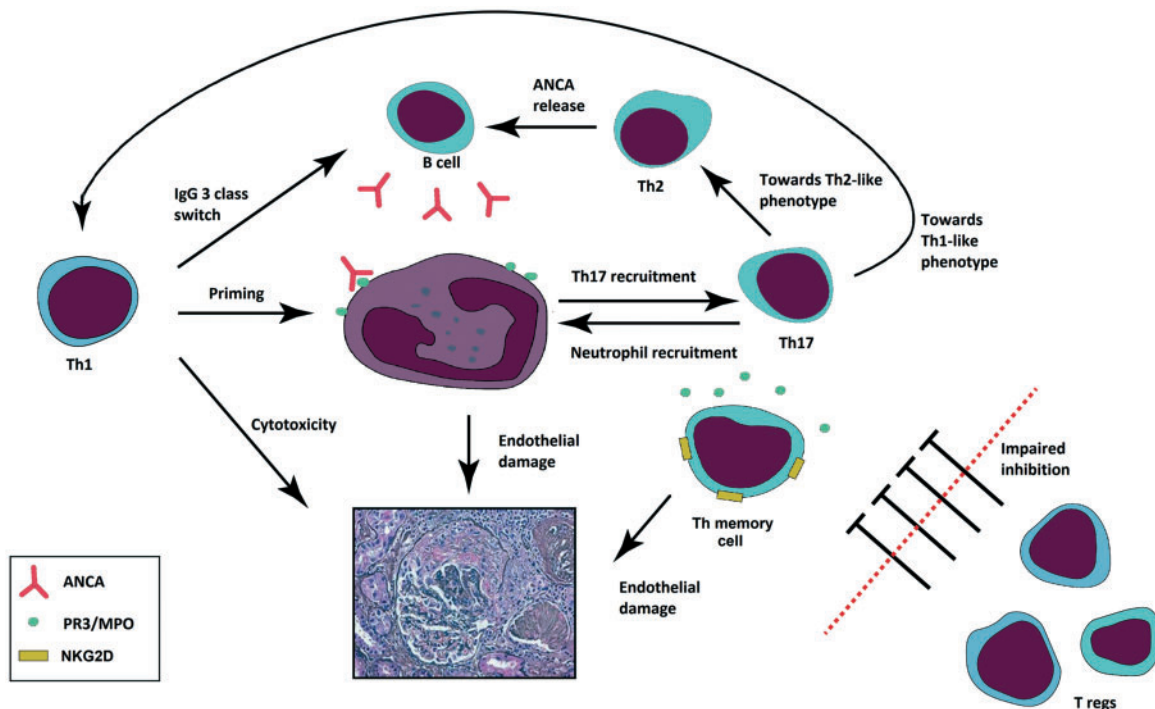
### Th1/Th2 subset imbalance

Th1 cells release IL-2, IFN- $\gamma$  and TNF- $\alpha$  after activation, trigger macrophages and cytotoxic T-cells, as part of the intracellular pathogen defence, and stimulate immunoglobulin G (IgG) class switch by B-cells [10]. On the other hand, Th2 cells release IL-4, IL-5, IL-10 and IL-13 after activation and stimulate B-cells, constituting the main defence against extracellular pathogens.

Available data point to a predominance of the Th1 immune response in AAV, especially during the acute phase. Masutani et al. found a higher peripheral Th1/Th2 ratio in AAV acute patients compared with controls, that significantly decreased in remission, and a higher tissue expression of IFN- $\gamma$  in kidney biopsies [17]. Abdulahad et al. described a predominance of the Th1 subset during the acute and remission phases of the disease, but in particular they observed a polarization towards Th2 response in remission in the memory cell population [18]. Popa et al. stimulated PBMCs from AAV remission patients with proteinase 3 and found a predominance of Th2 ILs in the supernatant, with very low IFN- $\gamma$  levels [19]. Szczeklik et al. also found higher peripheral Th2 cell counts during remission in AAV patients [20], in line with the higher plasma levels of CCL22 produced by this subset, as observed by Eriksson et al. [21].

Th1 polarization in the acute phase is related to an altered co-stimulation pattern. CD28 co-stimulation promotes the differentiation of naïve T-cells to a Th2 profile, whereas the absence of this co-stimulatory signal and an increased B7 (CD80/86) expression, both features present in AAV, as will be later discussed, should lead to differentiation to a Th1 profile [22]. Interestingly, IgG3 is the strongest immunoglobulin subclass inducing neutrophil activation, and the IgG switch to IgG3 mainly depends on Th1 induction [23]. On the other hand, authors hypothesize that polarization to a Th2 response in the remission phase represents an imbalance recovery [20] or a poorer susceptibility to immunosuppressive therapy of this T-cell subset [24].

Additionally, differences in Th1/Th2 imbalance have been described, depending on the clinical phenotype or extent of the disease. Müller et al. suggested that in granulomatosis with polyangiitis (GPA), a predominant Th1 response is found in patients with localized disease, based on the findings of higher IFN- $\gamma$  expression both in nasal biopsies and in PBMCs in patients with localized disease compared with those with



**FIGURE 1:** Proposed role for T-lymphocytes in AAV. The neutrophil is the key cell in AAV. After neutrophil priming and surface exposure of proteinase 3 or MPO, ANCA produced by B-lymphocytes are able to bind to their target antigen. ANCA binding fully activates neutrophils and causes degranulation. Endothelial damage and further inflammation are promoted by substances contained inside neutrophilic granules. Th1 response also results directly in cytotoxicity. In addition, Th1 response causes neutrophil priming and IgG3 class switch in B-cells, which constitutes the main antibody class found in AAV. Th2 response is less prominent in the acute phase of AAV and promotes antibody formation. Th17 stimulates further neutrophil recruitment to sites of tissue damage, thus promoting and amplifying the inflammatory response. Cross-talk between Th17 and neutrophils results in a feedback loop, with neutrophils, in turn, stimulating Th17 infiltration. Depending on the cytokine milieu, Th17 cells can acquire either a Th1- or a Th2-like phenotype, thus exhibiting high plasticity. Persistent exposure to antigen promotes the expansion of the T-helper lymphocyte memory subset. Aberrant expression of NKG2D in a subgroup of these effector memory T-cells has been described, conferring NK-cell-like cytotoxicity against endothelial cells, thus contributing to tissue damage. Treg inhibition of the immune response is impaired during the acute phase of AAV and tends to recover during remission, thus allowing an uncontrolled auto-inflammatory response.

generalized disease [25]. The authors hypothesized that in generalized GPA, a delayed-type hypersensitivity response related to Th2 is of greater importance [26], even though these findings have not been reproduced in other GPA cohorts [18]. Regarding eosinophilic GPA (EGPA), predominance of the Th2 response has been documented, which may indicate underlying aetiopathogenic differences with a major role of allergic response [27].

In summary, the bulk of the evidence points to a predominance of Th1 response, which probably enhances neutrophil activation that occurs early in the development of the disease. Meanwhile, in the remission phase, this polarization reverts, such that Th2 is the main participating T-cell subset.

### Th17 subset

The Th17 T-helper subset is involved in defence against extracellular bacteria and fungi, and is implicated in autoimmune diseases [28]. Th17 cells express the transcription factor ROR $\gamma$ 3 and produce IL-17A-F, mainly IL-17A. IL-23 is required by the Th17 subset to expand and mature [29]. In turn, Th17 cells are inhibited by Th1 and Th2 cytokines.

Various authors proved the role of IL-17-producing cells in a murine model of crescentic glomerulonephritis. IL-17 induces the renal expression of C-X-C Motif Chemokine Ligand 5 (CXCL5) by the tubular epithelium that is responsible for neutrophil attraction [30]. Gan *et al.* compared the histological lesions and kidney function parameters in IL-17 and IL-17 receptor knock-out mice with wild-type specimens in an MPO-induced

glomerulonephritis model. Knock-out animals showed less histological damage and better renal function compared with wild-type mice, suggesting a pivotal role of Th17 cells in the disease [31].

Given that neutrophils have a prominent role in AAV, the Th17 cell subset has also been a focus of attention in recent years because of the interplay between these two cell types. Expansion of the Th17 subset has been described in the setting of the acute phase of AAV, with a decline in early remission. Nogueira *et al.* demonstrated significantly higher levels of serum IL-17A in a cohort of 28 AAV acute patients and 65 AAV convalescent patients compared with healthy controls, with serum IL-23 levels following the same pattern [32]. Szczeklik *et al.* found higher proportions of circulating Th17 cells in AAV patients compared with healthy controls, by flow cytometry [20]. Specificity of this Th17 response against proteinase 3 was evaluated by Abdulahad *et al.*, who found a higher percentage of activated Th17 cells after stimulation with proteinase 3 in a cohort of 29 AAV patients in remission compared with healthy controls, as well as higher levels of IL-17 in the supernatant of stimulated AAV lymphocytes [33]. Additional results from Nogueira *et al.* were in agreement with these studies, describing higher percentages of IL-17-producing cells after stimulation with proteinase 3 or MPO, using the ELISPOT method, in a group of 17 AAV patients compared with healthy controls [32]. By contrast, other authors did not find evidence of this expanded Th17 response in AAV [20, 21, 34]. It may be hypothesized this is due to a high susceptibility of this cellular subset to steroids, as a

possible explanation [35], or due to the variety of cytokines in the IL-17 family. For instance, Krohn *et al.* found no differences in serum IL-17A levels from 70 AAV patients compared with healthy controls, but interestingly they found a significant elevation in IL-17C levels [36].

Whether the Th17 subset is the main source of production of IL-17 in AAV is also controversial. Velden *et al.* demonstrated the presence of IL-17 in an immunohistochemistry study including 22 AAV diagnostic kidney biopsies. Co-localization of the cytokine with other cellular markers identified neutrophils as the main source of IL-17, followed by mast cells and lastly by Th17 lymphocytes [37].

Under certain circumstances and in certain cytokine milieus, Th17 cells undergo epigenetic modifications, thus showing high plasticity and a capability of altering their phenotype and function. Under stimulation by IL-12 or IL-1 $\beta$  (produced by antigen-presenting cells in the presence of danger molecules), Th17 cells express IFN- $\gamma$  and acquire a Th1-like phenotype. If stimulated by IL-4, they convert into cells with a Th2-like phenotype. Along the same line, under IL-1 $\beta$  and IL-6 stimulation, T-regulatory lymphocytes (Tregs) (see T-regulatory subset section) can acquire a Th17-like phenotype and produce IL-17 [38]. However, it seems that in immune-mediated kidney diseases, Th17 cells display minimum plasticity compared with other autoimmune conditions [39]. In AAV, the proportion of Th1-like Th17 cells is higher in the acute phase of the disease or in the remission phase of the disease with no treatment compared with healthy controls, but the significance of this subset needs to be further studied [40].

Knowledge of the existence of the Th17 subset is relatively recent. The role of Th17 cells in the development of AAV and their interaction with other immune cells remain to be fully elucidated, although knock-out murine models prove that this role certainly exists. Linking with the pathogenesis of AAV, what is clear at the moment is that by induction of a variety of chemokines, the Th17 subset enhances the recruitment of neutrophils to the inflammation site and contributes to organ damage in AAV. This cross-talk is reciprocal, and neutrophils also are able to induce chemotaxis of Th17 cells, which makes this cellular axis even more interesting as a possible target in the treatment of AAV, similar to other inflammatory conditions [41].

### T-regulatory subset

Tregs inhibit T-effector cells and are responsible for antigen-specific peripheral tolerance and autoimmunity prevention [5]. Tregs are defined as CD4+CD8-CD25 (IL-2R)+FOXP3+ T-cells. FOXP3 is a transcription factor indispensable for Treg development and function. The role of Treg disturbances in the pathogenesis of crescentic glomerulonephritis has been proven in animal models. Tan *et al.* reduced the Treg population in a murine model of MPO-induced glomerulonephritis by the administration of an anti-CD25 antibody. They found a higher proportion of MPO-specific Th1 and Th17 cells, higher kidney functional impairment and more severe histological lesions compared with vehicle-treated animals [42]. Along the same line, Paust *et al.* generated mice lacking CXCR3 specifically in Tregs, which is crucial for Tregs to migrate to inflammation sites. These mice showed enhanced kidney impairment and more severe histological lesions with lower Treg infiltration compared with wild-type mice, after induction of nephrotoxic nephritis [43].

Various authors described a higher proportion of Tregs in patients during the acute phase of AAV, with a decrease when

remission was reached [34, 44]. In addition, Morgan *et al.* found that patients with a higher Treg count at presentation entered into earlier remission compared with those with a lower Treg count, as well as a negative correlation between Tregs and disease relapse rate, suggesting that higher numbers of Tregs suppress AAV activity [45]. Opposite results have been reported by other authors, who observed a depletion of Tregs in the acute phase and progressive expansion in the remission phase, together with a negative correlation between Tregs and activity markers [20]. At tissue level, Yoshimura *et al.* correlated a lower FOXP3 expression by immunostaining of AAV renal biopsies with the requirement for maintenance dialysis, suggesting that lower Treg infiltration translates into higher disease activity [46].

Various proliferation studies proved diminished suppressor function of Tregs in AAV patients compared with healthy controls [34, 45, 47], although with occasional increase in the proliferation of T-effector cells after interaction with Tregs [47]. Morgan *et al.* found that Treg function was impaired, particularly in patients who remained persistently ANCA-positive over time compared with those who became ANCA-negative [45], consistent with a higher degree of activity of the disease. The mechanistical explanation for Treg impairment lies in the higher resistance of T-effector cells to Tregs and Treg hypofunctionality, associated with the expression of a FOXP3 isoform lacking exon 2, which contains a crucial domain for its suppressive properties [44].

In summary, probably both population imbalances and functional impairment of Tregs in AAV lead to an uncontrolled inflammatory state that relieves and stabilizes in the remission phase of the disease. Controversy regarding the number and functionality of Tregs in AAV is, in part, due to the changing gating strategies used to identify those cells, the heterogeneity of the disease stages and the differences in treatment of the patients included in the studies [34], so conclusions should be drawn cautiously.

### Memory T subset

Memory T-cells are a subset of T-cells that previously encountered their specific antigens, and thus they are also called 'antigen-experienced T-cells'. They have the capability of quickly expanding in number and activating after re-exposure to the antigen. They are CD4+ or CD8+, and usually express CD45RO [48].

Of interest is the finding of an increased population of effector memory T-cells with a parallel decrease in the naïve T-cell population, reflecting hyperactivation in the setting of continuous exposure to auto-antigens in AAV [33]. During the remission phase of the disease, persistent expansion of CD4+ T-cells with an effector memory phenotype has been described, together with a decrement in the amount of circulating CD4+ naïve cells. Strikingly, during a disease flare-up, a decrease in CD4+ effector memory T-cells has been observed [49, 50]. In line with this finding, Abdulhad *et al.* hypothesized that this phenomenon is due to migration of those cells to sites of tissue damage. They observed the presence of infiltrating CD4+ effector memory T-cells in renal biopsies and also in urinary sediments from patients who had experienced a disease flare-up, which disappeared when remission was reached again [51].

In summary, regarding T-cell subsets, virtually all have been found to be expanded. Various authors have reported an expansion of Th1 and Th17 effector memory T-cells in GPA and microscopic polyangiitis, and an expansion of Th2 and Th17 effector



memory T-cells in patients with EGPA [52]. Aberrant expression of Natural Killer Group 2D (NKG2D) in a subgroup of these effector memory T-cells has been described, conferring a natural killer (NK) cell-like cytotoxicity against endothelial cells, and thus contributing to tissue damage [53].

### Follicular T-helper subset

Follicular T-helper (T<sub>fh</sub>) cells are required for the development of the germinal centre where B-lymphocyte antigenic affinity undergoes maturation. The role of these cells is being investigated in autoimmunity and seems to be more prominent in diseases with a higher participation of autoantibodies. Altered T<sub>fh</sub> cells may lead to the generation of aberrant autoantibodies and the formation of ectopic follicles [54].

Abdulahad *et al.* identified increased circulating T<sub>fh</sub> cells, identified as BCL6+ IL-21-producing cells, compared with healthy controls. The percentage of these cells was significantly higher in ANCA-positive, compared with ANCA-negative, patients. Moreover, IL-21 enhanced spontaneous ANCA production in cultures of PBMCs from ANCA-positive patients compared with ANCA-negative patients [55]. This way, the expanded population of circulating T<sub>fh</sub> cells may be collaborating in the production of ANCA autoantibodies, which constitutes the trigger for the initiation of an inflammatory response in AAV.

## ALTERATIONS IN T-LYMPHOCYTE ACTIVATION IN AAV

T-lymphocytes express TCR. Ninety-five per cent of lymphocytes express  $\alpha\beta$ -chain TCR, and 5% express a variant  $\gamma\delta$ -chain TCR [5]. The first signal for T-cell activation consists of TCR recognition of peptides presented by MHC molecules. The second signal for activation of effector T-cells consists of the binding of CD28 to B7. By contrast, CTLA-4 binding to B7 inhibits this activation.

Differential expression of TCR genes in the variable domain of the  $\alpha$  and  $\beta$  chains has been found in AAV patients [56], with an increased expression of the V2.1 gene in the  $\beta$  chain compared with healthy controls [57]. T-cells carrying  $\gamma\delta$ -chain TCR are involved in innate host defence and can produce IL-17. The role of these IL-17-producing,  $\gamma\delta$ -chain TCR-expressing T-cells in AAV has been addressed in murine models [58, 59]. In  $\gamma$  and  $\delta$  chain knock-out murine models, structural and glomerular injury is attenuated. Limited evidence exists on the role of the  $\gamma\delta$  T-cell subset, but interestingly the presence of infiltrating  $\gamma\delta$  T-cells in biopsies from AAV patients has been observed, in contrast to healthy controls [60], and the authors hypothesized this could lead to disturbances in antigen recognition. In the case of IL-17-producing,  $\gamma\delta$ -chain TCR-expressing T-cells, it may be related to the neutrophil attraction function of this IL.

CD28 expression decreases in AAV patients, thus resulting in resistance to anergy [6, 61]. A negative correlation of the percentage of CD28+ cells with disease activity has been established [61]. CD4+CD28- T-cell infiltrate is present in the nasal tissue of AAV patients with upper respiratory tract involvement [62]. CTLA-4 is overexpressed in AAV patients compared with healthy controls [63], and certain CTLA-4 polymorphisms have been correlated with a higher susceptibility to the development of AAV [64]. Whether it also preserves inhibitory functions remains to be elucidated [65].

By contrast, IL-2 binding to IL-2R is the second signal for activation of Tregs [5] and is critical to their survival and functionality. Wilde *et al.* showed a lower expression of the  $\beta$  subunit of IL-

2R (CD122) compared with Tregs from healthy controls [66]. Altogether, altered co-stimulation of T-cells in AAV promotes effector T-cell anergy and causes impaired activation of Tregs, which, in turn, contributes to a more pronounced pro-inflammatory state.

## T-LYMPHOCYTES AS A THERAPEUTICAL TARGET IN AAV

T-lymphocytes constitute an interesting therapeutic target, given their prominent role in the pathogenesis of the disease. Reported therapies along this line are directed to abolish the T-cell response, either by cell depletion or by targeting their cytokines. Classical therapies such as use of cyclophosphamide or corticosteroids affect T-lymphocytes. Cyclophosphamide causes decreased B- and T-helper lymphocyte counts, and consequently an increased CD4/CD8 ratio that can persist for years [67]. Naïve T-lymphocytes are especially sensitive to cyclophosphamide [68]. Similarly, prednisone decreases the circulating T-cell count, particularly CD4+ T-cells, with a minor effect on B-cell count [69].

### Lymphocyte-depleting antibodies

The humanized anti-CD52 monoclonal antibody alemtuzumab selectively depletes lymphocytes and has been shown to be effective in other systemic vasculitides such as Behçet's disease [70]. Incomplete recovery of the CD4+ population and predominance of Tregs occur in remission and may benefit disease control [71]. Walsh *et al.* treated 71 patients with refractory or relapsing AAV with alemtuzumab and found it useful to achieve remission with a lower relapse rate [72].

ATG is a mix of polyclonal antibodies against lymphocytes. ATG is useful in the setting of refractory or relapsing AAV [16, 73]. The largest cohort described showed 13 partial or complete disease responses among 15 AAV patients [73]. Thus, ATG may be considered in non-responders to conventional therapies despite its side effects.

### Cytapheresis

Cytapheresis removes specific leucocytes from blood, using special columns that precisely deplete these cells [74], and is effective in AAV [75, 76]. Hasegawa *et al.* compared cytapheresis plus low-dose corticosteroid treatment in AAV with kidney involvement against treatment with corticosteroid pulses, and they found a similar renal recovery rate with lower 1-year mortality in the cytapheresis group [77]. In a Japanese study, 53 out of 715 AAV patients were treated with cytapheresis. Survival benefit was observed in those with pulmonary renal syndrome and higher C-reactive protein (CRP) levels, as well as a faster ANCA titre decline [78]. Reduction in pro-inflammatory cytokines is the underlying mechanism for the usefulness of cytapheresis [79].

### Haematopoietic stem cell transplantation

Haematopoietic stem cell transplantation (HSCT) is an emerging therapy in low-responding patients with autoimmune diseases. Low mortality and high efficacy have been reported. This therapy alters the disease course and leads to *de novo* T-cell reconstitution. Four refractory AAV patients achieved partial or complete response after HSCT, without treatment-related mortality, as reported by Daikeler *et al.* [80].

## Rituximab

Rituximab is an anti-CD20 monoclonal antibody that causes B-cell depletion and consequently acts on the pathophysiology of the disease, leading to a dramatic fall in ANCA titre. The efficacy and safety of this therapy in the induction of disease remission have been assessed in the RITUXVAS (Rituximab versus cyclophosphamide in ANCA-associated vasculitis) and RAVE (Rituximab in ANCA-Associated Vasculitis) trials [81, 82].

Rituximab leads also to T-cell impairment. B-cell depletion causes a reduction in T-cell-stimulating cytokines produced by B-cells, and consequently a reduction in peripheral circulating T-cells [83]. Moreover, a small population of T-cells also express CD20, constituting a direct target for this therapy [84]. Neél et al. found that rituximab inhibited CD8+ memory T-cell expansion in a group of AAV patients, in contrast to conventional immunosuppressive therapy, with no effects on the CD4+ T-cell population [85]. Regarding Tregs, Zhao et al. demonstrated a repopulation of this subset in patients treated with rituximab compared with AAV patients treated with conventional therapy, which may also contribute to remission [86].

## Abatacept

Abatacept contains the extracellular domain of CTLA-4, which blocks CD28-B7 interaction, given CTLA-4 affinity for B7. This co-stimulation blockade inhibits T-cell activation.

Abatacept was administered to 20 patients with non-severe relapsing AAV, showing a low relapse incidence rate during follow-up [87]. A randomized controlled trial is currently ongoing, evaluating the efficacy of abatacept in sustaining AAV relapse in a steroid-free regimen compared with conventional therapy [88].

## Etanercept

TNF- $\alpha$  is involved in the pathogenesis of AAV. The WGTE (Wegener's Granulomatosis Etanercept Trial) trial evaluated the efficacy of etanercept in maintaining disease remission. Etanercept was not useful as maintenance therapy and showed a worse adverse effect profile [89], particularly neoplasms [90].

## Th17 blockade

Antibodies directed against IL-17 and IL-23 (secukinumab and ustekinumab, respectively) are effectively used in other autoimmune conditions such as psoriasis [91], but no current studies are being conducted in vasculitis yet, despite the existence of a clear rationale, as described above.

## Immunomodulatory therapy

Induction of tolerance by nasal inhalation of MPO at low doses increases Treg response and attenuates kidney damage in a murine model of MPO glomerulonephritis. Although no evidence exists in human vasculitis, an immunomodulatory therapy may be effective, based on the high homology between mouse and human MPO protein [92].

## CONCLUSIONS

Characterization of the role of T-cells in AAV has helped to further clarify the pathogenesis of AAV. Although multiple inflammatory pathways are involved, auto-reactive T-cells are implicated both in the initiation and in the organization of the

immune response, with population imbalances, as well as functional impairment. T-cells constitute an attractive therapeutic target for the treatment of AAV.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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