

Study on CD4+CD25+Foxp3+T cells and clinical status of patients after renal transplantation.

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Abstract

Objective: To investigate the ratio change rule and its clinical meanings of peripheral CD4+CD25+Foxp3+T cells of patients under various immune status after renal transplantation.

Methods: This study involved 59 patients receiving renal transplantation who were assigned into stable kidney function group (n=20), acute rejection group (n=23), and pulmonary infection group (n=16), selected 18 healthy volunteers in control group and tested the change of peripheral T cell subsets of all participants with flow cytometry.

Results: The proportion of peripheral CD4+CD25+T cells, CD4+Foxp3+T cells, and CD4+CD25+Foxp3+T cells of patients in stable kidney function group was much lower than that in control group, with a statistical difference (P<0.05), but higher than that in acute rejection group and pulmonary group in which the ratio of CD4+CD25+T cells also exceeded that in the healthy group, having a significant difference (P<0.05).

Conclusion: Carefully monitoring the change of peripheral regulatory T cells of patients who underwent renal transplantation plays an essential role in preventing and treating post-renal transplantation rejection and local infection.

Keywords: Regulatory T cells, CD4+CD25+Foxp3+T cells, Renal transplantation, Rejection, Infection.

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Introduction

At present, renal transplantation is one of method which is commonly used in treating end-stage renal failure and improving patients' life quality [1]. However, patients receiving renal transplant have to chronically take immunosuppressive agents. Presently, along with the advancement of renal transplant technology and wide application of new immunosuppressive agent, favorable prognosis has been achieved for the patients receiving renal transplant and the post-operation immunosuppression strategies have been early inductive immunological tolerance scheme and chronic immunosuppression maintenance scheme for the patients after operation. Immunosuppressive agents include Cyclosporine A, Tacrolimus, MMF, prednisone and so on. The purpose is to restrain the activation and proliferation of T lymphocytes, of which aims are to control the graft rejection as much as possible and maintain the equilibrium of body immune system [2]. If the immunosuppression is too much, it will induce cytomegalovirus infection and cancers, while, if

insufficient, it will cause postoperative rejection, so objectively evaluating the rejection of patients has clinical significance for rationally selecting immunosuppressive agents and raising success rate of renal transplantation. Some recent studies verify that regulatory T cell (Treg) is closely associated with immune tolerance and immune status of patients who undergo renal transplantation and plays an important role in renal graft rejection and post-operation infection [3]. Some studies revealed that CD4+CD25+Treg with high-expression IL-2 recipient can cause transplantation immunologic tolerance; CD4+CD25+Foxp3+T lymphocyte is the most important T cell subgroup with immunosuppressant function, in which transcription factor *Foxp3* is a specific regulatory gene for Treg cell differentiation and functional maintenance. As the patients have to take immunosuppressant for a long period after the operation of renal transplant, it may direct or indirectly affect the role of CD4+CD25+Foxp3+T lymphocyte, resulting in co-existence of rejection and infection risks. Therefore, studying susceptible factors of rejection and infection will have

significant effect on prognosis of more and more renal transplant patients.

This study is to explore the relationship between regulatory T cell and clinical immune status of renal transplantation recipients through detecting the change in percentage of peripheral CD4+CD25+Foxp3+T lymphocytes of those with various immune status.

Methods

Clinical data

59 patients who underwent renal transplantation in our hospital for suffering end-stage renal failure from May 2010 to

September 2016 were enrolled in the study, aged from 38 to 77 y, 50.63 ± 10.18 in average, 36 were males, and 23 females. Serum creatinine of all patients decreased to normal in ten days after operation.

These patients, on the basis of their diverse postoperative conditions, were assigned to stable kidney function group (n=20), acute rejection group (n=23), and pulmonary infection group (n=16).

In addition, 18 healthy participants were enrolled in control group. General data of patients in three groups were in accordance without statistical difference, $P > 0.05$, which were comparable (Table 1).

Table 1. Analysis on patients' general data.

	Stable kidney function group	Acute rejection group	Pulmonary infection group
Amount of patients (cases)	20	23	16
Age of patients (y)	48.74 ± 9.33	50.17 ± 11.26	50.89 ± 11.84
Gender (male/female)	42929	43021	42896
Transplantation time (months)	16.91 ± 8.67	15.13 ± 10.59	14.52 ± 9.67
Source of renal (living related donors/dead donors)	43018	41883	42985

Inclusive criteria

If patients, who were admitted in our hospital with full medical records and didn't suffer infectious diseases, such as hepatitis, syphilis, and HIV virus; if they hadn't got diseases which affected their immune status during study; if the donor and recipient had same blood type; if the lymphocytotoxicity test was less than 10%, the panel reaction antibody of recipients lowered than 15%, and the mismatch number of human leucocyte antigen was less than 4; if patients or their families signed an informed consent, they were enrolled in the study.

Diagnosis criteria

Stable kidney function group (Group A): patients had normal creatinine level (44-133 $\mu\text{mol/l}$), no rejection which was confirmed through renal tissue biopsy, no infection and postoperative complications.

Acute rejection group (Group B): patients suffered kidney distending pain, and their body temperature was little higher than the normal (37.0°C - 38.5°C), but their urine volume less than the normal. Laboratory data showed that Scr was more than $133 \mu\text{mol/l}$, proteinuria positive, red blood cells more than $5.5 \times 10^{12}/\text{mm}^3$, and haemoglobin decreased. Kidney tissue biopsy indicated that they had acute or chronic rejection.

Pulmonary infection group (Group C): patients suffered chest tightness, tachypnea, cough, and rise of temperature. Images proved that the lung had clear infection signs.

Treatments

All recipients received 500 mg of intravenous methylprednisolone before transplantation and three days after operation, accepted triple immunosuppressive therapy (cyclosporin A or tacrolimus, mycophenolate mofetil, prednisone) from postoperative day 4, and underwent blood test for the plasma concentration of cyclosporin A or tacrolimus, twice a week.

Follow-up

All patients were followed up for 6 to 72 months after operation, 36.77 ± 14.15 months in average.

Collecting blood samples

5 ml fasting venous blood was obtained when patients got up in the morning, the day at admission, and then anticoagulant were added to it.

Isolating human peripheral blood mononuclear lymphocytes

The venous blood mixed with an equal volume of PBS was placed in centrifuge tubes with lymphocytes dissociation solution, centrifuged at 3000 rpm for 30 min. After the mixed solution layered, monocytes at the upper layer and middle layer were placed into another centrifuge tube with a pipette, washed twice with PBS, and re-centrifuged. With discarding supernatant, the re-centrifuged solution was suspended with

PBS, and then quantitated, setting cell concentration at $5 \times 10^5/\text{ml}$.

Flow cytometry

Took 100 μm cell solution, put FITC-CD4 monoclonal antibody, APC-CD25 monoclonal antibody, Percp-CD45RA monoclonal antibody in it. The mixed solution was incubated for 1 h at 37°C, washed with PBS, centrifuged, and then suspended with PBS to test the cell phenotype with the supernatant discarded.

The data was analyzed by software SPSS 19.0. The difference was tested with chi-square test, t-test was adopted for comparing measured data between groups and the single factor analysis of variance was used for comparing mean values among various groups. $p < 0.05$ indicates statistical significance.

Results

The change of CD4+CD25+T lymphocytes proportion of patients in each group

Flow cytometry showed that the proportion of peripheral CD4+CD25+T lymphocytes in stable kidney function group was much lower than the control group, having statistical difference, $P < 0.05$.

However, that in acute rejection group and pulmonary group was much higher than that in control group and stable kidney function group, with a significant difference, $P < 0.05$ (Table 2).

The variation of CD4+Foxp3+T lymphocytes proportion of patients in each group

The outcomes of flow cytometry indicated that the percentage of peripheral CD4+Foxp3+T lymphocytes of patients in stable kidney function group was much lower than that in control group, having statistical difference, $P < 0.05$.

Nevertheless, that in acute rejection group and pulmonary group was $8.48 \pm 0.59\%$ and $8.31 \pm 0.52\%$ separately, which had no statistical significance when compared with control group, $P > 0.05$, but was much higher than that in stable kidney function group, with statistical difference, $P < 0.05$ (Table 2).

The change of CD4+CD25+Foxp3+T lymphocytes of patients in each group

The results of flow cytometry revealed that the percentage of peripheral CD4+CD25+Foxp3+T lymphocytes of patients in stable kidney function group was much less than that in control group, with a statistical difference, $P < 0.05$.

While compared the percentage of CD4+CD25+Foxp3-T lymphocytes with control group, there was no difference, $P > 0.05$.

The difference of CD4+CD25+Foxp3+T lymphocytes of patients in acute rejection group and pulmonary group has no significance, compared with that in control group, but it was

much more than that in stable kidney function group, with a statistical difference, $P < 0.05$.

That pointed out those immunosuppressive agents could restrain CD4+CD25+Foxp3-T lymphocytes.

But when the body suffered rejection or infection, the proportion of CD4+CD25+Foxp3-T lymphocytes regulating immunity would increase, which explained that CD4+Foxp3+T lymphocytes are close related with the immune status of recipients (Table 2).

Discussion

Past researches prove that, the acute rejection of local infection of patients receiving renal transplantation is associated with the variation of peripheral T lymphocyte subsets proportion, and regulator T lymphocytes play an essential role in this process [4]. Since researchers like Sakaguchi firstly proposed that CD4+CD25+T lymphocytes have close relationship with immune resistance in 1995, a growing number of studies have deemed that this kind of suppressive T cells is vital for maintaining and inducing the immunological tolerance of organ transplantation [5]. But, are regulator T cells directly induce immunological tolerance or indirectly prevent grafts from resistance? Some researchers find that allotypic antigens, for patients with acute rejection or infectious symptoms after operation, activate not only effector T cells to repel transplant recipients but also regulator T cells to oppose grafts [6]. Therefore, it is also proven that regulator T cells protect grafts through controlling the lasting proliferation and activation of effector T cells [7].

Under the stimulation of external antigens, CD4+ cells can express CD25+ surface antigen a short time but have no physiological activity [8,9]. After analyzing the results of flow cytometry, it is known that in this study, the proportion of peripheral CD4+CD25+T lymphocytes of patients in stable kidney function group is largely below that in control group, with a significant difference, $P < 0.05$, but that in acute rejection group and pulmonary group much exceeds that in control group and stable kidney function group. Why? Is it caused by the increase or activation of peripheral CD4+CD25+T lymphocytes?

Renal graft biopsy is a golden standard for detecting renal rejection, but there are some derivations in estimating kidney function through lymphocytes infiltrating into renal graft, because renal graft contains cytotoxic T-lymphocytes but also Foxp3+Treg [10-12]. As a gene sequence that encodes transcription blocking proteins, Foxp3 specifically expresses at the surface of CD4+CD25+T lymphocyte to impact its production and physiological function regulating, it hence is regarded as a specific testing molecular marker for Treg [13,14]. In this study, we further explore the proportion of peripheral CD4+Foxp3+T lymphocytes of patients in control group, stable kidney function group, acute rejection group, and pulmonary group, which testifies that the proportion of peripheral CD4+Foxp3+T cells of patients in stable kidney function group is much less than that in control group, with a

statistical difference, $P < 0.05$, and that in acute rejection group and pulmonary group has no statistical significance when compared with control group, $P > 0.05$, but is much higher than that in stable kidney function group. These hints that immunosuppressive agents can decrease the proportion of

CD4+Foxp3+T lymphocytes, but when the patient suffer rejection or infection, the proportion of CD4+Foxp3+T lymphocytes that have immune-regulation still increase, which explains that CD4+Foxp3+T lymphocytes are closely related with the immune status of recipients.

Table 2. The percentage of Treg and lymphocytes subsets in each group (mean \pm SEM).

Group	CD4+CD25+	CD4+Foxp3+	CD4+CD25+Foxp3+	CD4+CD25+Foxp3-
Control	8.15 \pm 0.73	8.37 \pm 0.45	4.41 \pm 0.30	4.12 \pm 0.38
Stable kidney function	6.32 \pm 0.69*	6.52 \pm 0.61*	2.36 \pm 0.29*	3.96 \pm 0.55
Acute rejection	9.67 \pm 0.85*#	8.48 \pm 0.59#	4.09 \pm 0.23#	6.64 \pm 0.63*#
Pulmonary	9.44 \pm 0.81*#	8.31 \pm 0.52#	4.02 \pm 0.26#	6.26 \pm 0.59*#

Note: *Compared with control group, $P < 0.05$; #Compared with stable kidney function group, $P < 0.05$.

Through testing the proportion of peripheral CD4+CD25+Foxp3+T lymphocytes of participants, we know that the percentage of peripheral CD4+CD25+Foxp3+T lymphocytes of patients in stable kidney function group is much less than that in control group. While compared the percentage of CD4+CD25+Foxp3-T lymphocytes (with control group, there is no difference, $p > 0.05$). And the percentage of CD4+CD25+Foxp3-T lymphocytes of patients in acute rejection group and pulmonary group is much more than that in stable kidney function group, when compared them with control group, the difference has no significance. These point out that taking immunosuppressive agents for a long time can cut down the proportion of CD4+CD25+Foxp3-T lymphocytes but has little influence on the CD4+CD25+Foxp3-T lymphocytes with immune response effect, which is associated with the effect of drug suppression for IL-2 [15]. When the rejection or infection appears, CD4+CD25+Foxp3+Treg and CD4+CD25+Foxp3-effector T lymphocyte are activated simultaneously to immunize, and the balance between them decides the immune status of body.

To sum up, monitoring the variation of peripheral CD4+CD25+Foxp3+Treg of patients after renal transplantation is essential for the early diagnosis of rejection and inflammatory reaction. Meanwhile, for patients with acute rejection or local infection after renal transplantation, taking rational measures is able to raise the proportion of Treg to induce and maintain their immune tolerance for the renal graft, having vital significance in clinic.

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