Biochemical and Biophysical Research Communications 501 (2018) 320–327

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Recirculating Th2 cells induce severe thymic dysfunction via IL-4/STAT6 signaling pathway

Hui Shen [a], Chen Yin [a], Ya-Nan Gao [a], Xiao-Yan Pei [a], Xiu-Yuan Sun [a], Qing Ge [a], Wei Wang [a, **, 1], Yu Zhang [a, b, *, 1]

[a] Department of Immunology, School of Basic Medical Sciences, Peking University Health Science Center, Key Laboratory of Medical Immunology, Ministry of Health (Peking University), Beijing, China
[b] Institute of Biological Sciences, Jinzhou Medical University, Jinzhou, Liaoning, China

ARTICLE INFO

Article history:
Received 29 April 2018
Accepted 4 May 2018
Available online 9 May 2018

Keywords:
Thymocyte development
Thymic involution
Th2 cell
IL-4
STAT6

ABSTRACT

Thymic involution happened early in life, but a certain ratio of activated $CD4^+$ T cells will persistently recirculate into the thymus from the periphery and it have been suggested to be able to inhibit the development of embryonic thymocytes. Our present study was aimed to elucidate the specific mechanism how activated $CD4^+$ T cells could influence upon developing thymocytes by using fetal thymic organ culture (FTOC) and kidney capsule transplantation. Our results demonstrated that Th2 cells were found to play a fundamental role in the inhibition of embryonic thymocyte development since a very low concentration of Th2 cells could obviously reduce the total number of thymocytes. And this effect was not tenable in other Th cell type. Notably, IL-4, the major cytokine secreted by Th2 cells, was suggested the key factor playing the inhibition role. In addition to reduced cell population, the proportion of double positive (DP) T cells was also heavily decreased. Furthermore, we demonstrated that it was the downstream effector signal transducer and activator of transcription 6 (STAT6) of IL-4 partially manipulate this inhibition. Together, these findings reveal a novel influence of Th2 cells re-entering the thymus on thymic involution.

1. Introduction

The thymus is the primary site for the development of T lymphocytes. Hematopoietic progenitors seeding the thymus unfold an intrinsic developmental program, culminating in the generation of a T cell repertoire capable of responding to a diverse array of foreign antigens but tolerant to self-antigens [1–3]. This is vital to the development of a strong adaptive immune response against pathogens and tumours, without leading to autoimmune disease [4]. However, the thymus starts to undergo progressive regression since childhood [5,6]. This process – known as thymic involution – is characterized by reduced tissue mass and cellularity, disorganized morphology, and diminished production and exportation of naive T cells [4–7]. As the importance of the role of the thymus has grown, so too has the understanding that it is extremely sensitive to thymic involution associated with aging. The physiological significance of this seemingly undesired process remains elusive and our understanding of the mechanisms underlying thymic involution is still limited. Our previous studies have indicated that reduced thymic output favors the maintenance of the memory T cell pool [8].

Although mature T cells are exported to the periphery routinely, it has long been recognized that peripheral T cells are capable of recirculating into the thymus and reentry is largely restricted to activated or memory T cells [9]. It is estimated that the thymus of an adult mouse can accommodate about 10^5 recirculating peripheral T cells, accounting for 1–5% of SP thymocytes [10,11]. In addition, evidence is emerging that these cells may participate in the shaping of the T cell repertoire by delivering self-antigens into the thymus [10–14]. $CD4^+$ T cells consist of several subsets, including T helper 1 (Th1), Th2, Th17 and regulatory T (Treg) cells, as defined by their pattern of cytokine production and function. For example, Th2 cells

* Corresponding author. Department of Immunology, School of Basic Medical Sciences, Peking University Health Science Center, Key Laboratory of Medical Immunology, Ministry of Health (Peking University), Beijing, China.
** Corresponding author. Department of Immunology, School of Basic Medical Sciences, Peking University Health Science Center, Key Laboratory of Medical Immunology, Ministry of Health (Peking University), Beijing, China.
E-mail addresses: wangwei83427@bjmu.edu.cn (W. Wang), zhangyu007@bjmu.edu.cn (Y. Zhang).
[1] Tel: +0086 10 82802593; fax: +0086 10 82801436.

https://doi.org/10.1016/j.bbrc.2018.05.030
0006-291X/

secrete interleukin-4 (IL-4) as their signature cytokine and also produce IL-5, IL-13, and some produce IL-9 [15–17]. IL-4 and its downstream transcription factor STAT6 are identified as the key mediators in Th2 differentiation, and the transcription factor Gata-3 is discovered to be essential for in vivo Th2 development [16]. More recently, we have demonstrated that activated $CD4^+$ T cells homing to the thymus inhibits the development of T cells [18]. Nevertheless, the critical factor still need for clarification.

Using FTOC, the present study investigated the impact of different types of Th cells on the major cell population in the thymus, namely the thymocytes. Co-culturing with Th2 cells had a significant inhibitory effect on thymocyte development which was most likely due to the secretion of IL-4. Furthermore, we demonstrated that IL-4/STAT6 signaling pathway was a major contributor to the impaired development of thymocytes by using STAT6-deficient mice. These findings provide a new perspective on the role of recirculating T cells, especially Th2 cells, and its implication in age-related thymic involution.

2. Materials and methods

2.1. Mice

C57BL/6 (B6) mice were purchased from the Vital River Laboratories (Beijing, China) and raised in the animal breeding facility at Peking University Health Science Center under specific-pathogen-free conditions according to institutional guidelines. BALB/c mice with targeted disruption of the gene encoding STAT6 ($STAT6^{-/-}$ mice) were obtained as a gift from Peking University School of Life Sciences. The experimental procedures on use and care of animals had been approved by the ethics committee of Peking University Health Science Center.

2.2. Antibodies and reagents

PE-Cy7-, PE-CF594-, FITC-, and APC- conjugated anti-mouse CD4, CD8, CD45.1 and CD45.2; FITC- conjugated anti-mouse CD62L were purchased from BD Biosciences. PE- and APC- conjugated anti-mouse CD25 and CD44 were obtained from eBioscience. Purified anti-mouse CD3 and CD28 were obtained from ZSGB-BIO (Beijing). Recombinant mouse IL-4 and mouse IL-4 antibody were obtained from R&D systems.

2.3. Helper T cell differentiation in vitro

5×10^5 FACS sorted $CD4^+$ naive T cells from wild-type C57BL/6 (B6) mice lymphocytes in 0.25 ml culture medium were seeded into a well of the 48-well plate pre-coated with anti-CD3 (2 μg/ml) and anti-CD28 (1 μg/ml) in incubator (5% CO_2, 37 °C) for 48 h, stimulated in the presence of the following to promote differentiation: IL-12 (10 ng/ml) and anti-IL-4 (10 μg/ml) (Th1); IL-4 (20 ng/ml) and anti-IFN-γ (10 μg/ml) (Th2); IL-6 (20 ng/ml), TGF-β (5 ng/ml), anti-IFN-γ (10 μg/ml) and anti-IL-4 (10 μg/ml) (Th17); IL-2 (4 ng/ml), TGF-β (1 ng/ml), anti-IFN-γ (10 μg/ml) and anti-IL-4 (10 μg/ml) (Treg). RPMI 1640 supplemented with 10% FCS (Biochrom Ag, Berlin) was used as culture medium. Cytokines and anti-cytokine antibodies were purchased from R&D systems.

2.4. Fetal thymic organ culture (FTOC)

The hanging drop culture and FTOC were carried out as described in detail elsewhere [18,19]. Briefly, thymic lobes were obtained from fetal 16 d mice. Hanging drop cultures were prepared in Terasaki plates by adding 20 ml of culture medium containing a certain number of cells or recombinant protein of IL-4 together with fetal thymus each well. RPMI 1640 supplemented with 15% FCS (Biochrom Ag, Berlin) was used as culture medium. The plates were inverted to form hanging drops and incubated in incubator (5% CO_2, 37 °C) for 24 h. After incubation, the lobes were removed and washed in fresh RPMI 1640 to wash the cells away. Then the fetal thymuses were put on 0.45 μm Mixed Cellulose Ester Gridded Filters (HAWG 01300, Merck Millipore) on the top of cut Absorbable Gelatin Sponges (Jinling Pharmaceutical) in 12-well plates filled with culture medium. The lobes were cultured for 12 days in the incubator (5% CO_2, 37 °C) and then were subjected to further analysis. Neutralization antibody and recombinant protein of IL-4 were used in 10 μg/ml and 20 ng/ml respectively. The agents were given from the start of the hanging drop culture and throughout the whole FTOC. Cultures were fed every 2–3 days and the agents were given together with the fresh culture medium.

2.5. Flow cytometry analysis and cell sorting

Flow cytometry and cell sorting were performed as previously described [18]. Thymic lobes were carefully removed from filters at the indicative time and washed gently in PBS. After that, the lobes were digested in collagenase/dispase (Roche) and DNase I (Roche) to make the single-cell suspensions with the fine needles. Then the cell suspensions were incubated in 37 °C for 15 min with vortexing every 5 min. Cells were centrifuged with 0.5 mM EDTA in cold PBS added to them to prevent the aggregate formation. At last, cells were stained with fluorochrome-labeled antibodies on ice for 30 min. Flow cytometry was then conducted on a Beckman FACS Galios (Beckman Coulter) and data analysis was performed using Kaluza software.

To collect the naive $CD4^+$ T cells, lymphocytes were stained with fluorochrome-conjugated antibodies and $CD4^+CD8^-CD44^{lo}CD62L^{hi}$ cells were sorted using a BD FACSAria II.

2.6. Kidney capsule transplantation

The kidney capsule transplantation was performed as previously described [18,20]. Survival surgery was operated under sterile conditions after intra-peritoneal administration of the anesthetics, pentobarbital sodium (5 mg/ml) to 7 weeks old female 45.1 wild-type C57BL/6 (B6) mice. A small centrodorsal incision was made to expose the kidney and a small hole was made in the kidney capsule. FTOC 12 d thymic lobe treated with recombinant protein of IL-4 was placed under the left kidney capsule while thymic lobe without treatment was placed under the right kidney capsule. And then the incisions were closed with sterile sutures. The mice after surgery were kept in a specific pathogen free environment for 1–6 weeks. The thymus grafts were analyzed for total cell numbers and thymocyte phenotypes on indicated time after surgery by flow cytometry.

2.7. Statistics

Data are presented as mean ± SEM. Statistical significances were assessed by Student's *t*-test or two-way Anova (for multiple variant comparisons) using GraphPad Prism software (GraphPad).

3. Results

3.1. Th2 cells homing to the thymus inhibit T cell development

More recently, we have demonstrated that activated $CD4^+$ T cells homing to the thymus could inhibit the development of T cells [18]. In order to explore the mechanism of the inhibition, we examined the effects of several subgroups of $CD4^+$ T cells on the