T-Lymphocytes and Disease Mechanisms in Wegener's Granulomatosis

Fan Hua Benjamin Wilde Sebastian Dolff Oliver Witzke

Department of Nephrology, University Duisburg-Essen, Essen, Germany

Key Words

Anti-neutrophil cytoplasmic antibodies • T-cells •
Regulatory T-cells • Vasculitis • Wegener's granulomatosis

Abstract

The mechanisms underlying Wegener's granulomatosis (WG) are not well understood. The role of T-cells in the pathogenesis of WG has only recently come into focus of research. This review presents recent developments regarding the role of T-cells in WG. The occurrence of anti-neutrophil-cytoplasmic antibodies (ANCA) directed against proteinase-3 (PR-3) is a hallmark of WG. ANCA seem to mediate vasculitic damage in WG. Apart from ANCA, T-cells are involved in disease mechanisms. T-cells might participate in ANCA formation. Furthermore, T-cells are observed in affected tissue and granulomatous lesions. T-cells are indispensable for granuloma formation in other diseases and this might apply to WG too. In line with this, several aberrations of T-cell populations and alterations of the T-cell response were recently discovered in patients suffering from WG. Therefore, the impact of T-cell polarization, genotypic alterations modifying T-cell function and specific T-cell subsets on disease pathogenesis is discussed. Moreover, the influence of *Staphylococcus aureus* on T-cells and self-tolerance in WG is further elucidated. Finally, therapeutic options and implications with regard to T-cell-mediated pathogenesis are highlighted.

Copyright © 2009 S. Karger AG, Basel

Introduction

Wegener's granulomatosis (WG) is a granulomatous-necrotizing small vessel vasculitis [1]. WG forms together with microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS), a disease complex named ANCA-associated vasculitis (AAV). All forms of AAV share common features like the presence of anti-neutrophil-cytoplasmic antibodies (ANCA). In WG, ANCA are usually directed against proteinase-3 (PR3) whereas patients with MPA and CSS have myeloperoxidase-specific ANCA. However, there are also cases reported in which ANCA were not detectable [2, 3]. Although MPA and CSS show the same class of ANCA, the type of inflammation is different. Like WG, CSS is a granulomatous vasculitis whereas MPA is not. If and in what way this is related to the different types of ANCA is not clear. AAV is a life-threatening disease and adequate treatment is essential [4].

Both the innate and adaptive immune system play a role in disease mechanisms of WG, although the pathogenesis is not well understood [5]. Basically, it is believed that ANCA have a pathogenic role in disease mechanisms: the interaction of ANCA with its target antigen causes degranulation of neutrophils resulting in endothelial and finally vasculitic damage. There is evidence from in vivo experiments on the pathogenicity of MPO-ANCA [6]. Transfer of anti-MPO IgG in rodent models leads to necrotizing crescentic glomerulonephritis whereas administration of anti-PR3 IgG fails to induce granulomatous inflammation and glomerulonephritis [6–9]. Nevertheless, there is some evidence pointing at a patho-

Table 1. Overview on different T-cell subsets important in WG

T-cell subset	Properties and phenotype	Function in WG	References
Naturally occurring regulatory T-cells (T _{reg})	CD4+CD25 ^{bright} FoxP3+CD127 ^{low}	Presumably defective in function, do not suppress sufficiently	Abdulahad et al. [49]
Induced regulatory T-cells (iT _{reg})	CD4+FoxP3–IL-10+ CD4+FoxP3+TGF- β	Not studied yet, mainly limit immune responses in a physiologic context	Piccirillo [51] Roncarolo et al. [52]
Naive T-cells	CD4+CD45RO–CD45RA+	Diminished in WG, skewing towards memory phenotype	Marinaki et al. [55]
Effector T-cells (T _{eff})		Displaying effector function, e.g. IFN- γ or TNF- α production	
Effector memory T-cells (T _{em})	CD4+CD45RO+CCR7– CD4+CD25+CD45RO+CD134+ CD4+CD25+CD45RO+GITR+	Expanded T-cell population, maybe driver for granuloma formation and vasculitis	Abdulahad et al. [23] Wilde et al. [59]
Terminally differentiated effector memory T-cells (T _{TEM})	CD4+CD45RO+CD28–CTLA4+	Abundantly present in granulomas, main source of proinflammatory cytokines	Lamprecht et al. [25, 26, 64] Hoff et al. [96]

genic role of PR3-ANCA in humans. For instance, several reports have shown an association of anti-PR3 titers and disease activity [10–12]. However, this could not be confirmed in a recent controlled trial conducted by Finkielman et al. [13] and thus remains controversial. The expression level of membrane-bound PR3 on neutrophils is reported to have impact on the relapse rate in WG [14]. Last but not least, B-cell-targeted therapy with rituximab seems to be efficacious in refractory disease and is accompanied by a decrease of autoantibody titer [15]. However, apart from autoantibodies and B-cells, T-cells play an important role in disease mechanism. The IgG subclass of ANCA indicates that a T-cell-dependent subclass switch has occurred [16]. In addition, granuloma-like formations are usually found in affected tissue. These granulomas are a source of autoantibodies [17, 18]. Granuloma formation is driven by T-cells and T-cell-derived cytokines [19, 20]. T-cells are found in these lesions suggesting a role in the inflammatory process of WG [21]. Furthermore, Schmitt et al. [22] reported a strong correlation of disease activity and markers of T-cell activation such as soluble IL-2 receptor. T-cells are reported to be persistently expanded in WG [23]. Further evidence on the role of T-cells in AAV is provided by Ruth et al. [7] in an MPO animal model. Glomerular crescent formation was reduced after CD4+ T-cell depletion. These facts and the capacity of T-cells to control and induce immune responses gave rise to investigations of the role of T-cells in WG.

Granuloma Formation and T-Cells in WG

Fienberg [24] described WG-associated granulomas in detail. Macrophages, neutrophils and lymphocytes form granulomas usually observed in WG. Interestingly, most of the T-cells residing within granulomas display features of terminally differentiated effector memory T-cells (T_{TEM}) (table 1) [25–27]. T_{TEM} show a downregulation of CD28 whereas the cytotoxic T-lymphocyte antigen-4 (CTLA-4) is overexpressed (table 1) [28]. Despite the regulatory functions of CTLA-4 in CD28-dependent T-cell activation, this molecule seems to confer protection against induced cell death by upregulation of anti-apoptotic BCL-2 as shown by Pandiyan et al. [29]. T-cells found in granulomas mainly show a Th1-cytokine pattern with secretion of IFN- γ and TNF- α [30]. A recent report showed that PR3 antigen elicits strong Th1-responses via dendritic cell maturation and subsequent antigen presentation to T-cells [31]. As granuloma formation is driven by Th1 responses, DC maturation and activation pathways might be important in disease pathogenesis. Intriguingly, PR3 antigen is usually present in granulomas and antigen-presenting cells (APC) are found in these lesions as well. Therefore, T-cells might be primed in granulomas. B-cells are also located in these lesions [17].

Thus, the question arises whether granulomas sustain the immune response in WG or even provide conditions favorable to overcome tolerance.

Genotypic Alterations in WG

Genetic background is proven for some of the autoimmune diseases. Some findings point at a genetic disposition for WG as well. The coding gene for CTLA-4 was studied extensively as this molecule plays a key role in the T-cell activation processes and shows an aberrant expression pattern in WG [27, 28]. CTLA-4 is able to shut down and limit T-cell activation. It is expressed on T-cells and binds to CD80/CD86 on APC. Distinct polymorphisms of the CTLA-4 gene are associated with WG and correlate with the renal outcome of patients [32]. Some of the polymorphisms associated with WG were also shown to impair the regulatory function of CTLA-4 [33]. Dinucleotide expansions within the CTLA-4 gene in WG might cause instability of mRNA and hence interfere with the expression as well as functionality of CTLA-4 [33–35].

A study on polymorphisms of the protein tyrosine phosphatase non-receptor type 22 (PTPN22) was published in 2005 [36]. PTPN22 is a tyrosine phosphatase that is involved in T-cell receptor signaling [37]. Jagiello et al. [36] revealed that a particular allele of the PTPN22 gene is a risk factor for WG. This specific allele is supposed to be less functional [36]. Enhanced proliferation of memory T-cells and formation of ectopic germinal centers in rodent animal models with complete deficiency of PTPN22 was demonstrated [38, 39]. Since similar phenomena such as memory T-cell expansion are observed in WG, a role for PTPN22 has to be considered [23].

Polymorphisms of genes coding for cytokines were studied in the past. Although TNF- α and IL-2 levels are usually increased in WG, no association with polymorphisms was found [40]. However, gene analysis for IL-10 revealed an increased frequency of a specific allele within the promoter region of IL-10 in WG [41]. This specific allele is associated with decreased production of the immunoregulatory cytokine IL-10 and thus might promote break of tolerance [42].

Disease Pathogenesis and Specific T-Cell Subpopulations

Regulatory T-cells (T_{reg}) and effector T-cells (T_{eff}) are major players in T-cell-mediated immunity (table 1). The role of both T_{reg} and T_{eff} in the pathogenesis of WG will be discussed below. T_{reg} and T_{eff} have different phenotypes and functions (table 1). T_{reg} control immune responses and limit inflammatory processes that are initiated and sustained by T_{eff} . Thus, T_{reg} seem to have a key

role in maintaining self-tolerance [43–47]. T_{reg} are usually found within the CD25+ T-cell population and express high levels of FoxP3 allowing differentiation from activated effector lymphocytes. T_{reg} depletion in animal models leads to autoimmunity and defective regulatory function was also seen in patients suffering from multiple sclerosis and rheumatoid arthritis. The role of T_{reg} has been investigated in WG. Marinaki et al. [48] assessed FoxP3 mRNA expression in T-cells from patients with AAV but did not find differences as compared with healthy controls. Abdulahad et al. [23] analyzed FoxP3 expression of effector memory T-cells (T_{em}) defined as CD4+CD45RO+CCR7– in WG (table 1). It was shown that patients in remission have a slightly higher frequency of FoxP3+ T_{em} accompanied by an increase of FoxP3– T_{em} than patients with active disease. Active patients did not show any differences in frequency of FoxP3+ T_{em} . Calculated ratios for FoxP3–/FoxP3+ T lymphocytes did not differ in comparison to healthy controls. Again, these findings did not point at a role for T_{reg} in the pathogenesis of WG. The latest report in this matter was published by Abdulahad et al. An increased frequency of CD4+CD25^{bright}FoxP3+ and CD4+CD45RO+CD25^{bright}FoxP3+ cells was detected in peripheral blood lacking correlation with clinical features. These T_{reg} were less efficient in inhibiting proliferation of responder T-cells than T_{reg} from HC [49]. The authors concluded that T_{reg} from WG patients are impaired in function. It has to be stressed that FoxP3 was recently reported to be transiently upregulated in activated lymphocytes [50]. Therefore, these findings need to be confirmed. Nevertheless, this is a first hint pointing at a role of T_{reg} in disease pathogenesis. A different subset of T_{reg} , so-called ‘induced regulatory T-cells’ (iT_{reg}), have not been studied yet in WG but may be impaired in function, too (table 1) [51, 52].

Apart from T_{reg} , T_{eff} have been investigated as well. T_{eff} are controlled by T_{reg} and have the capacity to promote and initiate immune responses. In general, all T-cells displaying effector functions belong to T_{eff} [53]. Thus, T_{em} and T_{tEM} are subsets of T_{eff} (table 1). It was shown earlier that the circulating T_{tEM} with no or little CD28 expression are expanded. T_{tEM} were also found in granulomas and are a major source of IFN- γ and TNF- α . The expansion is associated with disease progression and organ involvement [25, 30, 54].

Recent reports show a significant increase of whole T_{em} along with a decrease of naive T-cells in WG patients [23, 55]. The cause for the expansion of T_{em} has been investigated by several groups. Data from Czernok et al. [31] suggest a role for dendritic cells in initial stages of WG,

but increased expression of CTLA-4 followed by protection from induced cell death might contribute to T_{em} expansion as well. Persistent antigen stimulation could also favor expansion of the T_{em} compartment. A role for T_{reg} should be considered. Furthermore, differences between patients in remission and those with active disease were revealed. Patients in remission show higher levels of circulating T_{em} than patients with active disease. It is speculated that these T_{em} migrate from peripheral blood to sites of inflammation causing a decrease in circulating T_{em} during active disease [23, 31]. Memory T-cells also express receptors belonging to the tumor necrosis factor receptor (TNFR) superfamily (CD134 and glucocorticoid-induced tumor-necrosis factor receptor, GITR); these receptors are known to confer resistance against immunoregulation [56–58]. In WG, CD134+/GITR+ T-cell subsets are expanded and associated with disease activity. These T-cell subsets are also present in inflamed tissue and might contribute to tissue damage in WG [59]. However, the importance of these T-cells as markers of disease activity needs further investigation. The pathogenic potential of these T-cells has to be elucidated further.

As T_{em} and T_{tem} respond rapidly and establish a powerful immune response, they might account for relapsing disease course; a contribution to granuloma formation seems to be likely. New therapeutic options might arise from targeting these T-cells.

T-Cell Cytokines and Chemokines in WG

It is stated that there are differences in Th1/Th2 polarization depending on disease and disease stage [60–62]. Some authors support the idea that localized disease is associated with a Th1 response whereas a Th2 response is observed in systemic disease. Schonermack et al. [63] assessed sCD26 plasma levels as a marker for Th1 response in patients with localized and systemic WG. Levels were elevated in patients with localized disease suggesting a preferential Th1 response. Lamprecht et al. [64] compared CCR5 (Th1) and CCR3 (Th2) expression on T-cells from patients with WG. In localized disease, there was a higher expression of CCR5 as compared to CCR3 whereas both markers were expressed at equal levels in systemic disease. This was confirmed by observations made in biopsy studies. Muller et al. [65] characterized the cell infiltrates from nasal biopsies. These cells were mainly positive for CD26 and IFN- γ in localized WG indicating a preferential Th1 response. In systemic disease, an increase of IL-4 mRNA was detected. Accordingly,

Balding et al. [60] found upregulation of IL-4 and failed to detect IFN- γ in biopsies suggesting a Th2 response in systemic WG. In contrast to these studies, Csernok et al. [61] detected high levels of IFN- γ mRNA and low levels IL-4 mRNA in granulomas in systemic WG. The majority of granuloma-derived T-cells were IFN- γ producers. Hence, data on Th1/Th2 polarization is inconclusive.

TNF- α levels are also increased in WG [30]. T-cells found in granulomas and in the circulation are sources of TNF- α [30]. TNF- α acts as a proinflammatory cytokine on lymphocytes and neutrophils. It primes neutrophils, making them susceptible for ANCA-mediated degranulation and oxidative burst. Furthermore, it promotes upregulation of adhesion molecules on endothelial cells thus facilitating migration to the tissue. Thus, TNF- α is of particular importance promoting vasculitic inflammation in several different ways.

IL-17-producing T-cells (Th17 cells) are major players in autoimmunity [66–68]. IL-17 is suggested to be involved in autoantibody formation as well as in recruitment and activation of neutrophils [69, 70]. Little data is available on the role of IL-17 in WG. However, a skewed Th17 response observed in WG might contribute to disease pathogenesis [71].

In conclusion, there is evidence for a disturbed cytokine axis promoting inflammation.

Staphylococcus aureus-Induced Immune Alterations and WG

Nasal carriage of *S. aureus* and the staphylococcal toxic shock syndrome toxin-1 (TSST-1) are risk factors for new onset and relapsing disease [72, 73]. Anti-*S. aureus* treatment with cotrimoxazole reduces the rate of relapses [74]. Therefore, the impact of *S. aureus* on pathogenesis in WG is discussed below. A study of outstanding interest was conducted by Pendergraft et al. [75], who investigated the role of complementary proteins in WG. In brief, the amino acid sequence of a specific protein is generally coded by the 'sense' strand of DNA [76]. Transcribing and translating this information results in a 'sense' protein. The opposite strand of the coding DNA is the so-called 'anti-sense' strand. The anti-sense strand carries genetic code of the complementary peptide that is the counterpart of the 'sense' protein [76]. Pendergraft et al. [75] hypothesize that the initial immune response in autoimmunity is directed against complementary PR3 (cPR3), resulting in the formation of antibodies against cPR3 (idiotypic response). Later on, an anti-idiotypic response

against anti-cPR3 antibodies evolves. The antibodies formed during this anti-idiotypic response react to the sense autoantigen PR3. Indeed, Pendergraft et al. demonstrated the presence of anti-cPR3 antibodies in a minority of WG patients. These antibodies were specific for cPR3 and also bound specifically to anti-PR3. After having shown that anti-cPR3 and anti-PR3 antibodies from human WG sera form idiotypic pairs, mice were immunized with recombinant or synthetic human cPR3 in order to test the hypothesis in vivo. After immunization, anti-cPR3 as well as anti-PR3 antibodies were detected in mice supporting the idea of idiotypic and anti-idiotypic responses. Furthermore, Pendergraft et al. [75] searched for sources of cPR3 in WG patients. cPR3 transcripts were found in none of the healthy controls, ANCA-negative or lupus patients. However, cPR3 mRNA could be detected in leukocytes of PR3-ANCA patients. The origin of the cPR3 transcripts has not been unraveled so far. It could be of endogenous origin or exogenous origin, the latter introduced by pathogens. Indeed, genetic sequences complementary to human PR3 gene were detected in pathogens like *S. aureus*, Ross River virus and *Entamoeba histolytica* [75]. In conclusion, Pendergraft et al. provided substantial evidence for an involvement of cPR3 in disease mechanisms of WG; the idea is supported that microbial pathogens are of importance in different stages of autoimmunity.

Apart from classic ANCA, a different type reactive to lysosomal membrane protein-2 (LAMP-2) has been described by Kain et al. [77]. Anti-LAMP2 was present in WG patients with active disease and renal involvement; having entered remission, anti-LAMP2 was no longer detectable in the majority of patients. Kain et al. also provide evidence for in vitro and in vivo pathogenicity of anti-LAMP2. Anti-LAMP2 showed similar pathogenic potential in vitro as compared to anti-PR3. Shape change and degranulation of neutrophils was induced by anti-LAMP2; in addition, apoptosis could be induced in human vascular endothelial cell culture by adding this antibody [77]. Furthermore, LAMP2 shows homology to the bacterial protein FimH, which is involved in adhesion to epithelial cells and is usually found on common bacterial pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*. Therefore, the in vivo pathogenicity of anti-LAMP2 was tested in two different ways. First, anti-human rabbit IgG with specificity to LAMP2 were injected into Wistar-Kyoto (WKY) rats [77]. These rats developed focal necrotizing glomerulonephritis (FNGN). Thus, anti-LAMP2 antibodies seem to be pathogenic in vivo and promote FNGN. Then, in a second approach, WKY

Fig. 1. The cause for the expansion of the memory T-cell compartment (T_{em} and T_{iEM}) is not known; however, there are some factors that might contribute to the persistent expansion of T-cells. T_{reg} defective in function might fail to limit T_{em} expansion. In addition, persistent antigen stimulation might lead to ongoing activation and expansion of T-cells. Furthermore, CTLA-4 is overexpressed on T-cells in WG followed by enhanced protection from activation induced cell death. Therefore, sustained activation and expansion of T-cells is favored in WG. Next to this, DCs might have a role in T-cell expansion. The autoantigen PR3 promotes maturation of DCs; these DCs are capable of stimulating and activating autoreactive T-cells. In conclusion, not a single but many different factors might cause persistent T-cell expansion in WG.

rats were immunized using recombinant FimH fusion protein. Subsequently, antibodies to FimH could be detected. However, standard ANCA assays were also positive. Antibodies to FimH cross-reacted with rat and human LAMP2. Furthermore, these rats developed FNGN and in some cases hemorrhagic pulmonary vasculitis occurred. Therefore, immunization with FimH induces antibodies to LAMP2 and provokes vasculitis. Finally, this study by Kain et al. [77] suggests that bacterial infections with common bacteria such as *E. coli* might trigger the development of autoantibodies and thus promote pauci-immune glomerulonephritis.

Exotoxins from *S. aureus* were suggested to be responsible for persistent activation and expansion of T-cells [78]. Some of the bacterial exotoxins are able to activate lymphocytes by binding to $V\beta$ regions of the T-cell recep-

Fig. 2. T_{em} trigger granuloma formation. Granulomas provide an inflammatory environment that sustains inflammation and might facilitate break of tolerance. Granulomas are sources for proinflammatory cytokines and autoantibodies; whether B- and/or T-cell maturation takes place is not fully proven. PR3 is also present in granulomas. PR3 acts on DCs and promotes maturation. Autoreactive T-cells might be activated by these DCs. ANCA produced by granuloma-resident B cells bind to primed neutrophils that express PR3 on their surface. Subsequently, degranulation of neutrophils is caused provoking vasculitic damage.

tor independent of antigen specificity; these exotoxins are so-called superantigens (SAg). SAg bind to MHC class II on APC and to T-cell receptor on T-cells outside the antigen-binding groove [79]. Especially expansion and activation of autoreactive T-cells could contribute to autoimmune disease. Popa et al. [78] analyzed the frequency of $V\beta$ -expressing T-cells. Although T-cell subsets expressing $V\beta$ regions with affinity to SAg were expanded, no correlation with presence of SAg or *S. aureus* was seen.

Therapeutic Implications

There is a rationale for therapeutic lymphocyte depletion as T-cells contribute to disease mechanisms. For this purpose, anti-thymocyte globulin (ATG) was administered to patients with refractory disease in the context of clinical studies. At least partial remission could be induced, but in view of severe side effects also known from transplant studies, ATG treatment is not the first choice

of therapy [80–82]. An antibody (alemtuzumab) directed against CD52 (displayed on mature lymphocytes) was also used for T-cell depletion in some small studies with patients suffering from WG. Responses and even sustained remissions were achieved in these patients. In line with the experience on ATG administration, alemtuzumab was used only in an experimental setting because of the risk for infections, malignancies and allergic reactions. No sufficient long-term data is available [83–86].

Targeting cytokines is a more specific therapeutic opportunity. Anti-TNF- α biologics were used abundantly in many different autoimmune diseases. It is also administered in WG in selected cases. There are different forms of anti-TNF- α biologics: (1) etanercept is a fusion protein with two TNF- α receptors bound to the Fc part of a human IgG; (2) infliximab is a chimeric monoclonal IgG-antibody able to bind soluble and membrane-bound TNF- α , and (3) adalimumab is a human monoclonal IgG antibody with two binding sites for soluble or already bound TNF- α .

Etanercept was tested against a placebo in a large, controlled trial named WGET [87]. Either etanercept or the placebo was added to the standard therapy. In this trial, both arms were comparable with regard to induction of remission or relapse rate. Interestingly, the incidence of malignancies was higher in the etanercept arm [88]. Some small studies were conducted with infliximab; a total of 48 patients were included in these uncontrolled trials [89–92]. 38 patients received infliximab in addition to a standard treatment regimen. 33 of these 38 patients entered remission. However, a relapsing disease course was observed frequently. In one of these trials, 10 patients received sole infliximab treatment with corticosteroids; complete remission (5) and partial remission (5) were in-

duced [89]. Right now, no data is available on adalimumab. The efficacy of anti-TNF- α agents in WG remains uncertain. Additional controlled clinical trials are needed to assess the potency and safety of anti-TNF- α agents in WG.

Attenuation of T-cell-dependent immune responses by blocking the CD28/CD80 pathway is effective in animal models. The CTLA-4Ig was tested recently in human clinical trials on rheumatoid arthritis (RA). So far, CTLA-4Ig seems to be a promising therapeutic option for refractory disease in RA [93, 94]. Even though disease pathogenesis is not comparable to WG, T-cell activation and CD80 expression pattern is altered in AAV [27]. Hence, modifying costimulatory pathways might be a new and effective therapeutic approach [95].

Conclusion

T-cells contribute to disease mechanisms in WG. Both the pathogenesis as well as the key events leading to the break of tolerance have not been uncovered yet. Potentially, T_{reg} are not able to control T_{em} sufficiently. Thus, defective T_{reg} function, persistent antigen stimulation, protection from induced cell death and activated DCs might cause an expansion of T_{em} (fig. 1). Hence, T_{em} might be major players in disease mechanisms by promoting granuloma formation (fig. 2). Thereafter, granulomas provide a proper environment for a break of tolerance and sustained autoantibody as well as T-cell-mediated inflammation (fig. 2). Further efforts in unraveling the disease pathogenesis of WG will finally result in new and effective therapeutic strategies.

References

- 1 Bosch X, Guilabert A, Font J: Antineutrophil cytoplasmic antibodies. *Lancet* 2006;368:404–418.
- 2 Eisenberger U, Fakhouri F, Vanhille P, Beauvais H, Mahr A, Guillevin L, et al: ANCA-negative pauci-immune renal vasculitis: histology and outcome. *Nephrol Dial Transplant* 2005;20:1392–1399.
- 3 Reinhold-Keller E, de Groot K, Holl-Ulrich K, Arlt AC, Heller M, Feller AC, et al: Severe CNS manifestations as the clinical hallmark in generalized Wegener's granulomatosis consistently negative for antineutrophil cytoplasmic antibodies. A report of 3 cases and a review of the literature. *Clin Exp Rheumatol* 2001;19:541–549.
- 4 Scott DGI, Watts RA: Systemic vasculitis: epidemiology, classification and environmental factors. *Ann Rheum Dis* 2000;59:161–163.
- 5 Kallenberg CGM: Pathogenesis of PR3-ANCA-associated vasculitis. *J Autoimmun* 2008;30:29–36.
- 6 Xiao H, Heeringa P, Hu PQ, Liu Z, Zhao ML, Aratani Y, et al: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 2002;110:955–963.
- 7 Ruth AJ, Kitching AR, Kwan RYQ, Odobasic D, Ooi JDK, Timoshanko JR, et al: Anti-neutrophil cytoplasmic antibodies and effector CD4+ cells play nonredundant roles in anti-myeloperoxidase crescentic glomerulonephritis. *J Am Soc Nephrol* 2006;17:1940–1949.
- 8 Brouwer E, Huitema MG, Klok PA, de Weerd H, Tervaert JW, Weening JJ, et al: Antimyeloperoxidase-associated proliferative glomerulonephritis: an animal model. *J Exp Med* 1993;177:905–914.

- 9 Pfister H, Ollert M, Frohlich LF, Quintanilla-Martinez L, Colby TV, Specks U, et al: Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood* 2004;104:1411–1418.
- 10 Segelmark M, Phillips BD, Hogan SL, Falk RJ, Jennette JC: Monitoring proteinase 3 antineutrophil cytoplasmic antibodies for detection of relapses in small vessel vasculitis. *Clin Vaccine Immunol* 2003;10:769–774.
- 11 Cohen Tervaert JW, Huitema MG, Sluiter WJ, The TH, van der Hem GK, Kallenberg CGM, et al: Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet* 1990;336:709–711.
- 12 Boomsma MM, Stegeman CA, van der Leij MJ, Oost W, Hermans J, Kallenberg CGM, et al: Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels – a prospective study. *Arthritis Rheum* 2000;43:2025–2033.
- 13 Finkielman JD, Merkel PA, Schroeder D, Hoffman GS, Spiera R, St. Clair EW, et al: Antiproteinase-3 antineutrophil cytoplasmic antibodies and disease activity in Wegener granulomatosis. *Ann Intern Med* 2007;147:611–619.
- 14 Rarok AA, Stegeman CA, Limburg PC, Kallenberg CGM: Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. *J Am Soc Nephrol* 2002;13:2232–2238.
- 15 Taylor SRJ, Salama AD, Joshi L, Pusey CD, Lightman SL: Rituximab is effective in the treatment of refractory ophthalmic Wegener's granulomatosis. *Arthritis Rheum* 2009;60:1540–1547.
- 16 Brouwer E, Tervaert JWC, Horst G, Huitema MG, Vandergiesen M, Limburg PC, et al: Predominance of IgG1 and IgG4 subclasses of antineutrophil cytoplasmic autoantibodies in patients with Wegener's granulomatosis and clinically related disorders. *Clin Exp Immunol* 1991;83:379–386.
- 17 Voswinkel J, Muller A, Lamprecht P: Is PR3-ANCA formation initiated in Wegener's granulomatosis lesions? Granulomas as potential lymphoid tissue maintaining autoantibody production. *Ann NY Acad Sci* 2005;1051:12–19.
- 18 Voswinkel J, Mueller A, Kraemer JA, Lamprecht P, Herlyn K, Holl-Ulrich K, et al: B-lymphocyte maturation in Wegener's granulomatosis: a comparative analysis of VH genes from endonasal lesions. *Ann Rheum Dis* 2006;65:859–864.
- 19 Co DO, Hogan LH, Il-Kim S, Sandor M: T cell contributions to the different phases of granuloma formation. *Immunol Lett* 2004;92:135–142.
- 20 Sato IY, Kobayashi K, Yamagata N, Shikama Y, Kasama T, Kasahara K, et al: Modulation of granuloma formation in vitro by endogenous mediators. *Immunopharmacology* 1991;21:73–82.
- 21 Aries PM, Lamprecht P, Gross WL: Wegener's granulomatosis: a view from the granulomatous side of the disease. *Isr Med Assoc J* 2005;7:768–773.
- 22 Schmitt WH, Heesen C, Csernok E, Rautmann A, Gross WL: Elevated serum levels of soluble interleukin-2 receptor in patients with Wegener's granulomatosis. Association with disease activity. *Arthritis Rheum* 1992;35:1088–1096.
- 23 Abdulahad WH, van der Geld YM, Stegeman CA, Kallenberg CG: Persistent expansion of CD4+ effector memory T cells in Wegener's granulomatosis. *Kidney Int* 2006;70:938–947.
- 24 Fienberg R: A morphologic and immunohistologic study of the evolution of the necrotizing palisading granuloma of pathergic (Wegener's) granulomatosis. *Semin Respir Med* 1989;10:126–132.
- 25 Lamprecht P, Moosig F, Csernok E, Seitzer U, Schnabel A, Mueller A, et al: CD28 negative T cells are enriched in granulomatous lesions of the respiratory tract in Wegener's granulomatosis. *Thorax* 2001;56:751–757.
- 26 Lamprecht P, Mueller A, Gross WL: CD28– T cells display features of effector memory T cells in Wegener's granulomatosis. *Kidney Int* 2004;65:1113–1114.
- 27 Moosig F, Csernok E, Wang G, Gross WL: Costimulatory molecules in Wegener's granulomatosis: lack of expression of CD28 and preferential up-regulation of its ligands B7-1 (CD80) and B7-2 (CD86) on T cells. *Clin Exp Immunol* 1998;114:113–118.
- 28 Steiner K, Moosig F, Csernok E, Selleng K, Gross WL, Fleischer B, et al: Increased expression of CTLA-4 (CD152) by T and B lymphocytes in Wegener's granulomatosis. *Clin Exp Immunol* 2001;126:143–150.
- 29 Pandiyan P, Gartner D, Soezeri O, Radbruch A, Schulze-Osthoff K, Brunner-Weinzierl MC: CD152 (CTLA-4) determines the unequal resistance of Th1 and Th2 cells against activation-induced cell death by a mechanism requiring PI₃ kinase function. *J Exp Med* 2004;199:831–842.
- 30 Komocsi A, Lamprecht P, Csernok E, Mueller A, Holl-Ulrich K, Seitzer U, et al: Peripheral blood and granuloma CD4+CD28– T cells are a major source of interferon- γ and tumor necrosis factor- α in Wegener's granulomatosis. *Am J Pathol* 2002;160:1717–1724.
- 31 Csernok E, Ai M, Gross WL, Wicklein D, Petersen A, Lindner B, et al: Wegener autoantigen induces maturation of dendritic cells and licenses them for Th1 priming via the protease-activated receptor-2 pathway. *Blood* 2006;107:4440–4448.
- 32 Spriewald BM, Witzke O, Wassmuth R, Wenzel RR, Arnold ML, Philipp T, et al: Distinct tumour necrosis factor- α , interferon- γ , interleukin-10, and cytotoxic T-cell antigen 4 gene polymorphisms in disease occurrence and end-stage renal disease in Wegener's granulomatosis. *Ann Rheum Dis* 2005;64:457–461.
- 33 Zhou YH, Huang DR, Paris PL, Sauter CS, Prock KA, Hoffman GS: An analysis of CTLA-4 and proinflammatory cytokine genes in Wegener's granulomatosis. *Arthritis Rheum* 2004;50:2645–2650.
- 34 Huang DR, Giscombe R, Zhou YH, Pirskanen R, Lefvert AK: Dinucleotide repeat expansion in the CTLA-4 gene leads to T cell hyperactivity via the CD28 pathway in myasthenia gravis. *J Neuroimmunol* 2000;105:69–77.
- 35 Ueda H, Howson JMM, Esposito L, Heward J, Snook H, Chamberlain G, et al: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003;423:506–511.
- 36 Jagiello P, Aries P, Arning L, Wagenleiter SEN, Csernok E, Hellmich B, et al: The PTPN22 620W allele is a risk factor for Wegener's granulomatosis. *Arthritis Rheum* 2005;52:4039–4043.
- 37 Mustelin T, Alonso A, Bottini N, Huynh H, Rahmouni S, Nika K, et al: Protein tyrosine phosphatases in T-cell physiology. *Mol Immunol* 2004;41:687–700.
- 38 Luzina IG, Atamas SP, Storrer CE, daSilva LC, Kelsoe G, Papadimitriou JC, et al: Spontaneous formation of germinal centers in autoimmune mice. *J Leukoc Biol* 2001;70:578–584.
- 39 Hasegawa K, Martin F, Huang GM, Tumas D, Diehl L, Chan AC: PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004;303:685–689.
- 40 Huang D, Giscombe R, Zhou YH, Lefvert K: Polymorphisms in CTLA-4 but not tumor necrosis factor- α or interleukin-1 β genes are associated with Wegener's granulomatosis. *J Rheumatol* 2000;27:397–401.
- 41 Murakozy G, Gaede KI, Ruprecht B, Gutzeit O, Schurmann M, Schnabel A, et al: Gene polymorphisms of immunoregulatory cytokines and angiotensin-converting enzyme in Wegener's granulomatosis. *J Mol Med* 2001;79:665–670.
- 42 Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV: An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997;24:1–8.
- 43 Scheffold A, Hühn J, Höfer T: Regulation of CD4+CD25+ regulatory T cell activity: it takes (IL-)two to tango. *Eur J Immunol* 2005;35:1336.
- 44 Wraith DC, Nicolson KS, Whitely NT: Regulatory CD4+ T cells and the control of autoimmune disease. *Curr Opin Immunol* 2004;16:695.
- 45 Chai JG, Xue SA, Coe D, Addey C, Bartok I: Regulatory T cells, derived from naive CD4+CD25– T cells by in vitro FoxP3 gene transfer, can induce transplantation tolerance. *Transplantation* 2005;79:1310.

- 46 Fontenot JD, Rudensky AY: A well-adapted regulatory contrivance: regulatory T-cell development and the forkhead family transcription factor FoxP3. *Nat Immunol* 2005;6:331.
- 47 Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL: X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001;27:18.
- 48 Marinaki S, Neumann I, Kalsch AI, Grimminger P, Breedijk A, Birck R, et al: Abnormalities of CD4 T-cell subpopulations in ANCA-associated vasculitis. *Clin Exp Immunol* 2005;140:181–191.
- 49 Abdulahad WH, Stegeman CA, van der Geld YM, Doornbos-van der Meer B, Limburg PC, Kallenberg CG: Functional defect of circulating regulatory CD4+ T cells in patients with Wegener's granulomatosis in remission. *Arthritis Rheum* 2007;56:2080–2091.
- 50 Ziegler SF: FoxP3: not just for regulatory T cells anymore. *Eur J Immunol* 2007;37:21–23.
- 51 Piccirillo CA: Regulatory T cells in health and disease. *Cytokine* 2008;43:395–401.
- 52 Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK: Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 2006;212:28–50.
- 53 Appay V, van Lier RAW, Sallusto F, Roederer M: Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A* 2008;73A:975–983.
- 54 Vogt S, Iking-Konert C, Hug F, Andrassy K, Hansch GM: Shortening of telomeres: Evidence for replicative senescence of T cells derived from patients with Wegener's granulomatosis. *Kidney Int* 2003;63:2144–2151.
- 55 Marinaki S, Kalsch AI, Grimminger P, Breedijk A, Birck R, Schmitt WH, et al: Persistent T-cell activation and clinical correlations in patients with ANCA-associated systemic vasculitis. *Nephrol Dial Transplant* 2006;21:1825–1832.
- 56 Huddleston CA, Weinberg AD, Parker DC: OX40 (CD134) engagement drives differentiation of CD4+ T cells to effector cells. *Eur J Immunol* 2006;36:1093–1103.
- 57 Lathrop SK, Huddleston CA, Dullforce PA, Montfort MJ, Weinberg AD, Parker DC: A signal through OX40 (CD134) allows anergic, autoreactive T cells to acquire effector cell functions. *J Immunol* 2004;172:6735–6743.
- 58 Valzasina B, Guiducci C, Dislich H, Killeen N, Weinberg AD, Colombo MP: Triggering of OX40 (CD134) on CD4+CD25+ T cells blocks their inhibitory activity: a novel regulatory role for OX40 and its comparison with GITR. *Blood* 2005;105:2845–2851.
- 59 Wilde B, Dolff S, Cai X, Specker C, Becker J, Totsch M, et al: CD4+CD25+ T-cell populations expressing CD134 and GITR are associated with disease activity in patients with Wegener's granulomatosis. *Nephrol Dial Transplant* 2009;24:161–171.
- 60 Balding CEJ, Howie AJ, Drake-Lee AB, Savage COS: Th2 dominance in nasal mucosa in patients with Wegener's granulomatosis. *Clin Exp Immunol* 2001;125:332–339.
- 61 Csernok E, Trabandt A, Muller A, Wang GCC, Moosig F, Paulsen J, et al: Cytokine profiles in Wegener's granulomatosis – predominance of type 1 (Th1) in the granulomatous inflammation. *Arthritis Rheum* 1999;42:742–750.
- 62 Sanders JS, Stegeman CA, Kallenberg CG: The Th1 and Th2 paradigm in ANCA-associated vasculitis. *Kidney Blood Press Res* 2003;26:215–220.
- 63 Schonermarck U, Csernok E, Trabandt A, Hansen H, Gross WL: Circulating cytokines and soluble CD23, CD26 and CD30 in ANCA-associated vasculitides. *Clin Exp Rheumatol* 2000;18:457–463.
- 64 Lamprecht P, Bruhl H, Erdmann A, Holl-Ulrich K, Csernok E, Seitzer U, et al: Differences in CCR5 expression on peripheral blood CD4+CD28– T cells and in granulomatous lesions between localized and generalized Wegener's granulomatosis. *Clin Immunol* 2003;108:1–7.
- 65 Muller A, Trabandt A, Gloeckner-Hofmann K, Seitzer U, Csernok E, Schonermarck U, et al: Localized Wegener's granulomatosis: predominance of CD26 and IFN- γ expression. *J Pathol* 2000;192:113–120.
- 66 Kurts C: Th17 cells: a third subset of CD4+ T effector cells involved in organ-specific autoimmunity. *Nephrol Dial Transplant* 2008;23:816–819.
- 67 Korn T, Oukka M, Kuchroo V, Bettelli E: Th17 cells: effector T cells with inflammatory properties. *Semin Immunol* 2007;19:362–371.
- 68 Bettelli E, Oukka M, Kuchroo VK: Th-17 cells in the circle of immunity and autoimmunity. *Nat Immunol* 2007;8:345–350.
- 69 Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J, et al: Interleukin-17-producing T-helper cells and interleukin-17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008;9:166–175.
- 70 Hoshino H, Laan M, Sjöstrand M, Lötvall J, Skoogh B-E, Lindén A: Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways in vivo. *J Allergy Clin Immunol* 2000;105:143–149.
- 71 Abdulahad WH, Stegeman CA, Limburg PC, Kallenberg CG: Skewed distribution of Th17 lymphocytes in patients with Wegener's granulomatosis in remission. *Arthritis Rheum* 2008;58:2196–2205.
- 72 Popa ER, Stegeman CA, Abdulahad WH, van der Meer B, Arends J, Manson WM, et al: Staphylococcal toxic shock syndrome toxin-1 as a risk factor for disease relapse in Wegener's granulomatosis. *Rheumatology* 2007;46:1029–1033.
- 73 Stegeman CA, Tervaert JWC, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CGM: Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. *Ann Intern Med* 1994;120:12–17.
- 74 Stegeman CA, Cohen Tervaert JW, de Jong PE, Kallenberg CGM, The Dutch Co-trimoxazole Wegener Study G: Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. *N Engl J Med* 1996;335:16–20.
- 75 Pendergraft WF, Preston GA, Shah RR, Tropsha A, Carter CW, Jennette JC, et al: Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med* 2004;10:72–79.
- 76 McGuire KL, Holmes DS: Role of complementary proteins in autoimmunity: an old idea re-emerges with new twists. *Trends Immunol* 2005;26:367–372.
- 77 Kain R, Exner M, Brandes R, Ziebermayr R, Cunningham D, Alderson CA, et al: Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med* 2008;14:1088–1096.
- 78 Popa ER, Stegeman CA, Bos NA, Kallenberg CGM, Tervaert JWC: Staphylococcal superantigens and T-cell expansions in Wegener's granulomatosis. *Clin Exp Immunol* 2003;132:496–504.
- 79 Müller-Alouf H, Carnoy C, Simonet M, Alouf JE: Superantigen bacterial toxins: state of the art. *Toxicon* 2001;39:1691–1701.
- 80 Schmitt WH, Hagen EC, Neumann I, Nowack R, Flores-Suarez LF, van der Woude FJ: Treatment of refractory Wegener's granulomatosis with anti-thymocyte globulin: An open study in 15 patients. *Kidney Int* 2004;65:1440–1448.
- 81 Hagen EC, Dekeizer RJW, Andrassy K, Vanboven WPL, Bruijn JA, Vanes LA, et al: Compassionate treatment of Wegener's granulomatosis with rabbit anti-thymocyte globulin. *Clin Nephrol* 1995;43:351–359.
- 82 Tarkowski A, Anderssonsgare B, Aurell M: Use of anti-thymocyte globulin in the management of refractory systemic autoimmune diseases. *Scand J Rheumatol* 1993;22:261–266.
- 83 Wertheim MS, Ross AH, Tole DM: The use of Campath in severe peripheral ulcerative keratitis associated with Wegener's granulomatosis. *Eye* 2006;20:1453–1454.
- 84 Lockwood CM: Refractory Wegener's granulomatosis: a model for shorter immunotherapy of autoimmune diseases. *J R Coll Physicians Lond* 1998;32:473–478.

- 85 Lockwood CM, Thiru S, Isaacs JD, Hale G, Waldmann H: Long-term remission of intractable systemic vasculitis with monoclonal-antibody therapy. *Lancet* 1993;341:1620–1622.
- 86 Mathieson PW, Cobbold SP, Hale G, Clark MR, Oliveira DBG, Lockwood CM, et al: Monoclonal-antibody therapy in systemic vasculitis. *N Engl J Med* 1990;323:250–254.
- 87 Stone JH, Hoffman GS, Holbrook JT, Meiner CL, Dodge J, Donithan J, et al: Etanercept plus standard therapy for Wegener's granulomatosis. *N Engl J Med* 2005;352:351–361.
- 88 Stone JH, Holbrook JT, Marriott MA, Tibbs AK, Sejismundo LP, Min YI, et al: Solid malignancies among patients in the Wegener's granulomatosis etanercept trial. *Arthritis Rheum* 2006;54:1608–1618.
- 89 Bartolucci P, Ramanoelina J, Cohen P, Mahr A, Godmer P, Le Hello C, et al: Efficacy of the anti-TNF- α antibody infliximab against refractory systemic vasculitides: an open pilot study on 10 patients. *Rheumatology* 2002;41:1126–1132.
- 90 Lamprecht P, Voswinkel J, Lilienthal T, Nolle B, Heller M, Gross WL, et al: Effectiveness of TNF- α blockade with infliximab in refractory Wegener's granulomatosis. *Rheumatology* 2002;41:1303–1307.
- 91 Huugen D, Tervaert JWC, Heeringa P: TNF- α bioactivity-inhibiting therapy in ANCA-associated vasculitis: clinical and experimental considerations. *Clin J Am Soc Nephrol* 2006;1:1100–1107.
- 92 Booth A, Harper L, Hammad T, Bacon P, Griffith M, Levy J, et al: Prospective study of TNF- α blockade with infliximab in anti-neutrophil cytoplasmic antibody-associated systemic vasculitis. *J Am Soc Nephrol* 2004;15:717–721.
- 93 Vincenti F, Luggen M: T-cell costimulation: a rational target in the therapeutic armamentarium for autoimmune diseases and transplantation. *Annu Rev Med* 2007;58:347–358.
- 94 Cunnane G, Chan OTM, Cassafer G, Brindis S, Kaufman E, Yen TSB, et al: Prevention of renal damage in murine lupus nephritis by CTLA-4Ig and cyclophosphamide. *Arthritis Rheum* 2004;50:1539–1548.
- 95 Levine SM, Stone JH: New approaches to treatment in systemic vasculitis: biological therapies. *Best Pract Res Clin Rheumatol* 2001;15:315–333.
- 96 Hoff H, Knieke K, Cabail Z, Hirsland H, Vratsanos G, Burmester GR, et al: Surface CD152 (CTLA-4) expression and signaling dictates longevity of CD28null T cells. *J Immunol* 2009;182:5342–5351.