Influence of genetic background of the host on Tregs following chlamydia muridarum respiratory tract infection

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Abstract: We discussed the influence of genetic background of the host on regulatory T cells (Tregs) following Chlamydia muridarum (Cm) respiratory tract infection. C57BL/6 (C57) and C3H/HeN (C3H) mice showing obvious differences in susceptibility to Chlamydia trachomatis (Ct) infection were intranasally administered with 1×10^3 IFU Cm. The mice were sacrificed at different days after infection. Intracellular cytokine staining was performed to detect the percentages of CD4^+CD25^+ T cells and Foxp3^+CD4^+CD25^+ T cells in the spleen. The mRNA expressions of Tregs-related cytokines IL-10 and IL-2 in lung tissues were detected by RT-PCR. The differences in Treg-mediated immune response between C57 and C3H mice at different stages after Cm respiratory tract infection were compared. Cm infection induced high levels of CD4^+CD25^+ T cells and Foxp3^+CD4^+CD25^+ T cells in both groups as well as high mRNA expressions of IL-10 and IL-2. The levels of CD4^+CD25^+ T cells and Foxp3^+CD4^+CD25^+ T cells in the spleen and mRNA expression of IL-2 in the lung were higher in C3H mice with a higher susceptibility than in C57 mice at 3 d and 7 d post-infection; the mRNA expression of IL-10 in C3H mice was obviously higher than that in C57 mice. Cm respiratory tract infection promoted Treg proliferation and production of IL-10 and IL-2 in C3H mice. As a result, the inhibition of Th1-mediated immune response specific to Chlamydia was enhanced. This mechanism plays a crucial role in the difference in susceptibility to Cm respiratory tract infection between the two mice.

Keywords: Chlamydia muridarum (Cm), regulatory T cell (Treg), IL-10, IL-2

Introduction

Genus Chlamydia encompasses a class of obligate intracellular bacteria parasiting on epithelial cells, endothelial cells, mononuclear cells and macrophages [1, 2]. A variety of human diseases, such as trachoma, inclusion conjunctivitis, genitourinary tract infection and lymphogranuloma venereum, are induced by Chlamydia. Many studies [3, 4] have shown that the genetic background can explain the difference in susceptibility to chlamydial infection. C57BL/6 (C57), C3H/HeN (C3H) and BALB/c mice display different susceptibility to chlamydial reproductive tract infection. C57 mice are most resistant to chlamydial infection, followed by BALB/c mice, while C3H mice are the most susceptible. As indicated by our preliminary study, C57 and C3H mice may differ in the course and outcome of Chlamydia muridarum (Cm) respiratory tract infection [5]. C57 mice had the shortened course of infection and mild pathological reactions in lung, while C3H mice had the longest course of infection with high mortality and severe pathological reactions in lung. Cm growth was seen in the lung at 2 d post-infection in the two groups of mice, accompanied by an increase in IFU. Cm growth reached the peak at 7 d post-infection, after which it gradually declined. C3H mice experienced a reduction in body weight at 3-4 d post-infection and died at d9 post-infection. The mortality reached 70% in C3H mice at 14 d post-infection; for C57 mice, the reduction in body weight after infection was less significant, and the body weight was restored at 7-8 d post-infection; all C57 mice survived [6].
Regulatory T cells (Tregs) are distinguished from Th1 and Th2 cell subgroups. Clinical trials demonstrate that the increased Tregs in the peripheral blood in patients infected by HCV and HIV inhibited the immune responses by antigen-specific CD4+/CD8+ T cells, and this led to the aggravation of infection [7]. Animal studies indicate that Tregs in the lung of mice infected by Bordetella pertussis secreted IL-10 and TGF-β to inhibit the Th1 cell-type immune responses [8].

We investigated the influence of Tregs and relevant cytokines on the difference in susceptibility to Cm respiratory tract infection between C57 and C3H mice. The purpose was to clarify the role of genetic background of the host in Cm respiratory tract infection.

**Materials and methods**

**Experimental infection**

Female C57 and C3H mice aged 6 to 8 weeks old (10 mice in each group) were intranasally administered with 40 µL of solution containing 1×10³ IFU Cm using microanesthesia technique. Mice were sacrificed at different days post-infection. Uninfected C57 and C3H mice (10 mice in each group) were taken as control.

**Detection of Tregs in the spleen by intracellular cytokine staining**

Mononuclear cell suspension derived from spleen was obtained and inoculated to 48-well plates (7.5×10⁶ cells/well). PMA (50 ng/ml, BD Corporation), ionomycin (1 µg/ml, BD Corporation) and Brefeldin A (5 mg/ml, BD Corporation) were added to culture the cells at 37°C for 5 h. The cells were harvested, incubated with APC-CD4, AF488-CD25, and/or PE-Foxp3 (Biolegend). Flow cytometry was performed using a FACS Calibur flow cytometer.

**Detection of mRNA expressions of IL-2 and IL-10 in the lung by RT-PCR**

Total RNA extraction was performed from the lung tissues using Trizol agent (Invitrogen) according to manufacturer's instruction. The extracted total RNA was reversely transcribed into cDNA (TaKaRa). The primers of IL-10 (193 bp) were: (forward) 5'-CTGAGGGCCTGTCACTGATT-3', (reverse) 5'-AGGTCTGGAGTCCAGCAGA-3'; the primers of IL-2 (428 bp) were: (forward) 5'-GATTACAGTGGCMTTGAA-3'; (reverse): 5'-GTTGAGTAGATGCMTTGACA-3'; primers of β-actin (582 bp): (forward) 5'-ATGGATGAGATATCAG-3', (reverse) 5'-ATGAGAGTACMG-TCAGGT-3'. PCR reaction was run in conditions as follows: 94°C 45 s, 35°C (53°C) 45 s, 72°C 1 min, 30 cycles, final extension at 72°C for 10 min. The products were identified by 1% agarose gel electrophoresis and then analyzed by automatic gel imaging system.

**Statistical analysis**

Statistical analysis was performed using the SPSS10.0 software. T-test was used for inter-group comparison. All data were expressed as mean ± standard deviation (SD), and P<0.05 was considered as statistically significant difference.

**Results**

**Levels of CD4+CD25+ T and CD4+CD25+Foxp3+ T cells in the spleen of C57 and C3H mice**

Mononuclear cells were isolated from the spleen and detected for CD4+CD25+ T cells and CD4+CD25+Foxp3+ T cells using a flow cytometer. The results are shown in Figure 1A and 1C. The percentage of CD4+CD25+ T cells (4.26%) in the spleen of uninfected C3H mice (day 0) was slightly higher than that in uninfected C57 mice (3.14%). Then Cm infection induced high level of CD4+CD25+ T cells in the two groups. Compared with C57 mice, the level of CD4+CD25+ T cells in C3H increased significantly at 3 d and 7 d post-infection, the former being about 1.5 times that of the latter. The change of level of CD4+CD25+Foxp3+ T cells was consistent with that of CD4+CD25+ T cells. The level of CD4+CD25+Foxp3+ T cells in C3H mice was considerably higher than that in C57 mice (Figure 1B and 1D). As indicated by the above results, C57 mice and C3H mice with Cm respiratory tract infection presented significantly different level of Tregs.

**mRNA expression of cytokines produced by Tregs in the lung in two groups of mice**

The mRNA expressions of IL-10 and IL-2 were detected in the lung for two groups of mice, so as to understand the influence of genetic background on Tregs-relevant cytokines following Cm respiratory tract infection. The mRNA expression of IL-10 in the lung of C3H mice was significantly higher than that of C57 mice at 14 d post-infection (P<0.01, Figure 2A and 2B),
but not at d7 post-infection (Figure 2B). The mRNA expression of IL-2 in the lung of C3H mice increased significantly compared with that in C57 mice at 3 d and 7 d post-infection (Figure 3A and 3B).

**Discussion**

It is generally recognized that mice of different species have different susceptibility to chlamydial infection. C57 mice are resistant to Cm respiratory tract infection, while C3H mice are susceptible to it. The functions and activity of Tregs at the site of inflammatory infiltration during different stages of infection are closely associated with the immune defense or immune-pathological process in host against infection. Tregs mediate immunosuppression in many infections, especially chronic, persistent infections. Reducing or eliminating Tregs can...
The effect of respiratory tract infection on the Treg after Chlamydia pneumoniae enhance the anti-infection immunity against a variety of pathogens (e.g., bacteria, viruses, fungi, parasites) [9].

C57 mice and C3H were experimentally infected with Cm in the respiratory tract, and the levels of CD4+CD25+ T cells and CD4+CD25+Foxp3+ T cells in the spleen were detected throughout infection. High levels of CD4+CD25+ T cells and CD4+CD25+Foxp3+ T cells were found in both groups at 3 d and 7 d post-infection; however, the increase of Tregs in C3H mice was considerably higher than that in C57 mice. This indicated that the level of Tregs after infection is related to the difference in susceptibility to infection between different species of mice. A significant proliferation of Tregs in C3H mice after infection led to the inhibition of Chlamydia-specific Th1 cell-type immune responses in the host. As a result, C3H mice are highly susceptible to chlamydial infection.

In vitro experiments indicated that IL-10 produced by Tregs plays a crucial role in immunosuppression [10]. IL-10 can induce the differentiation of CD4+CD25+ T cells into Tregs [11]. In severe malaria infection, CD4+CD25+Foxp3+ and CD4+CD25+Foxp3- T cells show a marked upregulation of IL-10, which is important for the clearing of pathogens [12]. Recent studies have found that Tregs secreted IL-10 through the antigen-presenting cells to produce an immunosuppressive effect [13, 14]. By neutralizing IL-10, the expression of B7-H4 molecule on CD14+ cells is greatly reduced, while introducing recombinant IL-10 can induce the expression of B7-H4. We detected IL-10 mRNA expression in the lung of C57 mice and C3H mice, and similar results were obtained. Compared with C57 mice, C3H mice showed a higher level of IL-10 mRNA expression in the lung following Cm infection. This suggested the role of IL-10 in the immunoregulatory activity of Tregs. C3H mice exhibited a higher level of Treg-mediated immune responses after Cm infection. Tregs inhibited the Chlamydia-specific CD4+Th1 immune response through IL-10 secretion. This may be one reason for the severe pathological reactions to Cm infection and high mortality in C3H mice.

IL-2 is an important stimulatory factor in the immunosuppressive activity of Tregs. It has been found that IL-2 signals have a regulatory effect on the growth and metabolism of Tregs. In mouse and human Tregs, IL-2-mediated JAK-STAT5 pathway are closely related to Foxp3 expression by Tregs [15]. IL-2 binding to receptor IL-2R causes the phosphorylation of STAT5 and hence promotes the Foxp3 gene transcription and Foxp3 synthesis. In this way, the proliferation, survival, differentiation and stability of Tregs are increased. Due to T-cell receptor (TCR)-mediated signaling and high concentration of exogenous IL-2, Tregs will be activated, causing the inhibition of CD4+ and CD8+ T cells. In the present experiment, the IL-2 mRNA expression of C3H mice was obviously higher.
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than that of C57 mice at 3 d and 7 d post-infection, which further promoted the activity of Tregs. More Tregs were recruited to the site of infection, leading to stronger inhibition of Th1 cell-type immune responses. This mechanism explains the severe pathological reactions and higher susceptibility to Cm infection in C3H mice.

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Disclosure of conflict of interest

None.

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References


