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Dynamic changes of DC and T cell subsets in mice during *Echinococcus multilocularis* infection

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Abstract

The aim of this study was to determine the dynamic changes of dendritic cell (DC) pheno-types and T cell in response to Echinococcus multilocularis (Em) infection in BALB/c mice. Mice comprised the control and Em-infected group. At day 0, 2, 7, 30, 60, 90 and 120 after infection, the size of larval cysts, the phenotype of DC and Th in splenocytes and the expression of CD40, CD86, TLR2 and TLR4, on DCs sulfur were examined. The results show that after 60 days' infection, larval cysts grow on the surface of liver, and they become larger over time. Compared with the control mice, MHC I and MHC II expressions on DC were significantly increased at day 7 ($p < 0.05$). At the same time, CD40, CD86, TLR2 and TLR4 increased rapidly, but after that they decreased gradually. At day 120, those markers were lower than in the control group. The ratio of CD4/CD8 was normal during 90 days of infection, while at day 120, a decline in CD4 T cell and increase in CD8 were foundleading to the inversion of the CD4/CD8 ratio. Our findings suggest that within the 120 days of Em infection, the major function of DC is to present antigens. Immune response is provided predominantly by Th1 cells, inducing host immune response against Em. However, after 120 days, DC matured and the function was suppressed. Furthermore, inversion of the CD4/CD8 ratio is beneficial to the growth of Em, thus favoring its immune evasion.

Key words: *Echinococcus multilocularis, dendritic cells, Th cell, TLR.*

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Introduction

Echinococcosis, also known as hydatid disease, is one of the most important zoonotic diseases in the world. Infection with the cestode *Echinococcus multilocularis* (Em) causes alveolar echinococcosis (AE), a life-threatening disease. The infection is like a slow-growing malignant tumor. Initially, it is located in the liver and then it may spread to any other organ through metastasis [1]. Despite effective medical and surgical treatment, the risk of recurrence remains the main problem in the treatment of the disease [2]. Previous studies have shown that without treatment, the 5-year and 10-year mortality rates will be up to 70% and 93%, respectively [3]. Parasite survival *in vivo* depends on efficient evasion mechanisms. However, these mechanisms are still unclear, especially with regard to how the parasite exploits the host immune response. Studies have shown that T cell-mediated immune response is the most important mechanism to clear hydatid infections, among them T helper (Th) cells play an important role. Dendritic cells (DC) are widely considered to be the major antigen-presenting cell (APC) type in immune responses [4, 5], DC subsets and maturation may affect the differentiation of Th cells. The aim of this study was to investigate the dynamic changes in DC cell phenotypes and the CD4/CD8 T-cell ratio in response to Em infections to provide further immunological evidence on the evasion strategies of this parasite.

Material and methods

Reagents and antibodies

Flow cytometric analysis for the determination of surface antigen expression on DC and T cell were performed using conjugated rat anti-mouse antibodies (mAbs) against the following surface markers: isotype control antibodies (Abs) (IgG1 or IgG2b), anti-CD3-PE, anti-CD4-FITC, anti-CD8-PECy5, anti-CD11c-FITC, anti-MHC I-PE, anti-MHC II-PECy5, anti-CD40-PE, anti-CD86-PECy5, anti-TLR2-PE and anti-TLR4- PEcy5, which were purchased from eBioscience (San Diego, CA). All other chemicals

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were purchased from Sigma (St. Louis, MO) unless stated otherwise.

Animal infection and groups

Echinococcus multilocularis (Sichuan strain) metacestodes were maintained in 6-month-old jirds (*M. meridianus*) and provided by the Key Lab of Hydatid Disease, the First Affiliated Hospital, Xinjiang Medical University, China. The rodents were killed by cervical dislocation. Larval cysts were collected in sterile physiological saline from the peritoneal cavity, minced and filtered through a 200 um pore size metal mesh to obtain the protoscoleces. Female BALB/c mice, weighing 16-20 g, were provided by the Experimental Animal Center, Xinjiang Medical University, China. The mice were infected with protoscoleces of *E. multilocularis* (100 protoscoleces per animal), and divided into seven groups ($n = 5$ per group), randomly, and sacrificed at days 0, 2, 7, 30, 60, 90 and 120. The Institutional Review Board at Xinjiang Medical University approved this study.

Preparation of splenocytes

Cells were isolated according to an established method with slight modifications. Briefly, the Em-infected mice were sacrificed at different time points and the single cell suspension was prepared by pressing the spleen between two glass slides. The cell suspensions were passed through a 300-gauge stainless steel sieve and then let to stand to remove tissue fragments. Contaminating red blood cells were lysed by suspending cells in 0.85% NH4 in Tris-HCl buffer. The cell suspensions were centrifuged (600 × g for 10 min) and then gently resuspended in FBS-RPMI 1640. These cells were counted and adjusted to the concentration of 2×10^6 cells/ml. All procedures were conducted under aseptic conditions.

Fluorescence-associated cell sorting analysis

Cells were washed with phosphate buffered saline (PBS) containing 0.5% BSA, suspension in concentration of 2×10^5 cells/0.1 ml and pre-incubated for 20 minutes with unlabeled isotype control Abs, and then labeled with either fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- or PE-cy5-conjugated Abs by incubation on ice for 30 minutes followed by washing with FACS buffer twice. Then, the cells were resuspended in 1% paraformaldehyde. Flow cytometry was performed using FACSCalibur (Becton Dickinson, San Jose, CA). Cellular debris was then eliminated from the analysis using a gate on forward and side scatter. Data were analyzed using CellQuest software (Becton Dickinson).

Statistical analysis

All data were analyzed with the statistical package SPSS 11.0 for Windows (SPSS incorporated, Chicago). One-way analysis of variance (ANOVA) was used to determine statistically significant differences among the groups, and means of every two different groups were detected with Student's *t*-test; $p < 0.05$ was considered statistically significant.

Results

Morphological observation after infection by Echinococcus multilocularis

Within early 30 days after infection of Em, there were no obvious growth of cysts in liver and abdominal cavity (Fig. 1A). 60 days later, larval cysts about (0.1-0.2) cm can be seen in the abdominal cavity and on the surface of liver, they can be easily separated from the surrounding tissue (Fig. 1B). At day 90 of mice inoculated with Em, hepatic lobule and abdomen shows cysts with different diameters, they were vesicles, translucent, fish roe shape clusters aggregation and could not be easily separated from the hepatic lobule and abdominal cavity (Fig. 1C). The cysts volume increased gradually, at day 120, vesicle center showed calcification and necrosis (Fig. 1D).

Dynamic changes in DC cells phenotype

FACS-sorted pure CD11c+ cells were used to exclude the contamination of other cell types (Fig. 2A). Compared with the 0-day group, the expressions of CD40, CD86, MHC I and MHC II were significantly increased at day 7 ($p < 0.05$, Fig. 2B), and then decreased gradually and remained lower than the 0-day group. As shown in Fig. 3, the expressions of TLR2 and TLR4 were significantly increased at day 7 ($p < 0.05$) compared to the 0-day group, and then decreased gradually to remain lower than the 0-day group.

Dynamic changes in CD4/CD8 ratio of T cells

The CD4/CD8 ratio of T cells was normal at the first 90 days after infection, followed by a decline in CD4 lymphocyte counts and an increase in CD8 lymphocyte counts, leading to inversion of the CD4/CD8 ratio. The CD4/CD8 ratio was lower than 1 at day 120 after infection. Compared with the CD4/CD8 ratio before day 90, the difference was significant ($p < 0.05$, Fig. 4).

Discussion

T cells are an important type of immunocompetent cells that can present a series of morphological and functional changes. They can produce a variety of cytokines after being stimulated by substances such as antigens [6, 7], and thus implement cellular immune functions. T lymphocytes and their subtypes show mutual coordination and mutual antagonism during an *in vivo* immune response to maintain their balance. A normal immunity depends on the regulation of T lymphocytes, especially on the mutual restraint and balance of CD4 and CD8 subtypes, since their

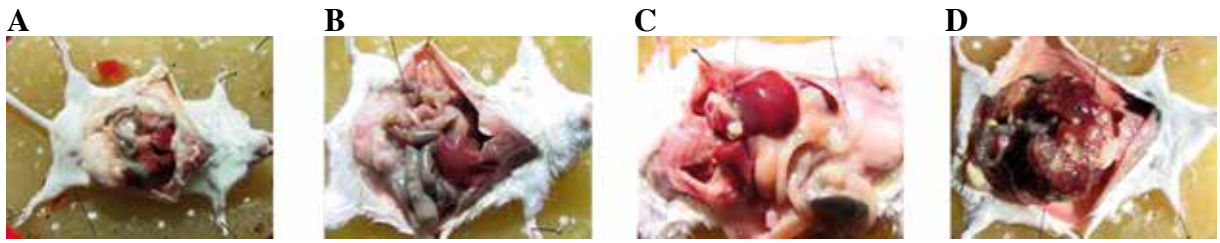
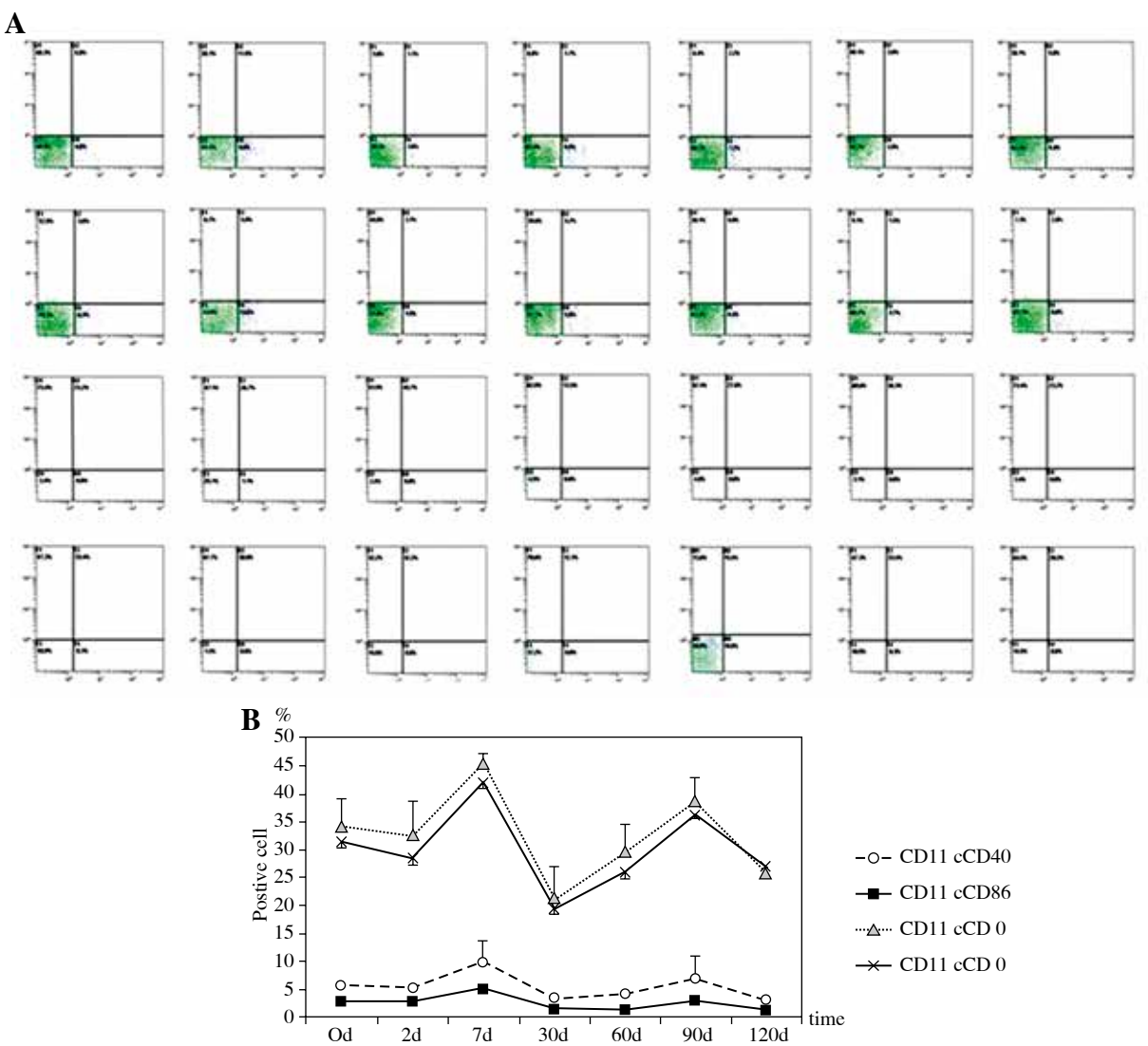
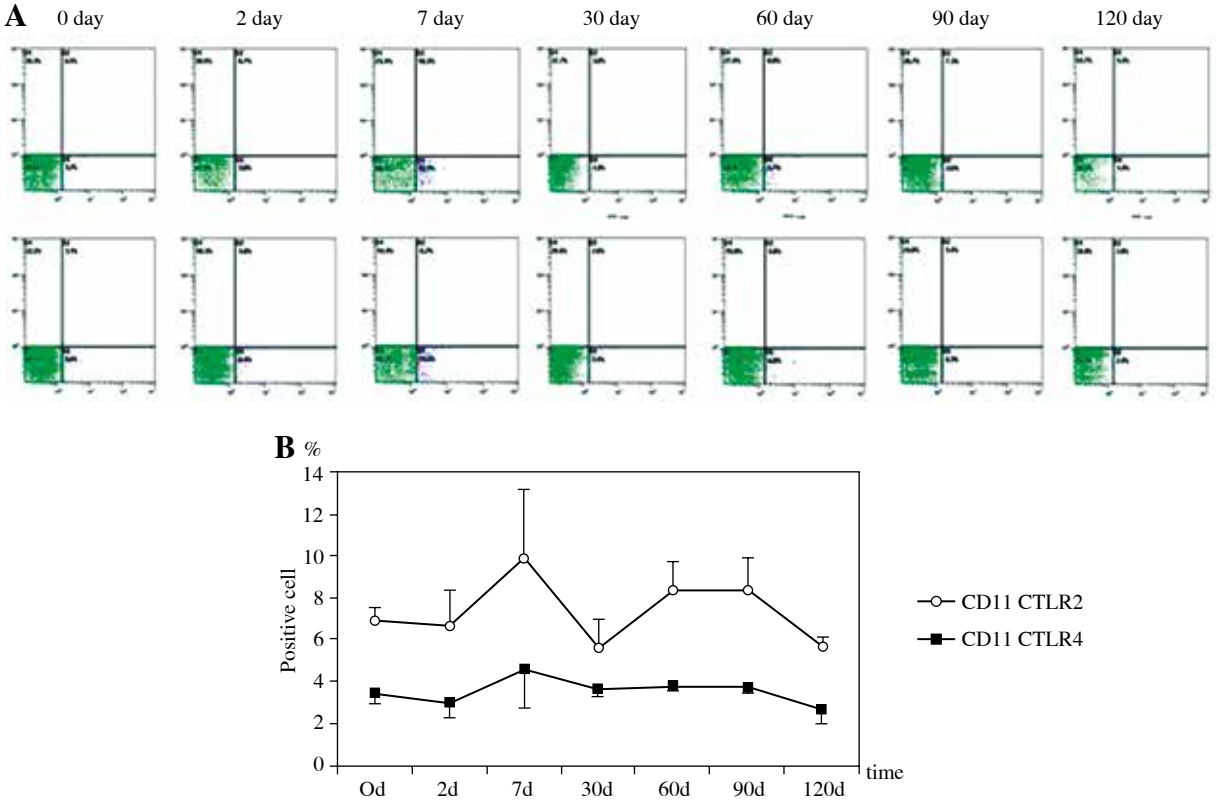


Fig. 1. Morphological observation after infected by Em Morphological observation after infection **A)** day 30, **B)** day 60, **C)** day 90, **D)** day 120.



*A) Representative individual FACS analysis of splenocytes isolated from Em-infected mice at different time points. The plots show side scatter/forward scatter (FSC/SSC) gated DC cells as well as CD40, CD80, MHC I+ and MHC II+. B) Cell surface CD markers and MHC expressions were compared with 0-day group. Mean values of gated cells are shown as percentage ± SE (n = 5). * compared with 0-day group $p < 0.05$.*

Fig. 2. Dynamic changes of CD40, CD80, MHC I+ and MHC II+ on DC cells



A) Representative individual FACS analysis of splenocytes isolated from Em-infected mice at different time points. The plots show FSC/SSC gated DC cells as well as TLR2 and TLR4.

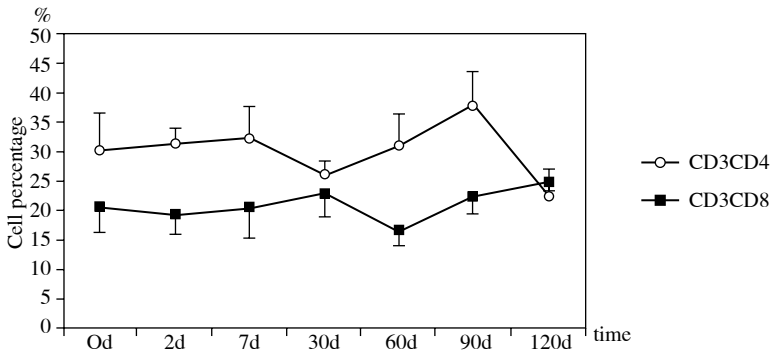
B) TLR2 and TLR4 expressions were compared with the 0-day group. Mean values of gated cells as percentage ± SE (n = 5).
** compared with the 0-day group $p < 0.05$.*

Fig. 3. Dynamic changes of TLR2 and TLR4 expression on DC cells

changes are better indexes reflecting cellular immunity. Studies have shown that T cell-mediated immune response is the most important mechanism of the body to clear hydatid infections, and that T helper (Th) cells occupy the central position [8]. AE progression is related to the cellular composition of granulomas around lesions and the proportion of T lymphocyte subsets [9].

Dendritic cells are the most powerful APC *in vivo*, and DC subsets and DC maturation may affect the differentiation of Th cells [10]. DC activate Th0 cells to differentiate into Th1 or Th2 cells, thus forming the link between the innate and the acquired immunity [11]. Dendritic cells provide three main signals to Th cells: an antigen-specific signal, a co-stimulatory signal and a differentiation or polarization signal. Dendritic cells have the unique feature of activating the initial T cells. Multiple receptors on the surface of DC mediate multiple biological effects to initiate natural immune responses. They also participate in immune regulation, in which pattern recognition receptors, such as DC-SIGN and Toll-like receptors (TLR), are expressed on the DC surface. The DC can therefore identify pathogen-associated molecular patterns (PAMPs) carried by pathogenic microorganisms invading the body, and present their antigens to T cells after processing them [12, 13]. DC activated by TLR play a defensive role mainly through two mechanisms. Firstly, the phagocytosis and the synthesis of type I interferons are enhanced; secondly, TLR activation can promote the release of cytokines and the up-regulated expression of co-stimulatory molecules and histocompatibility complex molecules, thereby improving DC maturation, and activating T cells and B cells to start the acquired immunity [14, 15].

MHC I and II are important parts of the first signal in T cell activation. In this study, MHC I and II expressions on the DC surface were detected at different times after Em inoculation, indicating that MHC I and II expressions reached their peak 7 days after Em inoculation, which might be related to the occurrence of a specific immune response after antigens entered the body (the body has experienced around 7 days' incubation period). Within 120 days after Em inoculation, MHC I and II expressions remained at a high level, while their expression decreased



*The splenocytes isolated from Em-infected mice at different time points. To determine the absolute counts of CD4+, CD8+ and CD3+ cells and their ratios by two color-immunophenotyping on the single FACS platform, fluorochrome-labeled monoclonal antibodies to CD4+/CD3+ and CD8+/CD3+ T-cells were used. The CD4/CD8 ratio is shown as percentage ± SE (n = 5). * compared with the 0-day group $p < 0.05$.*

Fig. 4. Dynamic changes in the CD4/CD8 ratio of T cells

after 120 days. This indicates that DC showed a fluctuation while presenting antigen signals to Th cells, which is closely related to Th activation and thus antiparasitic immunity [16]. Therefore, 120 days after Em inoculation, Th cell subsets showed cyclical dynamic changes. This might be related to the parasite biomass and the release of antigens, since antigens were massively released when the Em infection entered into the chronic phase. The chronic stimulation of T cells might produce a cell response dominated by Th2, while reduced damage of the parasites or reduced antigen release may induce Th1 cell activity [17]. Within 120 days after Em inoculation, the CD4/CD8 ratio was 1.5/1 in T cell subsets, showing a positive proportion, and the immune response was dominated by Th1. A highly efficient Th1 cell response is not only required by the stimulation of parasite specific cytotoxic lymphocyte (CTL) responses, but is also related to the production of neutralizing antibodies. After 120 days, CD8+T cell counts were relatively increased and CD4+T cells decreased, presenting an inversion of the CD4/CD8 ratio. Subsequently the body showed immunosuppression, which may be related to activation-induced apoptosis.

CD40 and CD86 are important co-stimulatory molecules on the DC surface, and their expressions are closely correlated to DC maturation. In this study, levels of the co-stimulatory molecules CD40, CD86, TLR2 and TLR4 expression on the DC surface were detected at different times after Em inoculation. It was found out that CD40, CD86, TLR2 and TLR4 expressions were significantly increased for the first 7 days after Em inoculation compared with day 0, indicating an increase in mature DC. This might be an acute-phase immune response triggered after the exposure of the body to antigens. With the persistent existence of the parasite in the body, CD40, CD86, TLR2 and TLR4 expressions changed in volatility, but increased before day 90, and decreased after day 120. This might be because mature DC could efficiently present antigens in an early Em infection, thereby inducing antiparasitic immunity. DC maturation was lowered after 120 days in the late Em infection, and DC presented antigens less effectively to T cells, so that the immunity of the body allowed the immune escape of hydatid.

TLR2 and TLR4 are important indicators reflecting DC function. Experiments showed that the expression of TLR2 and TLR4 on the DC surface are correlated with those of CD40 and CD86, thus confirming that until 120 days after Em infection, the main DC function was to present antigens and induce antiparasitic immunity. After 120 days, due to DC maturation and decreased DC activity, the immune response allowed the long-term infestation of Em in the body.

The immune escape mechanisms of Em are of great significance for the prevention and treatment of hydatid disease. In this study, *in vivo* DC maturation and expression of surface membrane receptors and Th cell subsets were detected at different times after Em infection, thus allowing the immune response and escape mechanisms of DC and Th cell subsets-induced AE to be examined further. We aim to study the mechanism of changes in DC and Th cells to reveal the immune escape mechanism of AE.

Authors declare no conflict of interest.

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References

1. Stojkovic M, Junghanss T (2013): Cystic and alveolar echinococcosis. Handb Clin Neurol 114: 327-34.
2. Health Guidelines for treatment of cystic and *aluvolar echinococcosis* in human. Bull world health organ 1996; 74: 231.

3. Ammann RW, Hirsbrunner R, Cotting J, et al. (1990) Recurrence rate after discontinuation of longterm mebendazole therapy in *Echinococcosus multilocularis*. Am J Trop Med Hyg 43: 506.
4. Salazar F, Ghaemmaghami AM (2013): Allergen Recognition by Innate Immune Cells: Critical Role of Dendritic and Epithelial Cells. Front Immunol 4: 356.
5. Fermin Lee A, Chen HY, Wan L, et al. (2013): Galectin-3 modulates Th17 responses by regulating dendritic cell cytokines. Am J Pathol 183: 1209-1222.
6. Boutennoune H, Qaqish A, Al-Aghbar M, et al. (2012): Induction of T helper 1 response by immunization of BALB/c mice with the gene encoding the second subunit of Echinococcus granulosus antigen B (EgAgB8/2). Parasite 19: 183-188.
7. Kim HJ, Jung BK, Lee JJ, et al. (2011): CD8 T-cell activation in mice injected with a plasmid DNA vaccine encoding AMA-1 of the reemerging Korean Plasmodium vivax. Korean J Parasitol 49: 85-90.
8. Amri M, Mezioug D, Touil-Boukoffa C (2009): Involvement of IL-10 and IL-4 in evasion strategies of Echinococcus granulosus to host immune response. Eur Cytokine Netw 20: 63-68.
9. Tuxun T, Wang JH, Lin RY, et al. (2012): Th17/Treg imbalance in patients with liver cystic echinococcosis. Parasite Immunol 34: 520-527.
10. Lewis KL, Reizis B (2012): Dendritic cells: arbiters of immunity and immunological tolerance. Cold Spring Harb Perspect Biol 4: a007401
11. Trumpfheller C, Longhi MP, Caskey M, et al. (2012): Dendritic cell-targeted protein vaccines: a novel approach to induce T-cell immunity. J Intern Med 271: 183-192.
12. Kaisho T (2012): Pathogen sensors and chemokine receptors in dendritic cell subsets. Vaccine 30: 7652-767.
13. Zanoni I, Granucci F (2010): Regulation of antigen uptake, migration, and lifespan of dendritic cell by Toll-like receptors. J Mol Med (Berl) 88: 873-780.
14. Watts C, West MA, Zaru R (2010): TLR signalling regulated antigen presentation in dendritic cells. Curr Opin Immunol 22: 124-130.
15. Frick JS, Grünebach F, Autenrieth IB (2010): Immunomodulation by semi-mature dendritic cells: a novel role of Toll-like receptors and interleukin-6. Int J Med Microbiol 300: 19-24.
16. Wei XL, Ding JB, Xu Y (2004): Change of cytokines in mice with Echinococcus multilocularis infection. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 22: 361-364.
17. Rigano R, Buttari B, De Falco E, *et al*. (2004): *Echinococcus granulosus*-specific T-cell lines derived from patients at various clinical stages of cystic echinococcosisc. Parasit Immunol 26: 45-52.