1. Introduction

Organ transplants were carried out gradually in recent years, but the postoperative autologous immune rejection become the most thorny problem[1-5]. Efficient immune inhibitor combined application can reduce the incidence of rejection after transplantation, obviously prolong survival time after transplantation[6-10]. The human body has the same reactive T cells of CD4+ CD25+ regulatory T cells (Tregs), it was showed that regulatory T cells content of the patients with immunosuppressant can be changed after transplantation[11-16]. Rapamycin (SRL) are one of macrolides families, studies have shown that SRL has stronger immune inhibition effect, which was widely used in the treatment of kidney transplant rejection[17-24]. The author aims to observe its influence on regulate T cell’s activation and function, SRL lavage was used on SD rats to observe its regulatory effect on CD4+, CD25+, FoxP3+ T cells, the results are reported as follows.

2. Materials and methods

2.1. Experimental animals

A total of 40 SD rats were selected, only our animal experiment center, clean level, male, weighting 210–220 g, aged 8 to 10 weeks, the rats were fed free with food and water, animal in the experimental were processed strictly according to the administration of experimental animals.

2.2. Instrument and preparation

Real time PCR instrument, FACS Aria TM flow cytometry instrument (BD co., United States); TGF–beta and IL–10 ELISA kit, RT–PCR kit (TaKaRa); Sirolimus (Wyeth Pharmaceutical co., LTD.)–the White House. Hemolysin, collagenase and hyaluronidase, Trizol reagent (Invitrogen Company). RPMI–1640 medium, PE–conjugated anti–CD25,
2.3. Methods

A total of 40 rats were randomly divided into experimental group and control group with 20 in each, the experimental group rats were given SRL lavage (0.4 mg/d) for 2 weeks; the control group given were given the same volume of saline lavage for 2 weeks. Pentobarbital was used for anesthesia (ip.), inferior vena venous blood was extracted for further use; Rats spleen and thymus were grinded using 200 mesh metal filter, for preparation of $10^6$ cells/mL suspension cells using RPMI-1640.

2.4. Observe project

A total of 100 $\mu$L spleen cells, thymus suspension and heparin anticoagulant was absorbed respectively, adding 1 $\mu$L APC-conjugated anti-CD4 (0.25 $\mu$L), LPE-conjugated anti-CD2, incubated away from light for 15 min, hemolysin, centrifugal 5 min, abandon supernatant, PBS wash twice, plus 200 $\mu$L PBS blending, computer detection of CD4+ CD25+ T cells accounted for the proportion of mononuclear cells; with double antibody sandwich ELISA method to detect the rat TGF-β and IL-10 in serum level; and the real-time PCR kits in the spleen cell SAP FoxP3 mRNA expression. The experiment was performed strictly in accordance with the above instructions.

2.5. Statistical processing

Using SPSS12.0 statistics software to process the experiment data, measurement data are expressed with (X±s), group comparision was analysed by t test, $P<0.05$ for the difference was statistically significant.

3. Results

3.1. CD4+ and CD25+ T cells detection in the peripheral blood, thymus and spleen

The experimental group in the peripheral blood, spleen and thymus of CD4+ and CD25+ T cells accounted for the proportion of mononuclear cells are higher than the control group with statistically significant ($P<0.05$), the results are shown in Table 1.

3.2. Level of TGF-β, IL-10 in serum

Experimental rats serum TGF-β, IL-10 were significantly higher than that of control group ($P<0.05$), as shown in Figure 1.

3.3. AP FoxP3 mRNA expression comparison in two groups

Experimental rats spleen FoxP3 mRNA was significantly higher than that of control group ($P<0.05$), as shown in Figure 2.

4. Discussion

CD4+ T cells were discovered in the thymus by Sakaguchi et al. in the 90 s, the new borne rats which accepted the thymus excision (d3Tx) injection of CD4+ CD25+ T cells can prevent autoimmune diseases[25–29]. The current universal view is that thymus is able to produce CD4+ CD25+ T in peripheral blood[30]. Studies have shown that[31–34], CD4+ and

<table>
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<th>Groups</th>
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<th>Peripheral blood</th>
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<tr>
<td>Control</td>
<td>20</td>
<td>5.35±1.36</td>
<td>8.31±2.56</td>
<td>6.69±2.63</td>
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*P<0.05 compare with control group.
CD25+ T cells can prevent autoimmune reactions and tissue damage caused by rejection and inflammatory response after organ transplantation.

Sirolimus is a potent immune inhibitor, has been widely applied in the immune rejection after kidney transplantation\[33\]. It works mainly by inhibiting the antigens and cytokines stimulation of T lymphocyte activation and proliferation of immune inhibition[35]. Sirolimus can selectively amplify CD4+ CD25+ and FoxP3+ T cells without blocking the CD4+ T cell expansion and activation to induce cell apoptosis\[37\]. CD4+ CD25+ and FoxP3+ regulatory T cells play an important role in maintaining immune tolerance. Studies have shown that[38-40], sirolimus can induce immune inhibition of regulatory T cells. In this study, the experimental group to sirolimus lavage (0.4 mg/d) after 2 weeks, the experimental group rats an increasing CD4+ CD25+ T cells proportion of mononuclear cells in peripheral blood, thymus and spleen significantly, much higher than that of control group (P<0.05); and the experimental group rats spleen foxp3 mRNA expression, TGF-β, IL-10 levels and cytokines stimulation of T lymphocyte activation were significantly higher than the control group (P<0.05), indicating that sirolimus can induce rats in vivo CD4+ CD25+ FoxP3+ regulatory T cell proliferation, leading to immune rejection tolerance.

According to the results of this study, immune inhibitors can increase the rat proportion of CD4+ CD25+ and FoxP3+ regulatory T cells in the mononuclear cells in vivo, shows that it can induce proliferation of CD4+ CD25+ and FoxP3+ regulatory T cells in rats, so it can play an important role in maintaining immune tolerance.

Conflict of interest statement

We declare that we have no conflict of interest

References


