Deficient CD4⁺CD25⁺ T Regulatory Cell Function in Patients With Abdominal Aortic Aneurysms

Mingdi Yin, Jian Zhang, Yong Wang, Shaoye Wang, Dittmar Böckler, Zhiquan Duan, Shijie Xin

Objective—Increasing evidence shows that autoimmune response contributes importantly to pathogenesis of abdominal aortic aneurysm (AAA). This work was aimed to assess the possibly altered function of peripheral CD4⁺CD25⁺ T regulatory cells (Tregs) that might breakdown immunologic self-tolerance in AAA patients.

Methods and Results—Peripheral blood from 22 AAA patients, 11 patients with abdominal aortic atherosclerotic occlusive disease (AOD), and 32 healthy controls (HCs) was analyzed to determine the percentage of CD4⁺CD25⁺ Tregs in the total CD4⁺ T-cell population and FOXP3 expression by means of flow cytometry. The frequencies of the CD4⁺CD25⁺ Treg population were not significantly different between groups (AAA, 5.69±0.99%; AOD, 5.52±1.13%; HC, 5.88±1.55%; P>0.05). However, the frequency of CD4⁺CD25⁺FOXP3⁺ T cells in AAA patients (2.45±0.57%) was significantly lower than that in AOD group (3.41±0.72%; P<0.01) or in HCs (3.69±0.82%; P<0.01). A comparison of FOXP3 mRNA and protein expression revealed significantly lower levels in CD4⁺CD25⁺ Tregs from AAA group than either of other 2 groups (P<0.01). Suppressive function assay showed that freshly isolated CD4⁺CD25⁺ Tregs from patients with AAA exhibited significantly less suppressive activity than those from AOD patients or HCs (P<0.01). Mixing cultures with CD4⁺CD25⁺ T cells and CD4⁺CD25⁻ T cells from AAA patients and HCs demonstrated that the primary regulatory defect is due to a dysfunction of CD4⁺CD25⁺ Tregs, and not a resistance of CD4⁺CD25⁻ responder T cells to suppression in AAA patients.

Conclusion—Our data demonstrate a reduced level of FOXP3 expression in peripheral CD4⁺CD25⁺ Tregs and decreased frequency of CD4⁺CD25⁺FOXP3⁺ T cells in a cohort of AAA patients enrolled in the study, which leads to a functional deficiency of CD4⁺CD25⁺ Tregs as a whole. This indicates an impaired immunoregulation by Tregs that may contribute to AAA pathogenesis. (Arterioscler Thromb Vasc Biol. 2010;30:1825-1831.)

Key Words: aneurysms ■ FOXP3 ■ T regulatory cell

Abdominal aortic aneurysms (AAAs) are a major cause of morbidity and mortality in the Western countries.¹ The incidence and prevalence in China have been increasing significantly during last decade.² The mechanism of this disease still remains unclear. Despite surgical advances, the recent interest in developing new therapies for treating small, asymptomatic, nonoperation-indicated AAA has led to greater efforts to investigate and define its cellular and molecular nature. Increasing evidence shows that immune response contributes importantly to aneurysmal disease, and AAAs are noted for many features of autoimmune disease, including genetic predisposition, organ specificity, and chronic inflammation.³⁴

See accompanying article on page 1679

Our previous study shows that activated T lymphocytes in the peripheral blood of AAA patients are resistant to Fas-induced apoptosis,⁵ and a study by Duftner et al shows that a certain subset of T lymphocytes (CD28⁻) is significantly expanded in AAA patients, and the T cells display a reduced spontaneous apoptosis.⁷ The process of apoptosis is important both for the elimination of autoreactive lymphocytes and the downregulation of appropriately generated immune responses. Thus these data suggest a deficiency of the immune response–silencing system where potentially pathogenic, autoreactive T cells have the capacity to survive and proliferate in AAA patients and induce a process of autoimmune injury related to the dilation of the abdominal aorta. On the other hand, recent studies of autoimmune diseases have provided strong evidence for the existence of a unique CD4⁺CD25⁺ population of naturally occurring regulatory T cells that actively prevent both the activation and the effector function of autoreactive T cells that have escaped tolerance mechanisms.⁸⁻¹⁰ A defect of this population, however, leads to the spontaneous development of various autoimmune diseases, both organ specific and systemic.¹¹ In human peripheral blood, CD4⁺CD25⁺ regulatory T cells (Tregs) constitute 3% to 10% of the CD4⁺ T cells.¹¹⁺¹² Although Tregs also include

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1825
other subgroups, CD4+CD25+ Tregs are the most important and represent a unique lineage critical for maintaining central tolerance, as well as exerting regulatory function in the peripheral immunity. In particular, the generation of CD4+CD25+ Tregs in the immune system is developmentally and genetically controlled, as recent studies have identified that forkhead/winged-helix family of transcription factor FoxP3 (forkhead box P3, Foxp3; FoxP3 for mouse, and FOXP3 for human) is essential for their thymic development and is sufficient to activate a program of suppressor function in peripheral CD4+CD25+ T cells by ectopic expression. Deficiency of FoxP3, which primarily affects the development or function of CD4+CD25+ Tregs, can be a primary cause of autoimmune and other inflammatory disorders in humans. Indeed, defects in CD4+CD25+ Treg cells, expression reduction, and mutation in FoxP3 gene have been identified in autoimmune liver disease, autoimmune thyroiditis, systemic lupus erythematosus, rheumatic arthritis, autoimmune diabetes, immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance, inflammatory bowel disease, and other autoimmune disorders. Importantly, a recent investigation using an animal model highly suggests that natural CD4+CD25+ Tregs are efficient inhibitors of AAA formation in mice and may even constitute an important therapeutic target.

Evidence to date suggests the possibility that the emergence of autoimmune disease in such as AAs could be related to deficient function of Tregs. An earlier study, focused on CD3+ T cells in AAA patients, indicated that the proportion of circulating CD4+CD25+ Tregs in AAA patients was not significantly different from that in control subjects. However, the function of CD4+CD25+ Tregs, including FOXP3 expression and regulation, which are critically important for initiation and development of autoimmunity, remains unknown. Therefore, the potential role of Tregs in AAs needs to be fully delineated.

In the current study, we evaluated expression of FOXP3 mRNA and protein and functional suppressive activity in CD4+CD25+ Tregs isolated from blood mononuclear cells of AAA patients and compared them with control groups of abdominal aortic atherosclerotic occlusive disease (AOD) patients and healthy controls (HCs). We found a functional defect in CD4+CD25+ Tregs, as well as a significant reduction of FOXP3 expression in AAA patients.

Materials and Methods

Human Subjects

Patients with a diameter of the abdominal aorta (measured by computer tomography scan or MRI) larger than 3.5 cm (AAA, n = 22, 17 males and 5 females, 50 to 82 years old) were prospectively recruited into the study as the AAA group. All AAs were accompanied by atherosclerotic change. Patients with Ehlers-Danlos syndrome, Marfan syndrome, other known vascular disorder, or connective tissue disorders were excluded, and patients in the study had no evidence or medical history of other autoimmune diseases.

For normal control group, age- and sex-matched HC individuals (n = 32, 23 males and 9 females, 45 to 73 years old) were selected. They had a relative healthy peripheral vascular system checked by ultrasoundography and no evidence or medical history of aneurysm, AOD, or other vascular disorder. Exclusion criteria also included cancer, infection, and any other immune-mediated disease.

Because AAs were also accompanied by prominent atherosclerosis, age- and sex-matched infrarenal AOD patients (n = 11, 8 males, 3 females, 49 to 70 years) were selected to be in the second control group. They had no evidence or medical history of aneurysmal disease, other vascular disorders, connective tissue disorders, or known autoimmune diseases. AOD was confirmed by CT scan or MRI. This control group served in part to exclude the interference of atherosclerosis to which recent attention has been given for the possible autoimmune mechanisms especially in early lesions.

All subjects gave informed consent before blood sampling. The study was approved by the Institutional Review Board at China Medical University and the local ethical committee.

Flow Cytometric Analysis

Peripheral blood mononuclear cells (PBMC) were isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation (TBD Sciences, Tianjin, China). For surface marker analyses, PBMC were incubated for 30 minutes at 4°C with a cocktail of 3 fluorescent monoclonal antibodies (BD Biosciences, San Jose, CA) directed to human CD25 (allophycocyanin), CD3 (peridinin chlorophyll protein), or CD4 (fluorescein isothiocyanate). For intracellular staining of FOXP3, cell surface staining having first been completed, cells were subsequently fixed and permeabilized according to the FOXP3 staining buffer set protocol before anti-human FOXP3 (clone PCH101; eBioscience, San Diego, CA) was added for 30 minutes at 4°C. Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences). Isotype-matched antibodies were used as controls. A minimum 20,000 events in the lymphocyte gate (forward scatter/side scatter) were acquired on a FACSCalibur flow cytometer and analyzed using Cell Quest software (BD Biosciences).

Isolation of T-Cell Subpopulations

PBMC were isolated as previously described. To further isolate T cell subpopulations, PBMC were incubated for 30 minutes with CD25-(allophycocyanin) – and CD4-(fluorescein isothiocyanate)-labeled antibodies (BD Biosciences) at 4°C. CD4+CD25+ Tregs were sorted as the top 2% of CD4+ T cells (showing the brightest expression of CD25) using a FACSAria cell sorter (BD Biosciences). In parallel, CD4+CD25- T cells were isolated as responder T cells. The purity of cell fractions sorted using a fluorescence-activated cell sorter was routinely >95% for each T-cell fraction.

FOXP3 Expression

Real-Time Polymerase Chain Reaction

T-cell subpopulations were analyzed for FOXP3 expression by using real-time polymerase chain reaction (PCR). Total RNA was extracted from frozen cell pellets with an RNeasy Kit (Takara Bio, Shiga, Japan). The purity of cell fractions sorted using a fluorescence-activated cell sorter was routinely >95% for each T-cell fraction.
Western Blot Analysis
For immunoblots, T cell subpopulations were lysed in lysis buffer (25 mMmool/L Tris-Ci, pH 8.8, 1 mmool/L ethylenediaminetetraacetic acid [EDTA], and 2% sodium dodecyl sulfate [SDS]) and analyzed by Western blotting with 10% SDS-polyacrylamide gel electrophoresis gels. The membrane was incubated with mouse anti-human FOXP3 monoclonal antibody (1:1000; Santa Cruz Biotechnology) overnight at 4°C in Tris-buffered saline with 0.1% Tween 20 plus 5% freeze-dried milk, horseradish peroxidase–labeled secondary antibody (1:1000 goat anti-mouse IgG, Santa Cruz Biotechnology) and the ECL Bio-Imaging systems (MF-ChemiBis 3.2, DNR, Jerusalem, Israel) were used for detection of FOXP3 protein. Actin was subsequently detected with mouse antianti antibody (Takara) and horseradish peroxidase–labeled secondary antibody (Santa Cruz Biotechnology) as an internal control.

Treg Suppression Assay
CD4+CD25+ Tregs purified from PBMC were used as suppressor cells, and the CD4+CD25+ fraction was used as responder cells. All suppression assays were performed in 96-well round-bottom plates in a final volume of 200 μL/well of RPMI 1640 medium (GIBCO, Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, Beijing, China), 10 mMmool/L HEPES, 1000/μL penicillin G, 100 μg/μL streptomycin (Solarbio, Beijing, China), and 20 U/mL recombinant human interleukin-2 (eBioscience). For assay setup, CD4+CD25+ Tregs were cultured with CD4+CD25+ T cells at different ratios (1:0.2, 1:0.4, 1:1, 1:2), and a maximum suppressive capacity of suppressor cells was obtained generally at a ratio of 1:1 (AAA, 31.1% to 73.4%; AOD, 67.4% to 85.7%; HCs, 76.5% to 91.3%). Therefore, isolated suppressor cells were usually cultured with responder cells at a ratio of 1:1 if not otherwise indicated. Before the assay, 9 wells each in the 96-well plates were coated with 100 μL of a final concentration of 5.0 μg/μL anti-CD3 (Santa Cruz Biotechnology), and the plates were incubated overnight at 4°C. All wells were washed before assay setup. The CD4+CD25+ T cells were plated at 2.0×10^4/well alone or in combination with CD4+CD25+ Tregs in triplicate at 2.0×10^4/well, and the CD4+CD25+ Tregs were cultured alone at 2.0×10^4/well. To the wells containing 5 μg/μL plate-bound anti-CD3 mAb, 1.0×10^5 irradiated (3,000 rads) PBMC were added as antigen-presenting cells. Cell proliferation was measured using a modified 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyltetrazolium bromide assay with a Premix WST-1 Cell Proliferation Assay System (Ktaka), which is based on the cleavage of the tetrazolium salt WST-1 to soluble formazan dye by mitochondrial dehydrogenases in viable cells. The formazan dye produced by viable cells can be quantified as OD value in a microplate ELISA reader (Sunrise Remote, TECAN, Austria) by measuring the absorbance of the dye solution at 450 nm with a reference wavelength at 630 nm. Percentage suppression was determined at a 1:1 ratio compared with responses of CD4+CD25+ Tregs (suppressor cells) and CD4+CD25− T cells (responder T cells) alone, as follows: [mean OD value (responder cells)−mean OD value (mixed cell culture)]/mean OD value (responder cells)−mean OD value (suppressor cells)×100%.

To determine any possibility of an increased resistance of CD4+CD25− T cells to inhibition in AAA patients, we also carried Tregs purified from PBMC were used as suppressor cells. As a result, the mean frequency of CD4+CD25+ Tregs in AAA patients, AOD patients, and HCs were nearly identical (median, 5.55% [5.69±0.99%] versus median, 5.50% [5.52±1.13%] versus median, 5.65% [5.88±1.55%]), with no significant difference between them (P>0.05; Figure 1C). However, the frequency of CD4+CD25+ FOXP3+ T cells in AAA patients (median, 2.45% [2.45±0.57%]) was significantly lower than that in AOD patients (median, 3.40% [3.41±0.72%]; P<0.01) or HCs (median, 3.80% [3.69±0.82%]; P<0.01; Figure 1D). Thus, AAA patients showed a reduced number of blood circulating CD4+CD25+ FOXP3+ T cells. AOD patients, as compared with HCs, showed a trend toward lower levels of CD4+CD25+ FOXP3+ T cells that did not reach statistical significance (P>0.05).

Expression of FOXP3 in CD4+CD25+ Tregs
We measured and compared the expression of FOXP3 in the CD4+CD25+ Tregs fraction of PBMC with mRNA detection assays in 17 AAA patients, 11 AOD patients, and 28 HCs. The comparison of FOXP3 mRNA expression by quantitative real-time PCR revealed lower message levels in CD4+CD25+ Tregs from AAA patients than those from AOD patients or HCs with significant difference (P<0.01; Figure 2). Similarly, study by Western blot showed a consistent significant reduction of FOXP3 protein expression in CD4+CD25+ Tregs from the 10 AAA patients compared with those from the 10 AOD patients or 10 HCs (AAA, 31.1±5.7% versus AOD, 48.4±10.6% or HC, 53.3±7.6%; P<0.01; Figure 3). AOD patients showed lower levels of FOXP3 than HCs but with no significant difference (P>0.05).

Suppressive Function of CD4+CD25+ T Cells
To evaluate functional suppression of CD4+CD25+ T cells from AAA, AOD, and HC groups, we stimulated CD4+CD25− responder cells with CD4+CD25+ Tregs at a ratio of 1:1 with the concentrations (5.0 μg/mL) of plate-bound anti-CD3 antibody. Freshly isolated CD4+CD25+ Tregs from AAA patients showed the same low proliferative response to immobilized anti-CD3 as the AOD patients or the HCs (P>0.05), whereas freshly isolated CD4+CD25− T cells from AAA patients showed the same high proliferative response to immobilized anti-CD3 as the AOD patients or the HCs (P>0.05; Figure 3A). The regulatory properties of CD4+CD25− T cells were investigated by testing their ability to suppress the proliferative responses of CD4+CD25+ T cells to immobilized anti-CD3. At a ratio of 1:1, proliferation of CD4+CD25− responder T cells from AAA was significantly higher than proliferation of those from AOD or HCs after coculture with

Statistical Analysis
The Mann-Whitney U test was used to evaluate differences in categoric variables. All values were expressed as mean±SD. Error bars in all figures indicate SD. Statistical significance was accepted at P<0.05.

Results
Frequency Analysis of Circulating CD4+CD25+ Tregs and CD4+CD25+FOXP3+ T Cells
We analyzed peripheral blood from 22 AAA patients, 11 AOD patients, and 32 HC donors to determine the percentage of CD4+CD25+ Tregs and CD4+CD25+FOXP3+ T cells in the total CD4+ T-cell population by means of flow cytometry. In AAA patients, the CD4+CD25+ Tregs represented 3.7% to 7.6% of CD4+ T cells; the CD4+CD25+FOXP3+ T-cell population comprised 1.4% to 3.4% of CD4+ T cells. In AOD patients, the CD4+CD25+ Tregs represented 3.8% to 7.3% of CD4+ T cells; the CD4+CD25+FOXP3+ T-cell population comprised 2.1% to 4.7% of CD4+ T cells. In HCs, the CD4+CD25+ Tregs represented 3.6% to 10.8% of CD4+ T cells; the CD4+CD25+FOXP3+ T-cell population comprised 1.9% to 5.6% of CD4+ T cells (Figure 1A and 1B). As a result, the mean frequency of CD4+CD25+ Tregs in AAA patients, AOD patients, and HCs were nearly identical (median, 5.55% [5.69±0.99%] versus median, 5.50% [5.52±1.13%] versus median, 5.65% [5.88±1.55%]), with no significant difference between them (P>0.05; Figure 1C). However, the frequency of CD4+CD25+ FOXP3+ T cells in AAA patients (median, 2.45% [2.45±0.57%]) was significantly lower than that in AOD patients (median, 3.40% [3.41±0.72%]; P<0.01) or HCs (median, 3.80% [3.69±0.82%]; P<0.01; Figure 1D). Thus, AAA patients showed a reduced number of blood circulating CD4+CD25+ FOXP3+ T cells. AOD patients, as compared with HCs, showed a trend toward lower levels of CD4+CD25+ FOXP3+ T cells that did not reach statistical significance (P>0.05).

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autologous CD4⁺CD25⁺ Tregs (P<0.01, Figure 4A). CD4⁺CD25⁺ Tregs from either AOD patients or HCs inhibited the proliferation of autologous CD4⁺CD25⁻ T responder cells (AOD, n=7, median, 77.26%; mean±SD, 78.16±6.23%; HC, n=10; median 82.83%; mean±SD, 81.97±6.93%; Figure 4B), indicating that CD4⁺CD25⁺ Tregs from AOD patients and HCs have similar direct suppressive effects on T cells (P>0.05). However, as shown in Figure 4B, freshly isolated CD4⁺CD25⁺ Tregs from AAA patients exhibited significantly reduced suppressive activity (n=10, median, 42.88%; mean±SD, 46.68±13.46%) than those from AOD patients or HCs (P<0.01).

To determine whether the deficiency of regulatory function in AAA was explained by a decrease in the intrinsic function of CD4⁺CD25⁺ T cells or by an increase in the resistance of CD4⁺CD25⁻ responder T cells to inhibition, we conducted mixing experiments with cells from 7 AAA patients and 7 HCs. CD4⁺CD25⁺ Tregs from patients with AAA failed to suppress the proliferation of autologous CD4⁺CD25⁻ T responder T cells as well as CD4⁺CD25⁻ responder T cells from HCs, whereas CD4⁺CD25⁺ Tregs from HCs readily suppressed the proliferative response of CD4⁺CD25⁻ responders from AAA patients (Figure 5). These data clearly indicate that the primary regulatory defect is due to a dysfunction of CD4⁺CD25⁺ Tregs isolated from PBMC of AAA patients and not to resistance of AAA CD4⁺CD25⁻ responder cells to suppression.

In study of AAA group, we also tried to detect the possible association of CD4⁺CD25⁺FOXP3⁺ T-cell prevalence, FOXP3 expression, and suppressive function as reported above with AAA size and patient age, but we found no definite correlation.

**Discussion**

It is now well established that a small population of CD4⁺ T cells, identified by the coexpression of CD25, has the ability to regulate immune responses. These Tregs have been found and characterized in humans and rodents, and FoxP3 expres-
sion is required for Treg development and is found to confer suppressive function on peripheral CD4^+CD25^+ Treg cells. Furthermore, studies in both humans and mice have demonstrated that defective regulatory T-cell function contributes to autoimmune diseases in both animal models and human disease. Importantly, Treg function in humans largely resides in the fraction of CD4^+CD25^+ T cells that express the highest density of CD25. Therefore, we sought to determine whether a defect in CD4^+CD25^+ Tregs occurs in patients with AAs. Although our finding reveals no significant alterations in the frequency of CD4^+CD25^+ Tregs in AAA patients as previous reports show, our data demonstrate for the first time a functional defect in CD4^+CD25^+ Tregs in AAA patients. To exclude any probability of an increased resistance of CD4^+CD25^+ T cells to inhibition in AAA patients, we also performed mixing cultures with cells from AAA patients and HCs. The results show that the primary regulatory defect is due to a decrease in the intrinsic function of CD4^+CD25^+ Tregs in AAA patients rather than the resistance of AAA CD4^+CD25^+ responder cells to suppression.

Autoimmunity arises in genetically susceptible individuals owing to the loss of self-tolerance and the expansion of autoreactive lymphocytes that lead to organ or tissue damage. The inflammatory infiltrate in the media and adventitia of the aortic wall in AAs is dominated by CD4^+ T-helper cells exhibiting a unique activated memory phenotype and producing proinflammatory cytokines. Local activation might therefore occur within the aortic wall, giving rise to a unique subset of infiltrating lymphocytes with an unrestricted repertoire and clonal expansion of T cells with T-cell receptors. In AAA patients, a higher level of T-cell subset (CD4^+CD28^-) has also been identified in peripheral blood. These cells, also appearing in AAA tissue and producing high levels of interferon-γ and perforin, could initiate and participate in aortic tissue injury. Our previous data suggest that expansion of autoreactive T cells might result from their resistance to Fas-induced apoptosis. This expansion and broken self-tolerance, on the other hand, may be due to deficiency or dysfunction of Tregs as well.

**Figure 3.** FOXP3 protein expression in CD4^+CD25^+ Tregs from AAA patients, AOD patients, and HCs. A, Upper panel shows Western blot results from 3 AAA patients, 2 AOD patients, and 2 HCs as a representative of Western blot analysis of subjects from the 3 groups. FOXP3 protein stains as 2 bands of approximately 50 kDa. B, Mean FOXP3 protein expression percentage±SD. There was a highly significant difference in the FOXP3 protein levels in AAA versus the AOD and HC groups (P<0.01).

**Figure 4.** CD4^+CD25^+ Tregs from AAA patients failed to suppress proliferation of CD4^+CD25^- responder T cells. CD4^+CD25^- responder cells and CD4^+CD25^+ Tregs were cultured with plate-bound anti-CD3 either alone or at a 1:1 ratio. A, After 72 hours, proliferation was measured by modified 3-[4,5-dimethyl-2-thiazol-yl]-2,5-diphenyltetrazolium bromide. Results are the mean±SD of separate experiments using AAA patients (n=10), AOD patients (n=7), and HCs (n=10). The proliferation of CD4^+CD25^- responder T cells from AAA was significantly higher than that from AOD patients or HCs after coculture with autologous CD4^+CD25^+ Tregs (P<0.01). B, Significantly different percentage of inhibition of proliferation between AAA and control groups (P<0.01).
investigated further. The polyclonal nature of infiltrating CD4+ T cells yields no definite result. This reflects a complicated process that involves the naïve autoreactive T cells. Studies have shown that CD4+ T cells from HCs readily suppress the proliferative response of autologous CD4+ responder T cells as well as CD4+ responder T cells from patients with AAA, whereas CD4+ T cells from patients with AAA fail to suppress the proliferation of autologous CD4+ T cells as well as CD4+ T cells from HCs, whereas CD4+ T cells from patients with AAA fail to suppress the proliferation of autologous CD4+ T cells.

Under normal physiological conditions, the immune system is maintained by deletion of autoreactive T cells from the developing repertoire within the thymus. However, some autoreactive T cells can enter the peripheral circulation, where they are normally kept in check by fail-safe mechanisms with Tregs, which suppress the proliferation of naïve autoreactive T cells. The defect of circulating Tregs in AAA patients, together with local and general T-cell activation and expansion, could explain the altered immunoregulation with autoimmune responses against abdominal aortic wall.

Interestingly, the reason why T cells initiate and propagate an immune response against components of the aortic wall remains unknown. Investigations aimed at identification of specific autoantigen responsible for T-cell activation and recruitment have so far raised a few possible candidates but yielded no definite result. This reflects a complicated process of aneurismal development, the polyclonal nature of infiltrating lymphocytes and the oligoclonal T-cell specificity, and argues against the previous dedication to a single specific antigen that invokes an autoimmune process in AAAs. Further molecular sequencing and structural identification of autoantibodies might facilitate the identification of certain individuals at risk of developing AAA. The CD4+ CD25+ Treg cells, however, have diverse T-cell receptor repertoires, suggesting that they are capable of responding to a wide spectrum of antigens. Studies have also shown that CD4+ CD25+ Tregs suppress proliferation and cytokine production to both self- and foreign antigens, and the depletion of Tregs combined with accumulated autoantigens caused severe autoimmune responses in a normal animal model. Thus, defective antigen-specific Treg cells may contribute to the pathogenesis of AAAs, and the antigen-specific suppression of CD4+ CD25+ Tregs deserves being investigated further.

A major mechanism to explain functional defect of CD4+ CD25+ Tregs is deficiency of upstream control by FOXP3. Foxp3 expression both drives the differentiation of Treg and controls regulatory function. It provides a precise marker for murine Treg identification. Mutant or knockout mice lacking foxp3 gene function develop fatal, multiorgan inflammatory infiltrates. Currently, the increasing prevalence and unsatisfactory treatment of autoimmune diseases have led to greater efforts to investigate and define the cellular and molecular nature of autoimmune diseases. How FOXP3 proteins are involved in maintaining peripheral immune tolerance and prevention of autoimmunity such as AAA is still long and not smooth, there are great promises and excitement over the horizon.

The present study also shows a significant difference between AAA and AOD patients in phenotypic and functional profile of CD4+ CD25+ Tregs. The deficient CD4+ CD25+ Treg function in AAA patients suggests the unique way of AAA development, which may be different from AOD and independent of atherosclerosis. This question has been debated for quite a long time. Furthermore, the significant difference observed between AAA and AOD in respect to inflammatory infiltration and immune response within the aortic wall might be an important factor to influence the direction in which the arterial disease develops: dilation or obstruction.

In conclusion, our study shows a reduced level of FOXP3 expression in peripheral CD4+ CD25+ T cells and decreased frequency of CD4+ CD25+ FOXP3+ T cells in patients with AAAs, which is related to a reduction in functional suppression in Tregs as a whole. This observation is the first to link a defect in functional immunoregulation to an established genetic marker that has been unequivocally shown to be involved in maintaining peripheral immune tolerance and preventing autoimmune diseases. How FOXP3 proteins are regulated in the Treg cells remains to be defined further, but it is possible that regulatory mechanisms are altered during the course of AAA.

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Disclosures
None.

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