Original Article

CD4+CD25+ T-cell populations expressing CD134 and GITR are associated with disease activity in patients with Wegener’s granulomatosis

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Abstract

Background. An increased CD4+CD25+ T-cell population is observed in Wegener’s granulomatosis (WG). This T-cell population is not well characterized yet and their contribution to the disease pathogenesis remains obscure.

Methods. Thirty patients with WG and 18 healthy controls (HC) were included in this study. The disease activity and extension were measured by the Birmingham Vasculitis Activity Score (BVAS) and the Disease Extent Index (DEI). Lymphocytes from peripheral blood were analysed by FACS for the expression of CD4, CD25, CD134 and GITR. Cytokine expression in these subsets was assessed too. Nasal, lung and renal tissues from WG patients were immunohistochemically stained for CD3 and CD134.

Results. The percentage of CD134+ as well as GITR+ expressing CD4+CD25+ lymphocytes was increased in patients as compared to HC (37 ± 12% versus 27 ± 8%, P = 0.005; 18 ± 9% versus 11 ± 6%, P = 0.003). The expression of CD134 and GITR showed a significant correlation with disease activity (r = 0.5, P = 0.009; r = 0.55, P = 0.001). Most of these displayed the phenotype of effector memory T-cells (94 ± 4% and 91 ± 6%). CD134 T-cells were found in tissues affected by WG.

Conclusions. CD4+CD25+ effector memory T-cells expressing CD134 and GITR seem to play a role in disease mechanisms, as suggested by their close association with disease activity and their participation in inflammatory process.

Keywords: ANCA; CD134; GITR; Treg; Wegener’s granulomatosis

Introduction

Wegener’s granulomatosis (WG) is a life-threatening autoimmune disease characterized by granulomas, necrotizing small vessel vasculitis and pauci-immune crescentic glomerulonephritis [1]. This disorder is associated with anti-neutrophil cytoplasmic antibodies (ANCA) [2]. Both the innate and the adaptive immune system contribute to disease pathogenesis [3]. T-cells seem to play an important role within disease mechanisms [4,5]. They are abundantly present in granulomatous lesions, and soluble T-cell activation markers such as sIl-2r have been found to be elevated in WG patients [6,7].

Especially, the CD4+CD25+ T-cell population came into focus of research as it is expanded in WG patients [8–10]. This population is supposed to have a crucial impact on immune regulation [11–13]. Abdulahad et al. recently demonstrated that regulatory T-cells (Tregs) found within this subset seem to be defective in WG patients [14]. Nevertheless, the role of the expanded CD4+CD25+ T-cells within disease pathogenesis is not clarified and further characterization is missing.

An altered expression pattern of co-stimulatory molecules belonging to the CD28/CD80-family, which regulates T-cell activation, has already been described [15,16]. Whilst CD80 and CTLA-4 were found to be up-regulated in WG, CD28 expression was significantly diminished [15–17]. Co-stimulatory receptors belonging to the tumour necrosis factor receptor (TNFR) superfamily, in particular CD134 (OX40) and the glucocorticoid-induced TNFR-related protein (GITR), were not assessed in detail. CD134 and GITR are mainly expressed on T-cells and were shown to have crucial impact on T-cell activation and regulation in other diseases [18–20]. CD134 and GITR were regarded as specific markers for regulatory T-cells in the past but this turned out to be false [20–22]. CD134/GITR signalling drives T-cell expansion and the generation of memory...
Some recent reports suggest that both receptors mediate resistance against Tregs and abrogate Treg function [18–20]. Administration of an agonistic CD134 antibody leads to expansion of self-reactive T-cells relieved from an anergic state [24]. Weinberg et al. showed that this mechanism leads to fatal autoimmunity in a mouse model. Similar observations were made for GITR [25–27]. Some clinical studies assessed CD134 and GITR in autoimmune diseases; e.g. CD134 and GITR expression patterns are altered in systemic lupus erythematosus (SLE) [28,29]. Moreover, CD134+ T-cells may participate in inflammation due to rheumatoid arthritis (RA) [30]. This study aims to further characterize the role of CD4+ CD25+ T-cells in WG and to determine their role for the course of disease.

Patients and methods

All patients admitted to the out- and inpatient care of the Department of Nephrology (University Duisburg-Essen) in 2007/2008 and meeting the ACR’s/Chapel Hill criteria defining WG were included in this study. Therefore, 30 patients (females 17, males 13, mean age 60 ± 11 years, range 33–80) and 18 healthy controls (HC) (females 10, males 8, mean age 56 ± 13 years, range 34–77) were enrolled (Table 1).

Table 1. Clinical features of WG patients

<table>
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<th>Treatment</th>
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<td>Prednisone</td>
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WG, Wegener’s granulomatosis; BVAS, Birmingham Vasculitis Activity Score; MTX, methotrexate; MMF, mycophenolate mofetil; AZA, azathioprine; CYC, cyclophosphamide.

All patients were suffering from systemic vasculitis with kidney involvement. The disease activity and extent were assessed by Birmingham Vasculitis Activity Score (BVAS) and Disease Extent Index (DEI) [31,32]. Patients were treated with methotrexate (MTX) (n = 9), mycophenolate mofetil (MMF) (n = 9), azathioprine (AZA) (n = 4) and cyclophosphamide (CYC) (n = 4). One patient received a combination treatment with CYC and MMF. Twenty-six patients received prednisone in addition or as single treatment. Two patients were without any treatment. The study protocol was approved by the institutional review board. All patients gave their informed consent.

Laboratory parameters

Creatinine and C-reactive protein (CRP) serum levels were measured with commercially available kits in our central laboratory. Antibodies to PR3 (anti-PR3) were measured using an enzyme-linked immunosorbent assay (ELISA) commercially available (Orgentec, Mainz, Germany).

Flow cytometry

Expression level of the molecules on lymphocytes was measured by four-colour surface staining. Phycoerythrin (PE), fluorescein isothiocyanate (FITC), peridinin chlorophyll protein (PerCP) and allophycocyanin (APC)-labelled antibodies were used: anti-CD3 (mouse Ig G1, PerCP), anti-CD4 (mouse Ig G1, PerCP), anti-CD25 (mouse Ig G1, FITC), anti-CD45RA (mouse Ig G1, APC), anti-CD45RO (mouse Ig G1, FITC), anti-CD127 (mouse Ig G1, APC, R&D Systems, Wiesbaden, Germany), anti-CD134 (mouse Ig G1, PE), CD197 (CCR7, rat Ig G2a, Alexa Fluor© 647), anti-FoxP3 (mouse Ig G1, APC), anti-GITR (mouse Ig G1, PE, R&D Systems, Wiesbaden, Germany), anti-TNFα, anti-IFNγ (both mouse Ig G1, APC and FITC, Ebioscience, Frankfurt, Germany), MultiTest CD3/CD16/56/CD45/CD19 and MultiTest CD3/CD8/CD45/CD4 were used for four-colour surface staining. All antibodies except anti-CD127, anti-GITR, anti-TNFα and anti-IFNγ were purchased from Becton Dickinson (Heidelberg, Germany). Appropriate isotype controls (Becton Dickinson) were used. The peripheral blood was stained with labelled monoclonal antibodies for 20 min in the dark at room temperature. The cell suspension was incubated with a lysis buffer for 15 min and prepared as indicated. For a complete blood count, MultiTest antibodies and 50 μl of EDTA-anticoagulated blood were added to bead-containing TruCount tubes (Becton Dickinson). Intracellular FoxP3 staining and cell preparation procedures were performed as indicated in the manufacturer’s manual (Becton Dickinson). Data acquisition was performed with a fluorescence-activated cell sorter (FACS) Calibur™ from Becton Dickinson. For all data analysis, Flow Jo™ 7.2.2 (Treestar Software Inc. Ashland, OR, USA) was used.

Immunostaining for intracellular cytokines

PBMCs of patients were separated by standard Ficoll-Paque density gradient centrifugation. The cells were resuspended in a RPMI 1640 medium (Gibco BRL, Karlsruhe, Germany).
supplemented with 10% heat-inactivated fetal calf serum (Biochrom, Berlin, Germany), 100 U of penicillin per ml and 100 μg of streptomycin per ml (both Gibco BRL). The cells were cultured in the absence or presence of PMA (5 ng/ml) and ionomycin (1 μM) (Sigma-Aldrich, Seelze, Germany) for 6 h. Cytokine secretion was inhibited by Brefeldin A (Ebioscience). Then, a surface staining was performed with anti-CD3, anti-CD134 or appropriate isotype controls. Cells were fixed by using a Cytofix/Cytoperm kit purchased from Becton Dickinson. Finally, the samples were intracellularly stained with anti-TNFα, anti-IFNγ or appropriate isotype controls.

**Immunohistochemistry**

Five renal biopsies, three lung specimens and three nasal biopsies from WG patients were provided by the Institute of Pathology and Neuropathology, University Hospital of Essen. All specimens were fixed in 10% neutral buffered formalin and paraffin embedded. Five-micrometre-thick sections were deparaffinized in xylene and rehydrated in a series of ethanol with different concentrations (100%, 95%, 70% and 50%). A citrate buffer pH 6.0 (Zytomed, Essen) was used for heat-induced epitope retrieval was applied, followed by neutralization of endogenous peroxidase with 0.3% H2O2. Primary antibodies (anti-CD3 obtained from DCS, Hamburg, Germany, and anti-CD134 obtained from Becton Dickinson) and HRP-conjugated secondary antibodies (Zytomed) were incubated on slides (each for 30 min) at room temperature. Washing with PBS was performed after each incubation step. A DAB substrate kit (Zytomed) was used for visualization. Finally, the slides were slightly counterstained with haematoxylin.

**Immunofluorescence double staining**

Tissues were fixed, embedded in paraffin and sectioned as indicated above. Epitope retrieval was performed with a citrate buffer pH 6.0 (Zytomed). Primary antibodies against CD3 (rabbit Ig G1, DCS) and CD134 (mouse Ig G1, Becton Dickinson) were used and incubated for 60 min at room temperature simultaneously. Secondary antibodies conjugated to Cy2 and Cy3 (Dianova, Hamburg, Germany) were applied for 30 min. Finally, the slides were mounted with Immu Mount™ (Thermo Fisher, Kehl, Germany).

**Statistics**

All values are expressed as mean ± standard deviation (SD). Significance for the differences between groups was determined using the Mann–Whitney U-test. To compare more than two groups, the non-parametric Kruskal–Wallis test was used. Spearman’s rank correlation was applied for detecting correlation between different study parameters. Two-tailed P-values <0.05 were considered as significant. For statistical analysis, GraphPad Prism™ 4.0 was used (GraphPad Software, San Diego, CA, USA).

### Table 2. Absolute number of lymphocyte subsets in WG patients and HC given as cells/μl

<table>
<thead>
<tr>
<th>Cells/μl</th>
<th>WG patients</th>
<th>HC</th>
<th>P-value</th>
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<tr>
<td>CD3+CD4+</td>
<td>531 ± 385</td>
<td>706 ± 213</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD3+CD4+CD25+</td>
<td>147 ± 101</td>
<td>134 ± 80</td>
<td>NS</td>
</tr>
<tr>
<td>CD3+CD4+CD25+GITR+</td>
<td>30 ± 12</td>
<td>15 ± 8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD3+CD4+CD25+CD134+</td>
<td>59 ± 12</td>
<td>36 ± 12</td>
<td>&lt;0.05</td>
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</table>

WG, Wegener’s granulomatosis; HC, healthy controls.

The data are expressed as mean ± SD. A P-value <0.05 was considered as significant.

**Results**

**Expression of GITR and CD134 within the CD4+ CD25+ T-cell population**

The percentage of CD4+ T-cell expressing CD25 was assessed in 30 patients and 18 HC. WG patients showed an increased frequency of CD4+CD25+ T-cells (32 ± 19% versus 16 ± 6%, P = 0.001) whereas the absolute amount of CD4+CD25+ T-cells did not differ from HC (Table 2). This T-cell subset was further analysed for expression of GITR and CD134. Expression levels for both markers were significantly higher in WG patients as compared to HC (GITR: 18 ± 9% versus 11 ± 6%, P = 0.003; CD134: 37 ± 12% versus 27 ± 8%, P = 0.005; Figures 1, 2 and 3). The absolute number of GITR+ and CD134+ T-cells within the CD4+CD25+ T-cell subset was also increased in comparison to HC (Table 2).

**Correlation between expression level of GITR/CD134 and disease activity and extent**

Next, a possible association between disease activity and GITR+/CD134+ T-cells was investigated. Data on CD134 and GITR expression were correlated with BVAS. This
Fig. 2. Expression of GITR on peripheral CD4^+CD25^+ T-cells in patients with WG (n = 30) and healthy controls (n = 18). The illustrated data are shown as mean values ± SD (19 ± 2% versus 10 ± 2%). Significant differences by the Mann–Whitney U-test are indicated: *P = 0.005.

Cross-sectional analysis demonstrated a significant correlation between GITR^+/CD134^+ T-cell subsets and disease activity (CD134: r = 0.5, P = 0.009; GITR: r = 0.55, P = 0.001; Figures 4 and 5). The DEI neither correlated with GITR nor with the CD134 expression level (r = 0.28, P = 0.21; r = 0.16, P = 0.49). The patients’ serum level of CRP and anti-PR3 did not show an association with GITR/CD134 expression (GITR/CRP: r = 0.12, P = 0.66; GITR/anti-PR3: r = 0.16, P = 0.58; CD134/anti-PR3: r = 0.26, P = 0.34; CD134/CRP: r = −0.25, P = 0.35). Furthermore, serum creatinine levels were not linked to GITR/CD134 expression levels (r = 0.1, P = 0.7 and r = 0.3, P = 0.3). To determine whether the kind of treatment had an impact on the frequency of CD4^+CD25^+GITR^+/CD134^+ T-cells, patients were grouped according to their treatment regime (AZA, CYC, MMF or MTX). The frequency of CD4^+CD25^+GITR^+/CD134^+ T-cells was compared and showed no significant differences between the groups (GITR: P = 0.8; AZA = 18 ± 11%, CYC = 21 ± 5%, MMF = 19 ± 7%, MTX = 18 ± 11%; CD134: P = 0.6; AZA = 36 ± 8%, CYC = 45 ± 5%, MMF = 39 ± 10%, MTX = 41 ± 14%). Moreover, no correlation of prednisone dose and GITR/CD134 was found (GITR: r = 0.06, P = 0.8; CD134: r < 0.05, P = 0.99). Thus, the kind of treatment did

Fig. 3. Representative dot plots of multichromatic flow cytometry analysis gated on the CD4^+CD25^+ T-cell population. Examples for one WG patient (A) and one HC (B) are shown. The percentage of CD4^+CD25^+ T-cells positive for GITR or CD134 is given in the upper right quadrant. The number of CD4^+CD25^+GITR^+/CD134^+ expressing T-cells is increased in the WG patient in comparison to the HC. Appropriate isotype controls (Fluorescence-Minus-One-controls according to Roederer [56]) were used.
not influence the number of CD4⁺CD25⁺GITR⁺/CD134⁺ T-cells.

**Longitudinal analysis of GITR and CD134 expression**

This analysis was repeated in a longitudinal manner for 10 patients. The GITR/CD134 expression as well as the BVAS score was assessed at two different time points. The GITR/CD134 levels and BVAS scores varied for each patient at the two different time points. Therefore, the differences in GITR/CD134 levels and BVAS score were calculated and expressed as ‘delta (Δ)’ for each individual patient (Figures 6 and 7). The Δ for CD134 and GITR expression correlated with the Δ calculated for BVAS significantly \((r = 0.81, P = 0.006)\) and \((r = 0.8, P = 0.007)\).

**Characterization of GITR⁺ and CD134⁺ T-cells**

Both T-cell subsets were further investigated in 20 patients and 10 HC. A staining for CD45RO and CCR7 was performed to determine whether these subsets were naive or already antigen-experienced T-cells. The majority of CD4⁺CD25⁺GITR⁺/CD134⁺ T-cells in patients and HC expressed CD45RO thus being memory T-cells (GITR:
CD4⁺CD25⁺ GITR⁺ T-cells: CD127 expression

CD25⁺CD134⁺ T-cells: CD127 expression

Fig. 8. Representative dot plots of multichromatic flow cytometry analysis gated on the CD4⁺CD25⁺ T-cell population. An example for one WG patient is shown. The quadrant gates were adjusted according to the given isotype controls [56]. Isotype controls were performed in each experiment for each patient and HC. Most of the CD4⁺CD25⁺GITR⁺/CD134⁺ T-cells displayed CD127 (84.28% and 83.95%).

98 ± 1% versus 96 ± 1%, P > 0.05; CD134: 99 ± 1% versus 99 ± 1%, P > 0.05). This was further confirmed by assessing CD45RA expression in 10 patients and 5 HC. The majority of CD4⁺CD25⁺GITR⁺/CD134⁺ T-cells lacked CD45RA expression and no significant differences in comparison to HC were detected (GITR: 85 ± 8% versus 87 ± 10%, P > 0.05; CD134: 79 ± 9% versus 80 ± 4%, P > 0.05). An additional staining for CCR7 was done to further distinguish different types of memory T-cells. As a result, an overwhelming amount of CD4⁺CD25⁺GITR⁺ and CD4⁺CD25⁺CD134⁺ memory T-cells displayed the phenotype of effector memory T-cells (94 ± 4% and 91 ± 6%). Therefore, only a minority could be assigned to central memory T-cells.

CD4⁺CD25⁺ GITR⁺ and CD134⁺ T-cells do not belong to the Treg compartment

CD4⁺CD25⁺GITR⁺/CD134⁺ T-cells were further characterized regarding CD127 expression in the same 20 patients and 10 HC as above. CD127 was used to distinguish between effector T-cells and regulatory T-cells. CD127lo T-cells were recently shown to bear regulatory functions whereas CD127⁺ T-cells mainly belong to the effector T-cell compartment [33]. GITR⁺/CD134⁺ T-cells mainly expressed CD127; only a minor part was deficient for CD127 (Figure 8). There was no difference in comparison to HC [%CD127lo (of CD4⁺CD25⁺GITR⁺ T-cells): 25 ± 14% versus 32 ± 17% P > 0.05; %CD127lo
(of CD4+CD25+CD134+ T-cells): 20 ± 8% versus 34 ± 28% \( P > 0.05 \). At present, FoxP3 is known as the most valuable marker for Tregs. Thus an additional staining for FoxP3 was performed. For this purpose, 10 patients and 5 HC were analysed. The analysis for FoxP3 revealed similar results as above. Within the CD4+CD25+CD134+ T-cell population, >86% lacked FoxP3 expression and within the CD4+CD25+GITR+/CD134+ T-cell subset, ~75% were negative for FoxP3 expression; no significant differences were detected between patients and HC (86 ± 5% versus 90 ± 5%, \( P > 0.05 \) and 75 ± 11% versus 76 ± 6%, \( P > 0.05 \)). Most of the CD234+ were mostly displayed by CD4+CD25+ non-regulatory T-cells.

**CD134+ T-cells as a source for IFNγ and TNFα**

To determine the function of the CD134+ T-cells, these T-cells were stimulated and stained for IFNγ and TNFα expression. A minority of CD134+ T-cells produced IFNγ after stimulation (20.1 ± 15% of CD134+ T-cells). No correlation of IFNγ secretion with disease activity was observed. Most of CD134+ T-cells were a source for TNFα (57 ± 28% of CD134+ T-cells). TNFα expression of CD134+ T-cells was evident even in patients with inactive disease (BVAS = 0).

**IHC staining for CD134 in inflamed tissue**

To determine whether CD134+ T-cells participate in organ inflammation in WG, renal biopsies, lung tissue and nasal biopsies were stained for CD134 and CD3. CD134+ cells were found in all tissues. CD134+ cells were located within or in the neighbourhood of granulomas especially in lung and nasal specimen. These cells were also found in small vessels that were inflamed. In all extra-renal biopsies, CD134+ cells were also found in mononuclear follicle-like cell aggregates. Single cells were found around the glomeruli and in the tubulointerstitium in renal biopsies. Serial sections revealed a co-localization with CD3+ T-cells; this could be confirmed by immunofluorescence double staining with CD3 and CD134. About 10% of the CD3+ T-cells were also positive for CD134 (Figures 9 and 10).

**Discussion**

The results of the present study demonstrate that the CD4+CD25+ population in WG consists of increased amounts of activated GITR and CD134 expressing non-regulatory T-cells. GITR and CD134 expression on CD4+CD25+ effector memory T-cells correlated with disease activity implying that these cells may contribute to disease pathogenesis. This is supported by our finding that CD134 expressing T-cells were localized within the inflammatory lesions of WG.

The expansion of the CD4+CD25+ T-cell subset was shown before by several other authors [10,34,35]. Goscombe et al. found specific expanded T-cell populations within the CD4+CD25+ T-cell subset that were associated with disease activity [34]. But so far CD4+CD25+ T-cell subsets expressing receptors belonging to the TNFR family were not studied in WG; thus the increased presence of CD4+CD25+GITR+/CD134+ T-cells and their tight association with disease course are novel findings. Moreover, until now it was rather unknown that these specific T-cell subsets do not belong to the Treg lineage in WG; GITR and CD134 as well were reported to be expressed on Tregs [11,12,36].

Circulating CD4+CD25+GITR+/CD134+ T-cell populations were characterized in a few other human autoimmune diseases. Li et al. detected an increase of CD4+CD25+GITR+ T-cells in patients suffering from autoimmune uveitis [37]. The GITR expression on CD4+CD25+ T-cells correlated with disease activity. Lee et al. made similar observations in patients with SLE; the percentage of CD4+CD25+ T-cells bearing GITR expression was increased [29]. Hence, both studies also demonstrated an alteration within CD4+CD25+ T-cell subsets regarding GITR/CD134 expression. However, a further characterization concerning the regulatory or non-regulatory origin was not performed; therefore it remains unknown whether effector T-cells or Treg account for these alterations. In contrast, there was no increased presence of CD4+CD25+GITR+/CD134+ T-cells in peripheral blood from RA patients [30]. However, an overwhelming amount of CD4+CD25+ T-cells located in synovial fluid of RA patients showed GITR and CD134 expression suggesting participation in inflammatory response [29]. Again, the authors did not assess whether these T-cells were of regulatory origin. Another interesting study conducted by Endl et al. revealed that autoreactive T-cells in patients with diabetes type 1 mainly displayed a CD25+CD134+ phenotype whereas autoreactive T-cells from HC did not express CD134 [38]. Thus, this study indicates that autoreactive T-cells causing autoimmunity might be delineated by CD134 expression [38].

Based on our data and the studies mentioned above, it is likely that activated CD4+CD25+GITR+/CD134+ T-cells facilitate organ damage caused by inflammation during the disease course. Furthermore, transplantation immunology studies showed that blocking the CD134 pathway ameliorated both organ rejection and inflammation [39,40]. In tumour immunology, GITR and CD134 were shown to be crucial for anti-tumour response and tumour tolerance [41]. Moreover, reports on graft-versus-host disease after bone marrow transplantation demonstrated a close association between CD134 and the disease course [42]. These findings strongly suggest that both molecules are playing a key role in self-tolerance and immunity; thus it is not surprising that they might contribute to disease pathogenesis in WG.

There are some hints at the mechanisms underlying immunomodulation by GITR and CD134. Both are able to mediate resistance against Tregs to effector T-cells [18–20,23,43–46]. In addition, these molecules abrogate the ability of Tregs to suppress immune responses [24]. In our study, GITR and CD134 expression was mainly found on activated effector T-cells of non-regulatory type. Thus, both molecules might facilitate the immune response during active disease by conferring resistance against Tregs’ suppressive function [18,48].

Most of these T-cells also showed an effector memory phenotype enabling them to respond rapidly to stimuli and
Fig. 9. Representative nasal biopsy of a WG Patient. The biopsy was stained for CD134 with IHC as stated before. (A) Granuloma formation and accumulation of mononuclear cells. Within this granuloma, CD134⁺ cells are located (dark membrane staining). (B) Active vasculitis in the same biopsy. CD134⁺ cells are located around the vessel.
Fig. 10. Representative biopsy of a WG patient. The biopsy was stained for CD3+ and CD134+ cells by immunofluorescence as stated before. CD3 (green) co-localizes with CD134 (red).

to migrate to peripheral tissue. Interestingly, Abdulahad et al. recently published two studies dealing with the memory T-cell subsets in WG [14,49]. In the first study, the authors reported a skewing from naive T-cell subsets towards the CD4+CD45RO+CCR7− effector memory T-cell subset. According to our results, an expanded effector memory T-cell compartment was observed in WG patients experiencing remission as compared to HC [49]. However, a significant decrease of effector memory T-cells was found in active WG patients as compared to patients in remission. In patients in an active state of disease, no expansion of effector memory T-cells was observed as compared to HC [49]. This is in slight contrast to our study. A higher amount of specific effector memory T-cell populations (CD4+CD25+GITR+/CD134+) was also evident in patients with active disease and the expansion of these T-cells was positively correlated with disease activity. Nevertheless, the results on active WG patients are not necessarily conflicting as Abdulahad et al. investigated the overall effector memory T-cell subset whereas our research work mainly dealt with special subpopulations within the effector memory T-cell subset. Abdulahad et al. also characterized the effector memory T-cells regarding their FoxP3 expression. As a result, most of these T-cells did not belong to the Treg compartment as they were FoxP3− and thus expansion was mainly due to non-regulatory memory T-cell subsets. This is in line with our results; an overwhelming amount of the expanded CD4+CD25+GITR+/CD134+ effector memory T-cell subset was FoxP3−. The second study by Abdulahad et al. assessed CD4+CD25+ memory T-cells in WG [14]. In short, they found an increase in the CD25highFoxP3− memory T-cell subset showing defective suppressive function. As explained by the authors, FoxP3 expression may be induced by persistent stimulation and thus may not exclusively delineate Tregs in this case [14]. According to our data, it might be worthwhile to discuss an additional mechanism. As demonstrated above, the expansion of CD4+CD25+ T-cells is also due to an increase of GITR+/CD134+ memory T-cells; in addition, up to 20% of these T-cells show a regulatory phenotype. Taking into account that GITR and CD134 were shown to abrogate the suppressive function of Tregs, it might be speculated that these receptors contribute to the observed impairment of Tregs [18,23,24,50]. Nevertheless, this remains speculative and needs to be confirmed.

Moreover, Lamprecht et al. recently emphasized the importance of the effector memory T-cell for granuloma formation and autoimmunity [5]. Taken together with the presence of CD134+ T-cells in affected tissue, these CD134+ T-cells might have a pivotal function in inflammatory processes in WG [5,51]. Circulating CD134+ T-cell subsets could be responsible for relapsing disease course; it might be that this population constitutes a ‘pool’ being the starting point for organ and tissue inflammation.

CD134+ T-cells produced IFNg upon stimulation and were a source for TNFalpha suggesting Th1-polarization. The importance of IFNg and TNFalpha was emphasized and highlighted in the past within the context of disease pathogenesis [34,52,53]. This cytokine pattern enhances granuloma formation as well as the inflammatory process [54]. Thus, apart from phenotypic properties and associations with disease activity, CD134+ T-cells seem to have the functional ability to promote and facilitate inflammatory responses.

All these findings may have therapeutic and clinical implications. As mentioned above, therapeutic agents
blocking the CD134 pathway were already used successfully under experimental conditions in other diseases [39]. As toxicity related to common therapy regimen is a serious problem in WG and limits the patients’ prognosis, more specific therapies are needed [55]. Hence, targeting the CD134 pathway in WG might arise as a new and specific therapeutic opportunity.

Furthermore, assessing the CD134 and GITR expression on T-cells may be helpful in determining disease activity or predicting relapses. In this study, both the longitudinal and the cross-sectional analysis revealed a tight correlation of disease activity and CD134/GITR expression, and may provide a hint at a possible clinical applicability. However, to prove the applicability as well as the efficacy of this method, more patients have to be assessed and included into this study.

In summary, CD134+ and GITR+ T-cells might be the key players in WG. This is supported by their close association with disease activity, their presence in inflamed tissue and their ability to secrete cytokines of major importance, thus promoting inflammatory responses. Further efforts are needed to evaluate the clinical relevance of these findings.

Conflict of interest statement. None declared.

References


