Immunoregulatory therapy in anti-neutrophil cytoplasmic antibody-associated vasculitis: Low-dose Interleukin-2 therapy increases the reduced Treg cells

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Research article

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Abstract

Objective: The breakdown of immune tolerance caused by dysfunctional CD4+T cells is one of the key mechanisms to participate in the pathogenesis and progression of autoimmune diseases, especially the reduced regulatory T (Treg) cell is associated with the breakdown of immune tolerance. Low-dose Interleukin-2 (IL-2) therapy has been confirmed to be a potential therapy to treat autoimmune diseases by selectively expanding Treg cells to restore the immune tolerance. However, it is still unclear whether Treg cells play an important role in the progression of anti-neutrophil cytoplasmic antibody-(ANCA)-associated vasculitis (AAV), and whether low-dose IL-2 therapy contributes to restore the immune tolerance and promote the remission of disease in AAV. The aim of our study is to explore the role of Treg cells in the progression of AAV and evaluate the efficacy of low-dose IL-2 therapy on AAV.

Methods: We collected the clinical data of 39 patients with AAV (including 12 who received subcutaneous low-dose IL-2 therapy combined with conventional therapies) and 65 healthy controls (HCs) to compare the differences of the absolute number of CD4 + T cell subsets, and then analyze the relationship between these cells and the clinical indicators of disease activity. In addition, we investigated the changes of CD4 + T cell subsets and clinical indicators before and after the treatment of low-dose IL-2 therapy.

Results: Patients with AAV had reduced peripheral Treg cells than HCs ($P<0.001$), which were negatively correlated with the clinical indicators of disease activity and organ injury, and those patients with high disease activity exhibited lower level peripheral Treg cells($P=0.002$). The low-dose IL-2 therapy significantly increased the reduced Treg cells in patients with AAV compared with the baseline values ($P=0.001$) to promote the remission of disease.

Conclusion: The reduced peripheral blood Treg cells participated in the imbalance of immune in patients with AAV and were related to the progression of disease. Low-dose IL-2, as an immunoregulatory therapy, increases the reduced Treg cells to restore the immune tolerance and promote the remission of AAV.

1. Introduction:

Anti-neutrophil cytoplasmic antibody-(ANCA)-associated vasculitis (AAV) is a life-threatening group of autoimmune systematic diseases that includes granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) [1, 2]. The pathological characteristic of AAV is necrotizing small vasculitis mediated by the production of the ANCAs. And these antibodies including c-ANCA targeting to proteinase 3 (PR3) and p-ANCA targeting to myeloperoxidase (MPO)[3–5], which can affect small blood vessels and cause systemic autoimmune inflammatory response leading to multiple organ injuries especially the kidneys, lungs and peripheral nerve. Therefore, it is particularly critical to achieve the early diagnosis and treat the disease. However, the pathogenesis of AAV remains unclear by now.

ANCAs, produced by B cells to target and attack the primary granule constituents of neutrophils and the lysosomes of monocytes [6], have been considered to be the initiator of AAV. However, the ANCA titer is not related to AAV disease activity or predictive of recurrence [7, 8]. Particularly, there are some patients whose clinical features and pathology are consistent with AAV but ANCA testing are negative, can still be diagnosed as AAV [5, 6]. Therefore, there may be other important factors involved in AAV. Recent years, it has been a rising area to study the role of T lymphocytes in AAV. T cells play an important regulatory role in helping B cells to synthesize high-affinity antibodies to exert humoral immune response, and the insufficient regulation of T cells can lead to the deficiency of adaptive immune and promote the autoimmune inflammatory response [9, 10]. And in animal models, T cells were observed to infiltrate into kidney and other tissues in patients with AAV [11].Thus, T cells may be involved in the development of AAV.

CD4 + T cell subsets, which are critical regulators of the adaptive immune response, are implicated in the pathogenesis of autoimmune diseases. Naïve CD4 + T cells can differentiate into several distinct subsets including T-helper (Th)1, Th2, Th17 and regulatory T (Treg) cells [12].After the in-depth study of CD4 + T cell subsets, it is found that Th17 and Treg cells can exert opposite effects. Th17 cells have the ability to produce many pro-inflammatory cytokines and stimulate specific B cells to produce autoantibodies, and the increased level of Th17 cells can promote the overproduction of pro-inflammatory cytokines and autoantibodies resulting in systemic inflammation and autoimmune response finally[13, 14]. By contrast, with the presentation of the novel concept of immune tolerance, CD4 + CD25 + Foxp3 + Treg cell has gradually become a field of interest attracted a lot of attention. It has been confirmed that Treg cells are important for suppressing excessive immune responses and maintaining immune tolerance by inhibiting the stimulatory capacity of antigen-presenting cells and producing anti-inflammatory cytokines [15–17]. The reduced levels of peripheral blood Treg cells have been reported in some autoimmune disease, which has been considered to be the more important factor leading to the breakdown of

immune tolerance and the development of autoimmune diseases [18, 19]. Thus, the imbalance of Th17 and Treg cells has been confirmed to be the main force in the development of autoimmune diseases [20–22]. However, the effect of Th17 and Treg cells exerted in the development of AAV has not been well defined now.

Interleukin (IL)-2, a T cell growth factor, has the unique ability to promote the development, proliferation and differentiation of T cells after combining with IL-2 receptors (IL-2Rs),which consists of IL-2Rα (also known as CD25), IL-2Rβ (also known as CD122), and IL-2Rγ (also known as CD132). Thus, IL-2Rs can be divided into low affinity receptor (only IL-2Rα), intermediate affinity receptors (containing IL-2Rβ and IL-2Rγ) and high affinity receptors (containing IL-2Rα, IL-2Rβ and IL-2Rγ) [23–25],which have different affinities for binding to IL-2. In these three categories, intermediate affinity receptor is present mostly on CD8 + T cells and NK cells, which has a low affinity for IL-2 and requires high-dose IL-2 to bind to it, while high affinity receptor is expressed on the activated lymphocyte such as Treg cells, which has a high affinity for IL-2 (dissociation constant (Kd) $\approx 10^{-11}$ M) and can be easily activated by low-dose IL-2 [26]. It has been confirmed that IL-2 has pleiotropic function act and the dose of IL-2 may be a driver of the imbalance between autoimmunity and immune tolerance, which means that IL-2 can act as a pro-inflammatory factor to promote autoimmune inflammatory response under the high-dose IL-2 on the one hand, and act as an anti-inflammatory factor to maintain immune tolerance under the low-dose IL-2 on the other hand [23, 24]. The therapies targeted the recovery of the number or function of Treg cells have become a hot topic in clinical trials [19, 27] and the effect of low-dose IL-2 has essential role in the proliferation, differentiation and function of Treg cells [27–29], which is a significant impact on immunology research. A single and open clinical trial have showed that low-dose IL-2 had a broad therapeutic potential to treat 11 autoimmune diseases effectively and safely by expanding and activating Treg cells[30], and which also has been confirmed in other autoimmune diseases [31–34].And we have demonstrated that low-dose IL-2 has potential therapeutic prospects in alleviating the disease activity of rheumatoid arthritis by expanding the number of Treg cells to restore the balance of Th17/Treg in the peripheral blood[25].But the effect of low-dose IL-2 therapy in AAV is still unknown, which needs further exploration.

Our study assessed the absolute number of CD4 + T cell subsets in patients with AAV and HCs by flow cytometry and analyzed the relationship between the cells and disease activity indicators to explore the role of Treg cells in the progression of AAV. And we compared the changes of CD4 + T cell subsets before and after the low-dose IL-2 therapy to evaluate the effect of it on CD4 + T cell subsets. In addition, we also compared the changes of disease activity before and after the low-dose IL-2 therapy to evaluate its efficacy on AAV.

2. Materials And Methods:

2.1. Patients and HCs

We enrolled 39 patients with AAV (18 females and 21 males) including 5 GPA, 18 MPA and 3 EGPA who were admitted to the Second Hospital of Shanxi Medical University from May 2016 to April 2020, and 65 sex and age matched HCs (27 females and 38 males).The patients all met the criteria of 2007 European Medicine Agency algorithms for AAV[35] (Table 1) and the exclusion criteria of patients included any other autoimmune/inflammatory disease, a malignant tumor, cancer and active HIV infections. This study was conducted in accordance with all relevant principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Second Hospital of Shanxi Medical University (Approval (2019) KY No. (105)).All subjects provided a written informed consent.

Table 1
A summary of baseline demographics and clinical characteristics of all enrolled patients with AAV and healthy controls

	Patients(n = 39)	HCs(n = 65)	P Value
Sex(Female/Male)	18/21	27/38	0.649
Age(years)[a]	60.13 ± 1.12	56.46 ± 2.24	0.107
GPA	5	-	-
MPA	18	-	-
EGPA	3	-	-
IL-2/non-IL-2 group	12/27	-	-
p-ANCA$^+$ (n, n%)	24(61.5%)	-	-
c-ANCA$^+$ (n, n%)	8(20.5%)	-	-
MPO$^+$ (n, n%)	11(28.2%)	-	-
PR3$^+$ (n, n%)	5(12.8%)	-	-
ESR(mm/h)[b]	95.00(33.00,120.00)	-	-
CRP(mg/L) [b]	24.85(6.00,100.325)	-	-
BUN(mmol/L) [b]	5.80(4.60,11.90)	-	-
Cr(μmol/L) [b]	79.00(59.00,121.00)	-	-
BVAS [b]	14.00(6.00,17.00)	-	-

a Results are expressed as the mean ± standard error. b Results are expressed as the median(Q1,Q3).

Normally distributed continuous variables were analyzed by the independent-samples Student's t-test. And nonparametric variables were analyzed by Mann–Whitney U test

2.2. Study design and clinical indicators

Clinical indicators for accessing the disease activity and organ injury included erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP, mg/L), blood urea nitrogen (BUN, mmol/L) and creatinine (Cr, μmol/L), which all obtained by routine laboratory examinations (Table 1).The patients were grouped into low disease activity group (BVAS < 16, n = 21) and high disease activity group (BVAS ≥ 16, n = 18) according to the Birmingham Vasculitis Activity Score (BVAS) [36] system, which has been thought as a more reliable indicator of disease activity by scoring the degree of involvement of organs in AAV. And the absolute number of CD4 + T cell subsets in different groups was detected by flow cytometry (Table 2). All patients received glucocorticoids and disease-modifying anti-rheumatic drugs (DMARDs), and in which 12 patients were received subcutaneous low-dose IL-2 treatment [500,000 international units (MIU)/day for 5 days].Based on it, patients were divided into IL-2 group (n = 12) and non-IL-2 group (n = 27) (Table 3).The laboratory examinations were collected before and after the treatment for all patients, but only the absolute number of CD4 + T cell subsets in the IL-2 group was reassessed within 2 days after treatment.

Table 2
The absolute number of CD4 + T cell subsets in different group

Cells/μL	HCs (A) (n = 65)	Patients (B) (n = 39)	Low disease activity (C) (n = 21)	High disease activity (D) (n = 18)	*p* value (A vs. B)	*p* value (C vs. D)
Th1 [a]	125.72(77.24,164.16)	84.43(52.36,168.78)	112.93(50.70,183.72)	82.60(48.73,128.42)	0.162	0.568
Th2 [a]	7.49(4.68,10.43)	5.57(2.75,8.51)	7.03(5.13,9.62)	3.23(2.10,6.39)	0.042*	0.012*
Th17 [a]	6.13(3.71,8.62)	4.57(2.76,7.15)	5.05(2.91,12.72)	3.84(2.49,6.33)	0.212	0.133
Treg [a]	33.58(25.75,46.19)	22.05(15.88,28.79)	25.32(20.34,35.65)	16.63(10.16,22.71)	P < 0.001***	0.002**
Th1/Th2 [a]	14.83(8.97,24.99)	13.12(6.97,26.32)	15.99(6.77,24.52)	16.18(10.59,33.63)	0.692	0.394
Th17/Treg [a]	0.17(0.11,0.26)	0.21(0.12,0.42)	0.21(0.11,0.50)	0.25(0.16,0.36)	0.03*	0.666
a Results are expressed as the median(Q1,Q3). Nonparametric variables were analyzed by Mann–Whitney U test						

Table 3
A summary of baseline demographics and medication of IL-2 group and non-IL-2 group

	IL-2 group(n = 12)	Non-IL-2group(n = 27)
Sex(Female/Male)	4/8	14/13
Age(years)[a]	59.42 ± 13.99	59.38 ± 14.51
GPA	2	3
MPA	10	8
EGPA	-	3
BVAS ≥ 16 (n, n %)	7(58.3%)	11(40.7%)
p-ANCA$^+$ (n, n%)	9(75.0%)	15(55.6%)
c-ANCA$^+$ (n, n%)	2(16.7%)	6(22.2%)
MPO$^+$ (n, n %)	8(66.7%)	3(11.1%)
PR3$^+$ (n, n %)	1(8.3%)	4(14.8%)
Glucocorticoid (n, n %)	12(100%)	27(100%)
Leflunomide (n, n %)	3(25%)	11(40.7%)
Hydroxychloroquine (n, n %)	4(33.3%)	5(18.5%)
Methotrexate (n, n %)	0(0)	1(3.7%)
Thalidomide (n, n %)	2(16.7%)	2(7.4%)
a Results are expressed as the mean ± standard error.		

2.3. Detection of the absolute number of CD4 + T cell subsets by flow cytometry

Peripheral blood sample of each patient was collected and placed in a heparin anticoagulation tube. Next, 200μl blood sample, 200μl RPMI-1640 and 1μl leukocyte activation kit regent were mixed in a test tube, and the tube was incubate at 37°C under 5% CO2 for 5 hours to detect the Th1, Th2 and Th17 cells. Anti-CD4-FITC was added into the tube to stain at room temperature in the dark for 30

minutes after the lysis of red blood cells and then added Fixation/Permeabilization to place at 4°C in the dark for 30 minutes. And then the tube was divided into tube A and tube B. Anti-IFN-γ-APC and anti-IL-17-PE were added into tube A for intracellular staining in the dark for 30 minutes to detect Th1 cells and Th17 cells respectively. And Anti-IL-4-PE was added into tube B for intracellular staining in the dark for 30 minutes to detect Th2 cells. As for the detection of Treg cells, anti-CD4-FITC and anti-CD25-APC were added into 100μl blood sample after the lysis of red blood cells and incubated at the room temperature in the dark for 30 minutes. Next, the tube was incubated at 4°C for 30 min after adding fixation/permeabilization buffer. And then anti-Foxp3-PE was added for intracellular staining for 30 minutes in the dark. All the tubes were washed by phosphate buffer saline (PBS) and tested on the flow cytometry (FACSCanto™ II; Becton Dickinson) within 2h finally (Fig. 1a and Supplement Fig. 1). All antibody reagents were all from BD Biosciences (Supplement Table 1). The relative percentages of CD4 + T cell subsets were analyzed by Cell Quest software, and the absolute number was calculated by multiplying the relative percentages by the absolute number of CD4 + T cells determined using BD Trucount™ tubes containing magnetic beads.

2.4. Statistical analysis

Statistical analysis was performed by SPSS 22.0 and GraphPad Prism 8.0 software. Normally distributed continuous variables were presented as the mean ± standard error and analyzed by the Student's t-test for comparing the differences of patients and healthy controls. And nonparametric variables were presented as median (Q1,Q3), and were analyzed by Mann–Whitney U test for comparing the two groups, Kruskal-Wallis test and one-way analysis of variance (ANOVA) for comparing the three groups. The Spearman's rank correlation was used to calculate the correlation between the absolute number of CD4 + T cell subset and the clinical indicators. All P-values were two-tailed and $P < 0.05$ was considered to be significant.

3. Results:

3.1. Patients with AAV had lower level of Treg cells than HCs

The absolute number of peripheral total CD4 + T cells was similar in patients with AAV (n = 39) and HCs (n = 65) (P = 0.174) (Supplement Fig. 2). Notably, by comparing the absolute number of CD4 + T cell subsets between the patients with AAV and HCs (Fig. 1a), we found that patients with AAV had significantly lower absolute number of Treg cells and Th2 cells in their peripheral blood than HCs ($P < 0.001$ and $P = 0.042$ respectively), while the absolute number of Th1, Th17 cells and the ratio of Th1/Th2 were similar. And the reduced Treg cells in patients with AAV resulted in the imbalance of Th17/Treg (P = 0.03) (Fig. 1b-g and Table 2).

3.2. Reduced peripheral blood Treg cells were correlated with the disease activity of AAV

The correlation analysis between the absolute number of CD4 + T cell subsets and the disease activity of AAV showed that Treg cells were the only cell with a significant difference between the low and high disease activity group, and the latter exhibited a lower level of Treg cells (P = 0.023), while the absolute number of Th1, Th2 and Th17 cells and the ratio of Th1/Th2 as well as Th17/Treg were similar between the two groups (Fig. 2a-f and Table 2). And by evaluating the relationship between the absolute number of CD4 + T cell subsets and the disease activity indicators of AAV, we found that only Treg cells was negatively correlated with all clinical indicators of disease activity and organ injury (Fig. 2g-h). The absolute number of Th1, Th2 and Th17 cells was only related with some clinical indicators and the ratio of Th1/Th2 and Th17/Treg were not related to any disease activity indicators (Supplement Fig. 3a-e).

3.3. Comparison of ROC Curves and AUC of Treg cells in AAV

To evaluate whether peripheral CD4 + T cell subsets are a good indicator for monitoring the occurrence and recurrence of AAV, we plotted the receiver operating characteristic (ROC) curves of CD4 + T cell subsets and compared the area under curve (AUC) of them(Fig. 3). We found that Treg cells and Th17/Treg were significantly different. And the area under curve of Treg cells and Th17/Treg was 0.7787 and 0.6264 respectively, and 95% confidence interval (CI) of them was 0.6847–0.8727 and 0.5116–0.7413 respectively. But Th1, Th2, Th17 cells and Th1/Th2 had no significant differences.

3.4. The effects of low-dose IL-2 therapy in the treatment of AAV

By comparing the changes of absolute number of CD4 + T cell subsets and disease activity before and after the low-dose IL-2 therapy, we found that the level of Treg cells were increased after the low-dose IL-2 therapy than before treatment (P = 0.001), which had no significant difference compared with that in HCs(Fig. 4d and Table 3). And the ratio of Th17/Treg showed a decreasing trend although it did not reach statistical significance (Fig. 4f). In contrast, the level of other cells had no significant differences among HCs, IL-2 and non-IL-2 group (Fig. 4a-c, e). And the level of ESR and CRP were significantly decreased after the low-dose IL-2 therapy, which exhibited significant improvements in disease activity (Fig. 4g-h).

4. Discussion:

4.1. Reduced peripheral blood Treg cell is associated with the progression of AAV

Current studies believe that the destruction of immune tolerance is one of the key mechanisms that promote the pathogenesis and progression of autoimmune diseases. Available evidence suggests that the balance of Th17 and Treg cells is critical for maintaining the immune homeostasis, but the dysfunction of Th17 or Treg cells may cause the disrupted dynamic balance of the human cytokine network, which in turn contributes to the pathogenesis and development of autoimmune diseases [20–22]. Especially, Treg cell, as an important negative regulatory cell to maintain immune tolerance, the reduction of it can destroy the immune tolerance leading to the progression of autoimmune diseases. And it is worth noting that increasing studies are more inclined that the development of autoimmune response is due to the breakdown of immune tolerance caused by the decreased Treg cells rather than the excessive immune inflammatory response caused by the increased Th17 cells [37, 38]. However, there is rare evidence supporting that reduced Treg cells play a critical role in the development of AAV.

Our study explored the role of T cells in the progression of AAV and the results showed that there was no difference in the level of CD4 + T cells between the patients with AAV and HCs, but the absolute number of these cells was different significantly. It was possible that some cells in the CD4 + T cell subsets were reduced while the level of others were increased, which led to a similar total absolute number of CD4 + T cells. Therefore, we further explored whether CD4 + T cell subsets played an important role in the development of AAV. Our study found that the patients with AAV particularly those with high disease activity had lower level of Treg cells, and Treg cells were significantly negatively correlated with disease activity indicators of AAV. In addition, Treg cells have the largest AUC, which is the best indicator for monitoring the occurrence and development of diseases. These above results indicated that reduced level of peripheral blood Treg cells could result in the further aggravation of the disease activity in AAV, which suggested that reduced peripheral blood Treg cell was involved in the progression of AAV. Previous studies have suggested that Treg cells were the suppressors of the inflammatory process in GPA and impaired function of Treg cell played an important role in the development of it [39, 40]. Consistent with the recent studies, the results of our study further supported that patients with AAV have an imbalance immune homeostasis caused by reduced Treg cell, which is the main factor to promote the progression of disease rather than the excessive inflammation caused by the increased Th17 cells.

Thus, based on the above we speculate that the destruction of immune tolerance caused by the decreased Treg cells can lead to the development of vasculitis and deteriorate the function of organs, which may be one of the main factors involved in the progression of AAV. However, the underlying mechanism of the development of AAV caused by the reduced Treg cells is still unclear, which may be associated with the abnormalities in genome, and regulations of epigenetics, transcription and metabolism. It requires further in vivo and in vitro experiments to explore the specific mechanism.

4.2. Low-dose IL-2 therapy promotes the recovery of AAV by increasing the reduced Treg cells

Immunosuppression is typically used to treat autoimmune diseases because the excessive immune inflammation was thought to be the important factor to lead to the development of these diseases. However, the increase of infection rate and the introduction of the concept of immune tolerance led to a reappraisal of the advantages and disadvantages of immunosuppressive therapy.

Accumulating studies present that IL-2 is at the crossroads of autoimmunity and immune tolerance because it can exert different effects by activating different cells in immune system [41]. IL-2 can activate effector T cells by high-dose to promote autoimmune response and can exert the anti-inflammatory function by low-dose especially preferentially act on Treg cells to maintain immune tolerance [42]. Treg cell constitutively expresses high affinity IL-2 receptor because it expresses high levels of CD25 (IL-2Rα) by itself on

the cell surface[43, 44], while other T cells only acquire CD25 upon activation, which makes it possible that Treg cell has the superior efficacy to compete with other cells to bind low-dose IL-2 [28]. And the combination of low-dose IL-2 and IL-2Rs can promote the expression of Foxp3 by activating the JAK-STAT5 signaling pathway, which is highly dependent on exogenous IL-2 to enhance the proliferation, differentiation and function of Treg cells. In addition, in the early stages of an immune response, Treg cells are able to quickly sense IL-2 produced by activated T cells to promote the survival of Treg cell, stabilize the expression of Foxp3 and enhance the suppressive activity [23]. Thus, low-dose IL-2 therapy can be used to treat autoimmune disease for its great advantages in restoring the number and function of Treg cells to exert anti-inflammatory effect and maintain immune tolerance.

In our study, after the treatment of low-dose IL-2 therapy, the number of Treg cells was significantly increased compared to that than before, which was similar to that in HCs. Interestingly, Th17 cells also increased slightly after low-dose IL-2 therapy, but the ratio of Th17 and Treg cells showed a decreasing trend, which suggested that low-dose IL-2 therapy mainly promoted the growth of Treg cells to maintain immune balance. And the disease activity was significantly alleviated after the low-dose IL-2 therapy. These above results further indicated that low-dose IL-2 therapy in AAV had therapeutic potential to expand Treg cells to restore the immune tolerance and promote the remission of disease. And our study strengthened the concept of immunoregulatory therapy in the treatment of AAV by low-dose IL-2 therapy, but not immunosuppression.

Of note, the appropriate dosage and administration schemes for maintaining a higher blood concentration of IL-2 while minimizing its toxicity is still a problem that needs further research. At present, the dose of low-dose IL-2 in clinical is mostly 1 MIU [30, 31] or 0.5 MIU [34], and there is few side effects, but the administration schemes are not the same. Numerous studies had explored the possible administration schemes for IL-2 by assessing the pharmacokinetics of IL-2 [28], but the application of low-dose IL-2 therapy for treating AAV is still a new attempt. Further clinical trials and animal experiments exploring the dose–response relationship will be required to determine the optimal dose and schedule of low-dose IL-2 on the specificity expansion for Treg cells in patients with AAV in order to achieve and maintain the balance between the autoimmunity and immune tolerance.

There are several limitations in our study. Next, we will conduct a larger follow-up study to measure the plasma cytokines secreted by Treg cells and detect the inhibition of Treg cells in order to better evaluate the function of Treg cells. And we will examine the tissue Treg cells to confirm the effect of Treg cells on organs and other tissues in patients with AAV. In addition, we will conduct in vivo and in vitro experiments to explore the specific mechanism of the pathogenesis of AAV due to the reduction or dysfunction of Treg cells. And in the future, we will start prospective studies to evaluate the long-term efficacy and safety of low-dose IL-2 and explore the IL-2-based approaches (such as IL-2 complexes) for inducing the expansion of Treg cells in vivo.

5. Conclusion:

Patients with AAV had reduced peripheral blood Treg cells, which may play an important role in the progression of AAV and provided a therapeutic target for relieving the disease, but the specific mechanism needs to be confirmed by further in vivo and in vitro experiments. Low-dose IL-2 therapy increased the reduced peripheral blood Treg cells in patients with AAV to restore the immune tolerance and achieve the remission of disease. Therefore, low-dose IL-2 therapy is a potential immunoregulatory therapy in the treatment of AAV. However, the optimal dose and treatment scheme to minimize the toxicity of IL-2 in the treatment of AAV still need further exploration by the clinical application.

Abbreviations

Treg: Regulatory T cells; IL-2: Interleukin-2; ANCA: anti-neutrophil cytoplasmic antibody; AAV: anti-neutrophil cytoplasmic antibody-associated vasculitis; HC: healthy control; GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; PR3: proteinase 3; MPO: myeloperoxidase; Th 1: T-helper 1 cell; Th 2: T-helper 2 cell; Th17: T-helper 17 cell; Foxp3: Forkhead Box P3; IL-2R: Interleukin-2 Receptor; RA: rheumatoid arthritis; DMARDs: disease-modifying anti-rheumatism drugs; MIU: million international units; BVAS: Birmingham Vasculitis Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BUN: blood urea nitrogen; Cr: creatinine; PBS: phosphate buffer saline; ROC: receiver operating characteristic; AUC: area under curve; CI: confidence interval.

Declarations

Ethics approval and consent to participate:

This study was conducted in accordance with all relevant principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Second Hospital of Shanxi Medical University (Approval (2019) KY No. (105)).All subjects provided a written informed consent.

Conflict of interest:

The authors all declare that there is no financial or commercial conflict of interest.

Availability of data and materials:

The dataset analyzed in our paper is available from the corresponding author on reasonable request.

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Author contributions:

RW performed the data analysis, drew illustrations and drafted the manuscript. YM, XZ and JZ participated in the research and data analysis. TD and HX participated in the collection and organization of samples and clinical data. CG and XL participated in the study design and revised of the manuscript. CW conceived the topic of the manuscript, critically revised the content of the manuscript and provided a substantial contribution throughout the study. All authors approved the publication of the manuscript.

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Figures

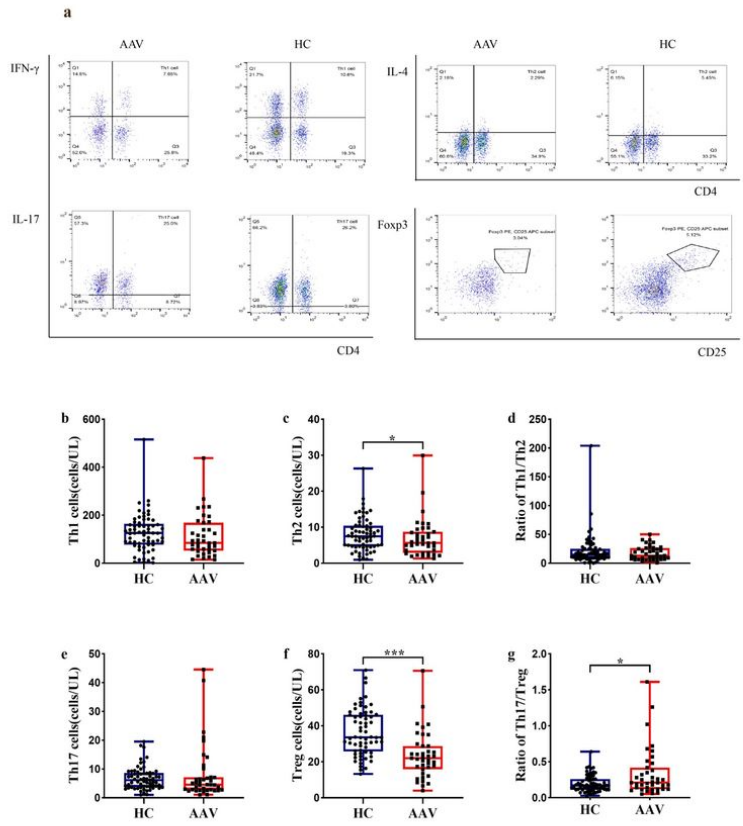

Figure 1

Absolute number of CD4+ T cell subsets detected by flow cytometry in patients with AAV (n=39) and HCs (n=65). (a) Representative fluorescence activated cell sorting diagrams of CD4+ T cell subsets from one patient with AAV and a sex- and age-matched HC. (b-g)Patients had lower Th2 cells (P=0.042) and Treg cells (P<0.001) compared with HCs. And the decreased Treg cells led to the imbalance of Th17/Treg cells (P=0.03). Data were presented as median (Q1, Q3) and were analyzed by Mann–Whitney U test. (*P<0.05; **P<0.01; ***P<0.001)

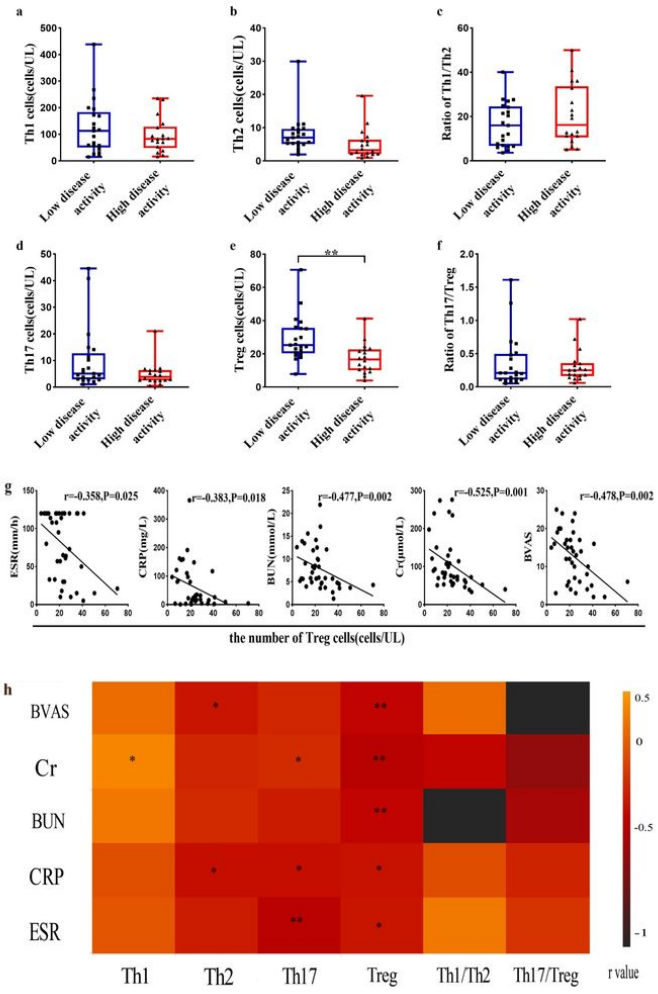

Figure 2

(a-f) Comparison of the absolute number of CD4+T cell subsets between low disease activity group (BVAS<16, n=21) and high disease activity group (BVAS≥16, n=18). The absolute number of Treg cells in high disease activity group was significantly lower than that in low disease activity group (P=0.002). (g) Correlation of the absolute number of Treg cells with the clinical disease activity indicators including ESR, CRP, BUN, Cr and BVAS. The level of Treg cells were negatively correlated with all clinical disease activity indicators. Data were presented as median (Q1, Q3), and were analyzed by Mann–Whitney U test. (h)Heatmap of correlation between the Th1, Th2, Th17, Treg cells and ratio of Th1/Th2 and Th17/Treg with ESR, CRP, BUN, Cr and BVAS. Data was analyzed by Spearman correlation test (Colors indicate the Spearman rank correlation). (*P<0.05; **P<0.01; ***P<0.001)

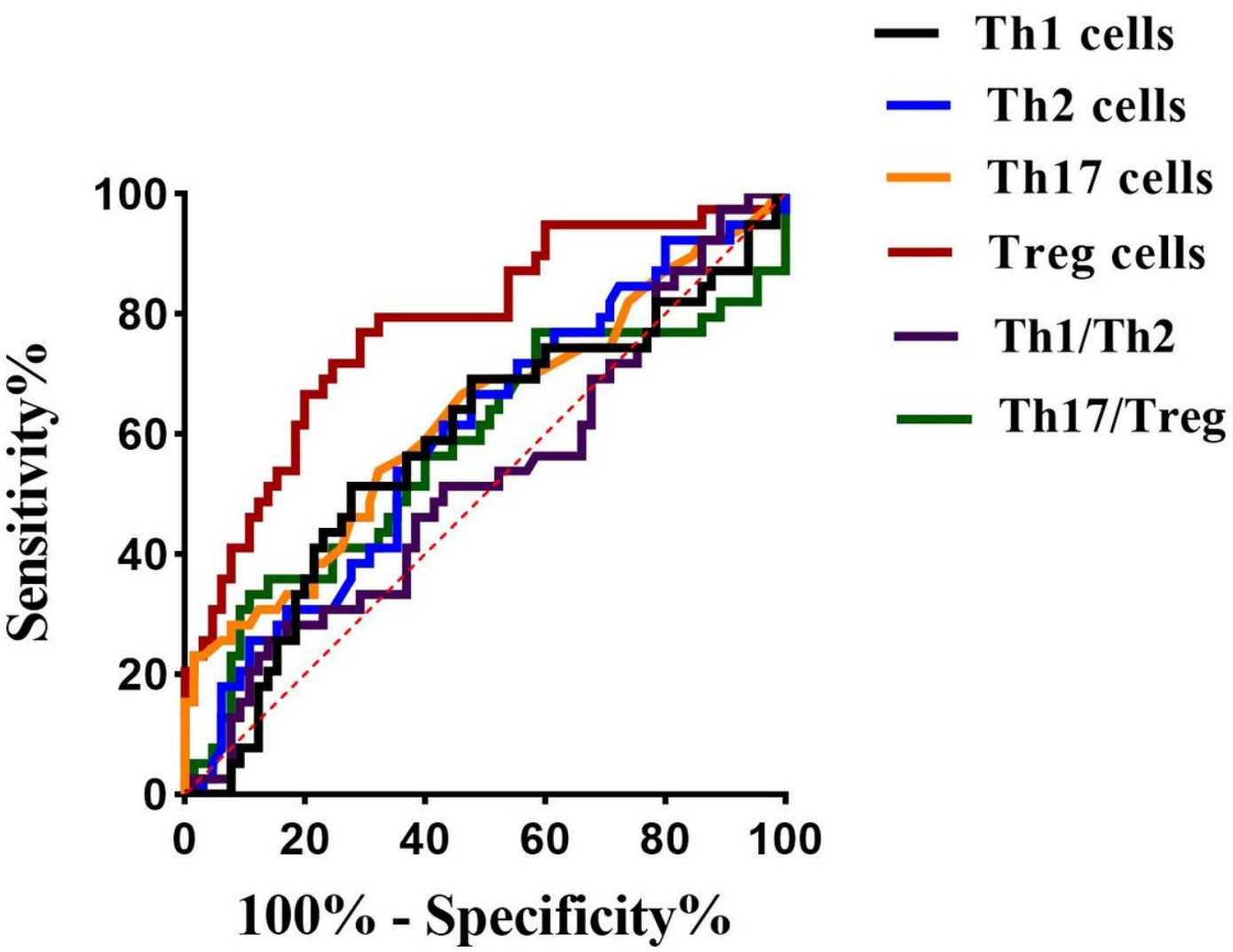

Figure 3

Comparison of ROC curves and AUC of Th1, Th2, Th17 and Treg cells and the ratio of Th1/Th2 and Th17/Treg. Treg cells have the biggest AUC, which are be used to monitor the occurrence and development of diseases.

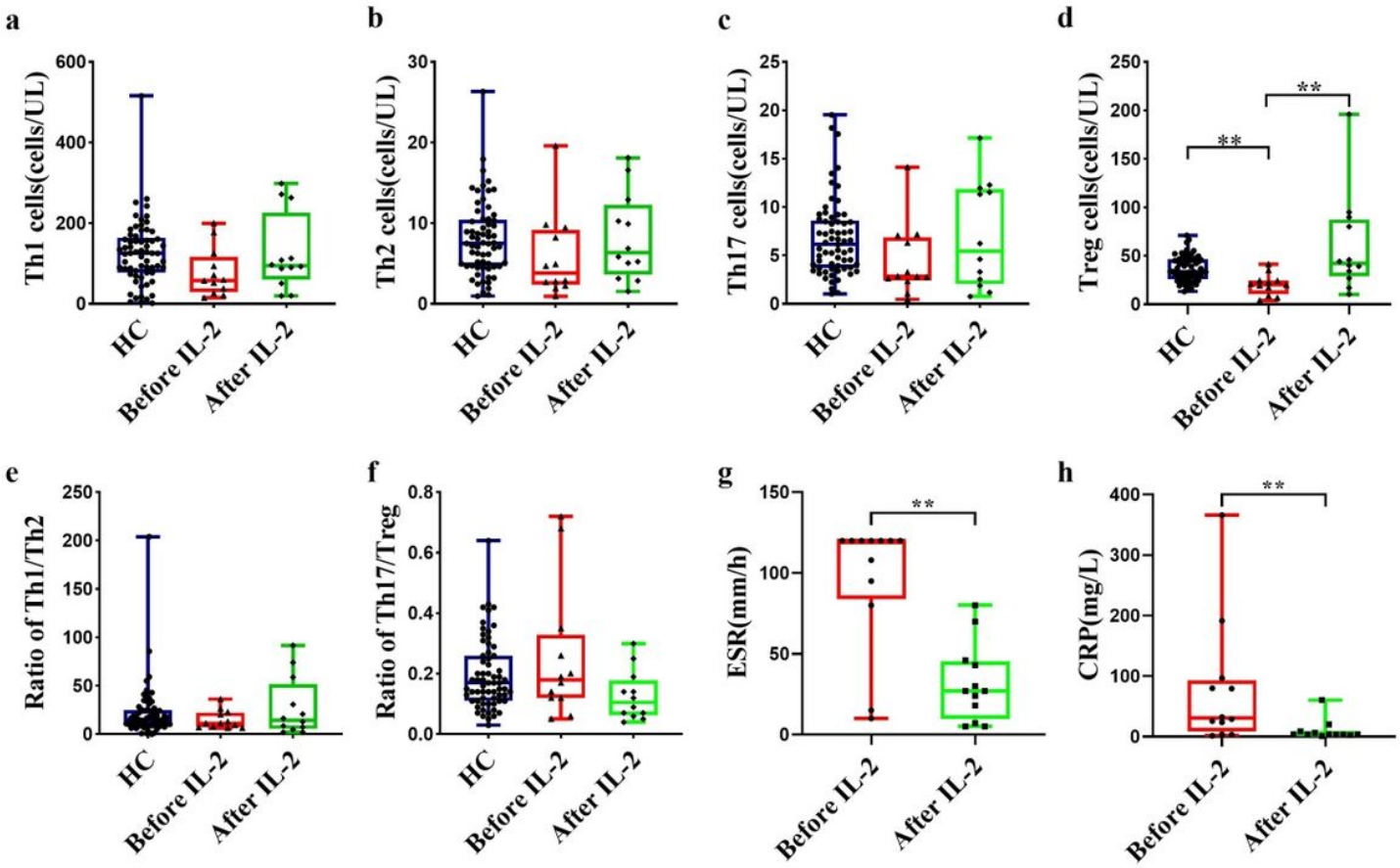

Figure 4

(a-f) Effects of low-dose IL-2 therapy on CD4+T cell subsets. The absolute number of Treg cells significantly increased (P=0.001) and closed to the level of HCs after the treatment of low- dose IL-2 therapy. In contrast, the level of Th17 cells and other cells had no significant difference. (g-h) The changes of ESR and CRP before and after the low-dose IL-2 therapy showed that the level of ESR and CRP were significantly decreased after the low-dose IL-2 therapy. Data were presented as median (Q1, Q3), and were analyzed by Mann–Whitney U test. (*P<0.05; **P<0.01; ***P<0.001)

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