Influence of Adoptive Transfer of Con A-activated T Cells on Serum IgG Levels and Effector Memory T Cells in Healthy BALB/c Mice

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Abstract

Background: Central memory T cells and effector memory T cells probably be affected by different inducing condition.

Objective: To observe the changes of serum IgG levels and memory T cells in the spleen mediated by adoptive transfer of Con A-activated T cells in healthy BALB/c mice.

Methods: The changes of serum IgG levels and the frequencies of memory T cells in the spleen were detected with ELISA and flow cytometry every week after adoptive transfer of Con A-activated BALB/c spleen T cells or untreated spleen T cells to syngeneic healthy BALB/c mice.

Results: The number of $CD3^+CD25^+$T cells increased after stimulation by Con A. The levels of serum IgG and the frequencies of effector memory T cells in the spleen of BALB/c mice which had been injected with Con A-activated BALB/c spleen T cells increased.

Conclusion: Adoptive transfer of Con A-activated T cells do have influence on serum IgG levels and the frequencies of effector memory T cells in healthy BALB/c mice.

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Introduction

Concanavalin A (Con A) is a lectin protein derived from sword beans, with molecular mass of 102 kDa. It is a typical mitogen that was widely used to stimulate the activation of T lymphocytes and promote the secretion of lymphocyte cytokines in vitro [1]. Con A can cause profound liver injury in mice by intravenous injection. Con A can activates T cells in the periphery. The activated T cells then can accumulate in the liver to mediate liver injury, either by direct cytotoxicity or by releasing pro-inflammatory cytokines [2]. As ConA has a high affinity towards liver sinusoidal epithelial cells, direct activation of lymphocytes in the liver may play a critical role in liver injury. However the influence of Con A-activated T lymphocytes on serum IgG and effector memory T cells has not yet been evaluated.

Representing approximately 75% of serum immunoglobulins, Immunoglobulin G (IgG) is the main antibody isotype found in blood and extracellular fluid allowing it to control infection of body tissues. IgG is produced by plasma B cells in the spleen and lymph nodes [3]. It is the most important immuneglobulin in primary immune response and participate predominantly in active and passive immunity [4].

In later stage of immune response, vast majority of effector T cells die by apoptosis, sparing a population of long-lived memory cells [5]. Recent studies indicated that mouse memory T lymphocytes contain distinct functionally subsets of central memory T cells (Tcm) characterized by $CD44^{+}CD62L^{+}$ and effector memory T cells (Tem) characterized by $CD44^{+}CD62L^{-}$ [6]. Protective memory is mediated by Tem that display characteristic sets of chemokine receptors and adhesion molecules, migrate to inflamed peripheral tissuees and exert immediate effector function. Whereas reactive memory is mediated by Tcm that home to T cell areas of secondary lymphoid organs, have little or no effector function, but readily proliferate and differentiate to effector cells in response to antigenic stimulation. When compared with Tcm, Tem are characterized by rapid effector function. Different inducing conditions may affect the formation of these two cell subsets [7].

In this study, we investigated whether the serum IgG levels and the number of Tem were significantly increased in BALB/c mice which had been immunized with homologous spleen T cells activated by Con A (Con A-T cells).The increased levels of serum IgG and Tem reached the peak in the third week and then gradually decreased to the basic level. In contrast, injection of untreated spleen T cells did not cause the change of serum IgG levels and Tem. The results demonstrated that adoptive transfer of ConA-T cells might lead to increases in serum IgG levels and the frequencies of Tem in the spleens of healthy BALB/c mice.

Materials and Methods

Animals

6-8 weeks old female BALB/c (H-2d) mice were purchased from Laboratory Animal Center of Guangxi Medical University.

Preparation of Activated T Cells

The mice were sacrificed by cervical dislocation method. The spleens were excised and cut into sections approximately with a size of 1 mm3. The sections were dilacerated and filtered with a sterile steel mesh and then the spleen cell suspension was collected and washed once for 5 minutes at 1200 rpm. Erythrocytes in the pellet were lysed using erythrocytelysis buffer. The cells were washed with PBS for three times and resuspended in

6-well cell culture plate within 1640 culture medium (Gbico, Life Technologies) consisted of 10% fetal calf serum, 10 U/ml penicillin and 10 μg/ml streptomycin [8]. Cells were activated with 10 μg/ml ConA or untreated for 24 h in environment of 37℃ with 5% CO2.

Characterization of Activated T Cells

CD25 is a T cell activation marker [9]. In this research, the frequencies of $CD3^+CD25^+$ T cells were used to evaluate the activation level of T cells induced by Con A [10]. The above-mentioned spleen T cells induced by Con A for 24 h and untreated T cells were collected respectively. 1 × 106 cells were incubated with 100 μL PBS containing the surface markers phycoerythrin (PE)-conjugated anti-mouse CD25 and fluorescein isothiocy -anate (FITC)-conjugated anti-mouse CD3 for 30 min on ice in the dark. After two washes in PBS, cells were detected with flow cytometer (Beckman Coulter® Epics XL-MCL). 10000 cells were collected for each test, and the data were analyzed with software Expo 32 (Beckman coulter). All antibodies for flow cytometry were purchased from BD Biosciences.

Adoptive Transfer of T Cells

The healthy BALB/c mice were randomly divided into two groups. Each mouse of experimental group was injected intravenously with 2 × 107 Con A-T cells, and for the control group, each mouse was injected intravenously with 2 × 107 untreated T cells.

Flow cytometry analysis of Tem in the spleen

After the initial injection, two mice were randomly selected from each group to sacrifice and separate spleen cells for detecting the frequencies of Tem every week. The preparation method for spleen cell suspension was indicated above. 1 × 106 cells were incubated with 100 μL PBS containing PE-conjugated anti-mouse CD62L, FITC-conjugated anti-mouse CD44 and Percp-conjugated anti-mouse CD3 for 30 min on ice in the dark. After two washes in PBS, cells were detected with flow cytometer (Beckman Coulter® Epics XL-MCL). 10000 cells were collected for each test, and the data were analyzed with software Expo 32 (Beckman coulter). All antibodies for flow cytometry were purchased from BD Biosciences.

Detection of Serum IgG Levels by ELISA

Before the mice were sacrificed and the retro-orbital blood was collected. The blood was kept at room temperature for 10 min before centrifuging at 3000 rpm for 10 min in order to obtain the serum. Serum IgG test was conducted according to the instruction on the assay kit. The assay kit was purchased from eBiosciences, San Diego, CA.

Results

Up-regulated Frequencies of $CD3^+$ $CD25^+$ T cells

After T cells were stimulated by Con A for 24 hours, the frequencies of $CD3^+CD25^+$ T cells were higher than those without stimulation, which proved that the T cell activation effect stimulated by Con A was obvious (Fig. 1).

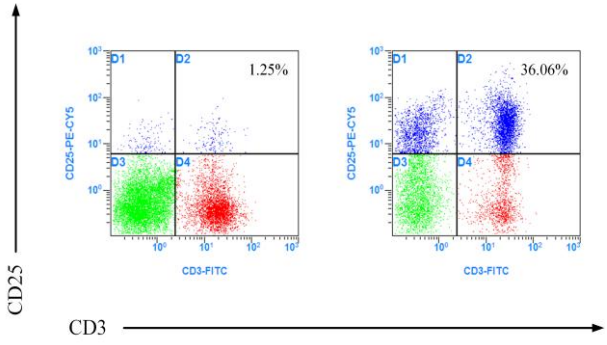

Figure 1: The frequencies of CD3+CD25+ T cells increased after stimulation by 10 ug/ml Con A for 24 h, and the frequencies of $CD3^+CD25^+$T cells

rose from 1.25%±0.6% to 35.9% ±2.4%.

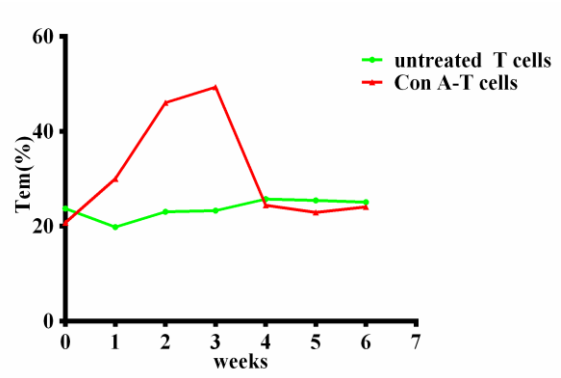

Figure 2: The injection of Con A-T cells caused a rise in Tem, which peaked on the third week and then decreased immediately. It dropped back to the basic level on the fourth week. Injection of untreated T cells did not cause obvious change in Tem

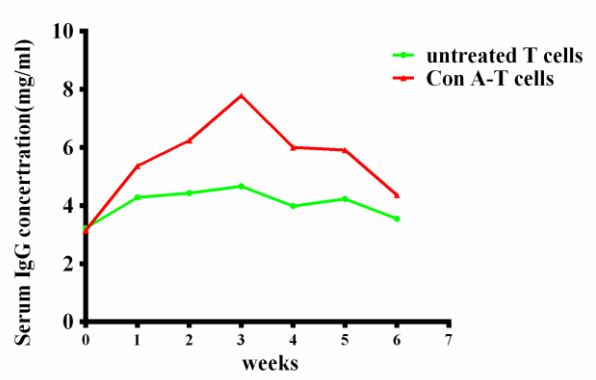

Figure 3: After injection of Con A-T cells, serum IgG levels of mice increased with time, peaked on the third week and then decreased gradually. It dropped back to the basic level on the sixth week. Injection of untreated T cells did not cause obvious change in serum IgG levels.

Adoptive Transfer of Activated T cells Resulted in Higher Frequencies of Tem

After injection of Con A-T cells, the frequencies of Tem in the spleen increased with time, peaked on the third week and then decreased thereafter. It dropped back to the basic level on the fourth week. Injection of untreated T cells did not cause obvious change in Tem (Fig. 2).

Adoptive Transfer of Activated T cells Resulted in Higher Serum IgG levels

ELISA result indicated that the trend of change in serum IgG was similar to that in Tem. After injection of Con A-T cells, serum IgG levels increased with time, peaked on the third week and then decreased gradually. It dropped back to the basic level on the sixth week. Injection of untreated T cells did not cause obvious change in serum IgG (Fig. 3).

Discussion

T lymphocytes are identified as key effector cells of concanavalin A-induced liver injury [11]. Activated T cells exhibit direct cytotoxicity or release proinflammatory cytokines that mediate hepatocellular death. The cytokines lead to further macrophage activation and accumulation in liver. Hepatocytes are found in direct contact with activated T cells and show severe damage on their sinusoidal surfaces. Activated liver macrophages primarily produce and release TNF-α, and by the action of this cytokine, they induce necrosis and/or apoptosis in hepatocytes. Previous studies have shown that the immunosuppressant could prevent con A-induced liver injury by inhibiting lymphocyte activation [12]. Nevertheless, few studies have been done on extrahepatic immune responses induced by Con A-activated T Cells. This research found out that Con A-activated T cells might generate the extrahepatic immune response in the body, as indicated by increases of serum IgG levels and memory T cells. Research showed that tissue damage was evident after administration of low-dose intravenous Con A for six weeks [13]. The period is similar to the changing cycle of serum IgG and Tem observed in this research, indicating that a possible relation exists between Con A-induced liver injury and increases of both serum IgG levels and Tem.

Current research asserts that Tem are a tissue-resident subset of memory T cells that display immediate effector function at the site of antigen deposition. Tem cells respond in nonlymphoid tissues, where they initiate a localized inflammatory immune response in reaction to antigen invasion [14]. Tem play an important role in the infection process of viral hepatitis, and they are the main effector cells for eliminating hepatitis virus [15]. However, the role of Tem in the liver injury induced by Con A is not clear yet. In our research, the observed change in Tem is in conformity with the current research progress. Tem showed a transient rise followed by injection of Con A-T cells and exerted their effector functions instantly upon the activation. This research will facilitate further clarify the role of Tem in Con A-induced liver injury. The observed increase in serum IgG levels is similar to the serological manifestations of autoimmune hepatitis, suggesting that Con A-induced liver injury may be related to the excessive activation of humoral immune respons.

This research observed that the increases of serum IgG levels and the frequencies of Tem in the spleen were caused by injection of Con A-T cells. These findings can be beneficial to further study on the relation between systemic immune response and Con A-induced liver injury, and they are valuable to explore the pathogenesis of human hepatitis.

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