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The Effect of T-Cell Transcription Factors on the Immunopathogenesis of Rheumatoid Arthritis

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Abstract: Objective: This looks at investigates the impact of T-cell transcription factors at the immunopathogenesis of rheumatoid arthritis (RA). Methods: An overall of 60 sufferers diagnosed with RA and handled in our health center between January 2022 and January 2024 had been selected because the remark organization, whilst 60 healthy those who underwent routine health test-usual through the equal length had been distinct as the manipulate group. Peripheral blood samples from each organization were analyzed to measure the proportion and proliferative interest of regulatory T cells (Tregs), as well as their inhibitory feature on effector T cells. Levels of the T-cell transcription component STAT3 and associated inflammatory factors were additionally tested. RA patients had been handled with a STAT3 inhibitor to take a look at changes in Treg proliferation, inhibitory function, and the secretion stages of seasoned-inflammatory factors. Additionally, synovial tissue from RA patients changed into received for histopathological evaluation the usage of mild microscopy to assess synovial infection and hyperplasia. Results: The peripheral blood of the remark organization confirmed substantially decrease Treg counts compared to the manipulate group ($P < 0.05$). The in vitro proliferation charge of Tregs in the remark group was additionally decrease than within the manipulate group ($P < 0.05$). Levels of p-STAT3, TNF-α, IFN-γ, and IL-17A in Tregs have been higher within the commentary group, and the expression tiers of TNF-α, IFN-γ, and IL-17A mRNA have been also improved in comparison to the manage group ($P < 0.05$). RA sufferers handled with 50 μg/L STAT3 inhibitor confirmed a substantially better Tresp suppression price in comparison to untreated sufferers ($P < 0.05$). However, there has been no statistically tremendous distinction in Treg proliferation charges among the treated institution and the control institution ($P > 0.05$). The expression of p-STAT3 protein in Tregs became lower in the treated group compared to untreated sufferers ($P < 0.05$), and not using a big difference as compared to the manage organization ($P > 0.05$). Similarly, TNF-α, IFN-γ, and IL-17A mRNA stages had been reduced in the dealt with organization compared to untreated sufferers ($P < 0.05$), but no great distinction turned into found whilst in comparison to the manage group ($P > 0.05$). Histopathological evaluation discovered mentioned inflammatory responses in the synovial tissue of the statement organization, inclusive of congestion and edema of the synovial stroma, full-size lymphocyte and plasma cellular infiltration, focal necrosis, epithelial hyperplasia, fibrinous exudation, and necrotic particles accumulation. Conclusion: RA patients show off synovial inflammation and hyperplasia, together with a lack of ability to efficiently suppress Tresp cells thru Tregs. The mechanism is intently related to peculiar STAT3 expression. Inhibiting aberrant STAT3 expression extensively influences the immunopathogenesis of RA, probably restoring Treg feature, assuaging seasoned-inflammatory thing secretion, and preventing the development of RA.

Keywords: T-cell transcription factor; rheumatoid arthritis; immunopathogenesis

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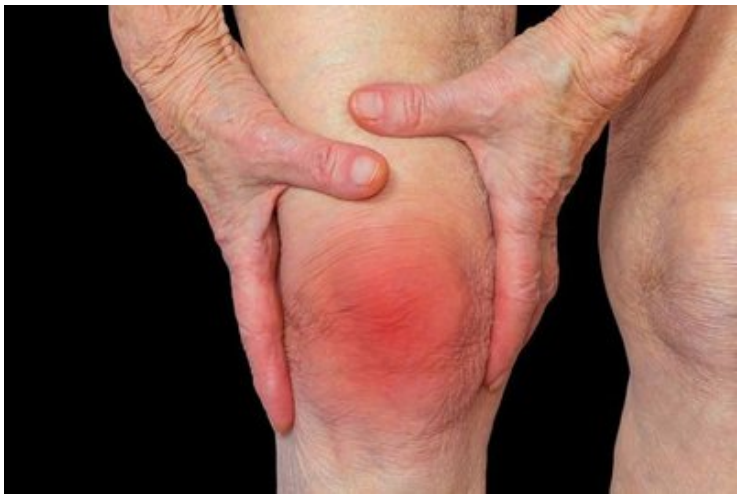
1. Introduction

Rheumatoid arthritis (RA) is an autoimmune-related disorder of uncertain etiology. Clinically, RA patients frequently gift with ache, stiffness [1,2], deformity, extraordinary temperature sensation, and modern destruction of affected joints and surrounding tissues, in the long run impairing joint characteristic and inflicting incapacity. Regulatory T cells (Tregs) play an important function in preventing and suppressing autoimmune diseases. Research on Tregs represents a key cognizance inside present day scientific immunology. Tregs, which exhibit bad immunoregulatory outcomes, keep immune stability and mediate immune tolerance. Studies indicate that RA sufferers may additionally enjoy bizarre ranges or function of Tregs, which might be strongly related to the onset and development of RA. However, the precise abnormalities and underlying mechanisms stay doubtful. This has a look at analyzed the impact of T-cell transcription elements at the immunopathogenesis of RA via inspecting 60 RA patients and 60 healthy controls handled at our sanatorium from January 2022 to January 2024. The findings are supplied as follows [3,4].

2. Materials and Methods

2.1. General Information

Sixty rheumatoid arthritis (RA) patients treated at our hospital between January 2022 and January 2024 were included as the observation group. Among these patients, 32 were male and 28 were female, aged between 30 and 65 years [4,5], with a mean age of （49.68±4.16） years. Disease duration ranged from 1 to 10 years, with a mean duration of （6.35±1.13） years. Additionally, 60 healthy individuals undergoing routine health check-ups during the same period were selected as the control group. The control group consisted of 35 males and 25 females, aged 28–67 years, with a mean age of （50.05±4.01） years[5-6]. There were no statistically significant differences in general demographic data between the two groups ($P > 0.05$).



2.1.1. Inclusion Criteria:

1) Diagnosis of RA confirmed through comprehensive evaluation;
2) Complete clinical data available;
3) Adults aged 18 years or older;
4) Signed informed consent from patients or their families.

2.1.2. Exclusion Criteria:

1) Presence of other autoimmune-related diseases;
2) Presence of infectious diseases;
3) Cognitive impairment or communication difficulties;

4) Psychiatric disorders;
5) Malignant tumors.

2.2. Methods

2.2.1. Detection of Treg Proportion in CD4+ Cells

Twenty milliliters of peripheral venous blood was collected from each participant. Peripheral blood mononuclear cells (PBMCs) were isolated using a lymphocyte separation solution treated with heparin as an anticoagulant. The cells were subjected to surface fluorescent antibody staining. Each flow cytometry tube was supplemented with CD3-FITC, CD4-APC/Cy7, and CD25-phycoerythrin (5 μL each). A blank tube was prepared with equivalent amounts of flow antibodies. The tubes were incubated at 4°C for 30 minutes, centrifuged with 2 mL sterile PBS, and fixed under light-protected conditions at room temperature [7,8]. After additional centrifugation and PBS washing, 5 μL of Foxp3-APC was added and incubated at 4°C for 1 hour. Flow cytometry was used to detect the proportion of regulatory T cells (Tregs) in CD4+ T cells [9,10].

2.2.2. Detection of Treg Proliferation

Isolated Tregs had been stained with CFSE dye. The cells have been resuspended in 1 mL of sterile PBS and incubated with 5 μmol/L CFSE for 15 mins within the darkish at room temperature. The reaction become terminated with RPMI-1640 medium, followed with the aid of centrifugation for five minutes to eliminate the supernatant. The CFSE-stained Tregs 5×10^4 have been seeded into 96-well U-bottom plates lined with five mg/L CD3, with 3 wells per institution. Tregs were activated and incubated for one week, and their proliferation ratio changed into assessed using glide cytometry [11,12].

2.2.3. Impact of Tregs on Tresp Cell Proliferation

CFSE-stained Tresp cells 5×10^4 were co-cultured or one after the other cultured with five×104 Tregs in ninety six-well U-bottom plates coated with five mg/L CD3. IL-2 (50 μg/L) was brought to set off Tresp cells. After one week, the proliferation ratio of Tresp cells turned into decided thru glide cytometry [13,14].

2.2.4. Proportion of p-STAT3 and Cytokine-Secreting Cells in Tregs

PBMCs have been cultured in six-properly plates with 1 μL of T-cell activation reagent for four–6 hours at 37°C. Surface antibody staining with CD4-APC/Cy7 and CD25-FITC was carried out. After permeabilization and fixation, five μL of Foxp3-APC, p-STAT3, IFN-γ, TNF-α, and IL-17A antibodies have been brought and incubated at four°C for 30 minutes. Flow cytometry was used to hit upon the proportions of p-STAT3-high quality and cytokine-secreting cells [15,16].

2.2.5. Detection of STAT3 Inhibitor Effects on Treg Proliferation and Suppression

STAT3 inhibitor (50 μg/L) became introduced to five×104 CFSE-stained Tregs cultured in ninety-six-properly plates. After one week, Treg proliferation rates have been measured via drift cytometry. Additionally, STAT3 inhibitor (50 μg/L) became introduced to co-cultures of 5×10^4 CFSE Tresp cells and five×104 Tregs. After one week, the suppression charge of Tregs on Tresp cells become measured the use of waft cytometry [17,18].

2.2.6. RT-PCR Detection of Cytokines Secreted by Tregs

Total RNA changed into extracted from Tregs using Trizell reagent. CDNA synthesis changed into completed the usage of a reverse transcription package. RT-PCR become carried out in line with the manufacturer's instructions to quantify cytokine mRNA tiers [19,20].

2.2.7. Detection of p-STAT3 Protein in Tregs

Western blot evaluation changed into used to assess p-STAT3 protein stages in Tregs. STAT3 inhibitor (50μg/L) turned into brought to 5×104 CFSE-stained Tregs cultured in ninety-six nicely plates. After one week, cells have been harvested, lysed with protease inhibitor-containing lysis buffer, and homogenized on ice. Cell lysates had been centrifuged at 14,000 rpm at 4°C for 20 mins, and the supernatant became gathered. Protein awareness became determined using the bicinchoninic acid (BCA) approach. Samples had been heated in a water bathtub for 15 minutes before loading. Proteins were separated by way of SDS-PAGE and transferred to nitrocellulose membranes. Membranes have been blocked with five% skim milk, incubated overnight with anti-STAT3 antibody (1:500) at four, and eventually incubated with HRP-categorized anti-rabbit IgG (1: a thousand) for 2 hours at room temperature. The signal became visualized the usage of diaminobenzidine (DAB) and analyzed via infrared scanning.

2.2.8. Histopathological Examination

Synovial tissues were aseptically obtained from RA patients, fixed in 10% formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin-eosin (HE). Synovial inflammation and hyperplasia were observed under a light microscope.

2.3. Statistical Analysis

Statistical analysis was conducted using SPSS 20.0 software. Continuous data were expressed as mean ± standard deviation （$\overline{x}$ ±s） and analyzed using t-tests. Categorical data were expressed as percentages (%) and analyzed using chi-square tests (X2). A P<0.05 was considered statistically significant.

3. Results

3.1. Changes in Peripheral Blood Tregs

The level of regulatory T cells (Tregs) in the peripheral blood of the control group was （6.50±0.27）% , significantly higher than （2.18±0.14）% in the observation group (P<0.05).

3.2. Effects of Tregs on Tresp Cell Proliferation

The in vitro proliferation rate of Tregs in the observation group was significantly lower than that in the control group (P<0.05), as shown in Table 1.

Table 1. Effects of Tregs on Tresp Cell Proliferation ($\overline{x}$ ±s)(%):

Group	Sample Size	Treg Proliferation Rate (%)	Treg Suppression Rate on Tresp (%)
Observation	60	24.34±5.80	21.36±4.54
Control	60	60.27±8.80	52.38±5.89
t	-	26.407	32.310
P	-	0.000	0.000

3.3. Proportion of p-STAT3 and Cytokine mRNA in Tregs

The observation group exhibited significantly higher proportions of p-STAT3, TNF-α, IFN-γ, and IL-17A in Tregs, as well as higher expression levels of TNF-α, IFN-γ, and IL-17A mRNA, compared to the control group (P<0.05), as shown in Table 2.

Table 2. Proportion of p-STAT3 and Cytokine mRNA in Tregs ($\overline{x}$ ±s):

Group	Sample Size	Percentage of p-STAT3 cells in regulatory T cells (%)				Inflammatory factor mRNA		
		p-STAT3	TNF-α	IFN-γ	IL-17A	TNF-α	IFN-γ	IL-17A
Observation	60	64.62±6.20	8.47±1.52	9.16±1.54	5.34±0.67	4.87±1.54	23.32±6.76	3.16±0.62
Control	60	37.52±1.84	1.03±0.12	0.73±0.06	2.28±0.72	1.00±0.16	1.00±0.10	1.03±0.06
t	-	32.458	37.797	42.369	24.100	19.361	25.573	26.487
P	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000

3.4. Effects of STAT3 Inhibitor on Treg Function

In the observation group, the application of 50 μg/L STAT3 inhibitor significantly increased the Treg suppression rate on Tresp cells compared to untreated patients (P<0.05). The Treg proliferation rate showed no significant difference compared to the control group (P>0.05). The expression rate of p-STAT3 protein in Tregs was lower than in untreated patients (P<0.05) but not significantly different from the control group (P>0.05). The expression levels of TNF-α, IFN-γ, and IL-17A mRNA were lower than in untreated patients (P<0.05) but not significantly different from the control group (P>0.05), as shown in Table 3.

Table 3. Effects of STAT3 Inhibitor on Treg Function($\overline{x}$ ±s):

Group	Sample Size	Treg Suppression Rate on Tresp (%)	Treg Proliferation Rate (%)	p-STAT3 Protein Expression	TNF-α mRNA	IFN-γ mRNA	IL-17A mRNA
Treated	30	61.60±6.34	42.42±5.40	0.26±0.04	1.18±0.02	1.16±0.03	1.20±0.02
Untreated	30	28.43±4.73	-	0.88±0.14	4.88±1.54	23.32±6.70	3.14±0.66
Control	60	-	42.40±4.28	0.24±0.03	1.12±0.03	1.08±0.02	1.14±0.03
F/t	-	22.968	0.019	791.182	267.506	498.892	406.742
P	-	0.000	0.985	0.000	0.000	0.000	0.000

3.5. Histopathological Results in RA Patients

Light microscopy revealed significant synovial inflammation in the observation group, including congestion and edema in the synovial stroma, as well as extensive lymphocyte and plasma cell infiltration. Tissue damage was evident, with focal necrosis observed. Hyperplasia of synovial epithelial cells was noted, along with localized fibrinous exudates and necrotic debris accumulation.

4. Discussion & Conclusion

Rheumatoid arthritis (RA) is a systemic immune-related disease with unclear pathogenesis, drawing significant attention from clinical researchers regarding its immunopathogenesis. RA patients commonly exhibit synovial inflammation. Pathological examinations reveal pronounced inflammatory responses in the synovial tissues of RA patients, often accompanied by hyperplasia of synovial epithelial cells. Regulatory T cells (Tregs), a subset of CD4+ T cells with immunosuppressive functions, play a crucial role in maintaining immune tolerance and response stability. In healthy individuals, Tregs account for up to 10% of CD4+ cells in peripheral blood. Despite their relatively low proportion, Tregs effectively inhibit the proliferation and activation of autoreactive T cells. Abnormal levels

or functions of Tregs are associated with the occurrence and progression of various autoimmune diseases, including RA. Research indicates that Tregs in RA patients exhibit reduced intrinsic proliferative activity and diminished suppressive effects on Tresp cell proliferation. These findings suggest a correlation between impaired Treg function and RA pathogenesis [21,22].

STAT3, a transcription factor for T-helper 17 cells, can be activated by external signals [23,24], translocating to the nucleus to regulate the transcription of target genes. Studies have shown that STAT3 modulates the growth and survival of abnormal synovial cells in RA patients, disrupting the development of Tregs and thereby affecting immune homeostasis, ultimately contributing to RA pathogenesis. This study observed that STAT3 expression in Tregs from RA patients was significantly abnormal, inhibiting Treg functionality. Moreover, the application of STAT3 inhibitors restored the suppressive effects of Tregs on Tresp proliferation. These results indicate that excessive STAT3 expression is a primary mechanism underlying Treg dysfunction in RA patients [25].

In conclusion, RA patients experience synovial inflammation and hyperplasia, leading to impaired negative regulation of Tresp cells by Tregs. The primary mechanism involves abnormal STAT3 expression. Inhibiting aberrant STAT3 expression can improve immune function in RA patients, restore Treg functionality, and potentially prevent the development of RA.

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