Int J Clin Exp Pathol 2015;8(2):1374-1383
www.ijcep.com /ISSN:1936-2625/IJCEP0004776

Original Article

PD-L1 blockade improves immune dysfunction of spleen dendritic cells and T-cells in zymosan-induced multiple organs dysfunction syndromes

Qian Liu*, Yi Lv*, Min Zhao, Yiduo Jin, Jiangyang Lu

*Department of Pathology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100048, China. *Equal contributors and co-first authors.*

Received December 12, 2014; Accepted February 5, 2015; Epub February 1, 2015; Published February 15, 2015

Abstract: This research is to investigate the role of tolerant spleen dendritic cells (DC) in multiple organs dysfunction syndromes (MODS) at late stage. Tolerant DC and MODS were induced by intraperotineal injection of zymosan. The immunity of DC was determined by examining interleukin (IL)-10, IL-12, IL-2, major histocompatibility complex (MHC), CD86, programmed death (PD-1), programmed death ligand 1 (PD-L1), paired immunoglobulin-like receptor B (PIR-B) or T-cell proliferation in serum, spleen homogenate, DC culture or DC/T-cell co-culture. The PD-L1/PD-1 pathway was blocked using PD-L1 antibody. The IL-12p70 in serum, spleen homogenate and DC culture supernatant were decreased at 5 d and 12 d after zymosan injection while the IL-12p40 and IL-10 were increased. The expression of MHC, cluster of differentiation 86 (CD86), PD-1 and PD-L1 in spleen DCs were increased at early stage after zymosan injection. At 5 d and 12 d, the expression of MHC and CD86 was reduced while the expression of PD-1, PD-L1 and PIR-B was increased, accompanied with decreased proliferation of T-cell and decrease of IL-2 in spleen and serum. Application of PD-L1 antibody improved the above changes. At late stage of MODS mice induced by zymosan, the expression of co-stimulators and inhibitors in spleen DCs was imbalanced to form tolerant DCs which reduced the activation of T-cells. PD-L1 antibody improved the immune tolerance of DCs through intervening PD-1/PD-L1 pathway, and attenuated the inhibition of T-cell activities by tolerant DCs and the immune inhibition.

Keywords: Dendritic cell, MODS, immune tolerance, zymosan

Introduction

As the major reason of death for patients in ICU, multiple organ dysfunction syndrome (MODS) is the most serious complication of sepsis [1, 2] which results in immune dysfunction due to imbalanced pro-inflammatory and anti-inflammatory mechanisms [3, 4]. The consumption and function change of immune cells induced by excessive inflammatory response at the early stage of sepsis attenuated the phagocytosis, proinflammatory cytokine release and antigen presentation of monocytes [5, 6], resulting in immune tolerance which is pathological feature of late stage of sepsis [3, 7-10]. Therefore, improvement of immune function is the critical step for prevention of MODS induced by sepsis.

The spleen plays important roles in the responses of congenital immunity and acquired immunity, and, therefore, has important effect on the prognosis of sepsis [11, 12]. The immune function of spleen depends on the synergistic effect between spleen dendritic cells (DC) and immunocytes including T-cells, B-cells and regulatory T-cells (Treg). As the strongest antigen presenting cells, DC is the bridge between congenital immunity and adaptive immunity, controlling the balance between immune tolerance and immune activation of T-cells [13]. Recent studies indicated that DC not only can induce immune response but also have ability of immune regulation [12, 14]. The apoptosis, number reduction and mature disorder of DC during sepsis attenuate the immune activation of DC, down-regulate the activities of T-cells and B-cells, and are closely related with the prognosis of sepsis [15, 16].

The positive and negative immune-regulation of DC is achieved through the interaction between

the superficial co-stimulators and co-inhibitors on DC and the ligands of lymphocytes [14, 17, 18]. As co-stimulators, the cluster of differentiation 80 (CD80), B7-2(CD86) and programmed death ligand 1 (PD-L1) were expressed on T-cell, B-cell, DC and macrophages. As one receptor of co-inhibitor, programmed death 1 (PD-1) was mainly expressed on the surface of activated and T-cells [19]. PD-1 was clarified as the major negative regulatory receptor of PD-L1 in PD-L1-knockout mice to play inhibitory effect [20]. Under continuous stimulation by antigens, PD-1/PD-L1 negatively modulated the balance of T-cell activation, tolerance and pathological immune reaction [21]. The PD-1 expression on T-cell, B-cell and macrophage and the expression of PD-L1 on B-cell and macrophage were found to be up-regulated in cecal ligation and puncture (CLP) mice. While PD-L1 blockade increased the survival of septic animals through inhibiting lymphocyte apoptosis and recovering monocyte function [22]. These studies suggest that blockade of the interaction between PD-L1/PD-1 pathway may be promising strategy for preventing immune tolerance at late stage of sepsis.

The biphasic MODS induced by intraperitoneal injection of zymosan is one experimental animal model having typical immunopathological features of sepsis and similar clinical progression of MODS [23]. In the present study, we investigated the changing pattern of spleen DC in zymosan-induced MODS mice, the relationship of DC with immune inhibition and the mechanism of improvement of immune disorders of DC and T-cell induced by intervening PD-L1/PD-1 pathway.

Materials and methods

Animals and MODS model

Male C57BL/6 mice (6-8 weeks, 20-25 g), purchased from the Laboratory Animal Center of Academy of Military Medical Science, were accommodated for 7 days at 12:12 light-dark circle with free access of food and water. After fasting 12 h, the mice were randomly divided into control group (n=10), zymosan group (n=64) and zymosan + PD-L1 antibody group (n=64). The zymosan group and zymosan+PD-L1 antibody group were further divided into subgroups of 12 h, 2 d, 5 d and 12 d (n=16 for each subgroup).

MODS model was induced by zymosan according to literature [23]. Briefly, 1 g zymosan powder (Sigma Chemical, St. Louis, MO, USA) was mixed with 40 ml medical paraffin oil to make 25 g/L zymosan suspension which was sterilized at 100°C water for 80 min and cooled to room temperature. After sterilization of abdomen of mice, zymosan suspension was intraperitoneally injected (800 mg/kg). At 12 h and 24 h after injection, saline (20 ml/kg) was injected for fluid infustion. In zysoman + PD-L1 antibody group, PD-L1 antibody (100 μg in 100 μl PBS) was injected via tail vein immediately and at 48 h after zymosan injection. Thereafter, the animals were bred as regular and the blood and spleen were collected at corresponding time points for measurement. All the animal experiments were approved by the Ethic Committee of Animal Care and Usage of the university.

Measurement of cytokines in serum and spleen

The blood collected from fundus artery of mice was settled for 30 min, centrifuged for 15 min at 3000 rpm to separate serum stored at -80°C. 100 g spleen tissue stored at liquid nitrogen was added with 1 ml PBS and homogenated at ice bath and centrifuged for 15 min at 3000 rpm (4°C) to collect the supernatant.

IL-2, IL-10 and IL-12 in serum and spleen homogenate were measured with ELISA, according to the manual (R&D, USA).

Separation and culture of spleen DC and measurement of related factors in culture supernatant

At the corresponding time points, the spleen was taken, placed at flat dish, removed the capsule, added with 1.25 ml collagenase IV (1 mg/ml, Sigma, USA) for invasion, and intra-tissue injected 500 μl collagenase IV. Then, the tissue was cut into small pieces, incubated at 37°C for 25 min and added 10 mmol/L EDTA for incubation of 5 min. The digested tissue was ground on 400 m metal mess and rinsed with PBS to collect cell suspension which was centrifuged at room temperature (2000 rpm, 10 min). After removal of supernatant and addition of 5 ml pre-cooled PBS, the sediment was pipetted evenly and added to centrifuge tube containing 10 ml lymphocyte isolating solution (Ficoll-

Papue) for centrifugation at room temperature (3000 rpm, 15 min). The content of middle layer was taken out, rinsed with PBS for 2 times and blew evenly with 2 ml PBS for cell number accounting and measurement of cell activities with trypan blue staining (>97%). DCs were purified with anti-CD11c magnetic beads and positive selection MS + columns according to the manual (Miltenyi Biotec, Auburn, CA, USA). PE-CD11c antibody and PE-IgG Isotype Control (BD Biosciences, USA) were used to label DC for measurement of purity (>99%). The purified DC was re-suspended with RPMI1640 (Hyclone, USA) and adjusted to concentration of 5×10^6/ml. Then the cells were inoculated in 96-well plate (1.25×10^6/well) and incubated in 5% CO_2 incubator at 37°C for 24 h and centrifuged (1500 rpm, 5 min). The supernatant was taken out for measurement of IL-10 and IL-12 with ELISA.

Measurement of superficial cytokines of DC

DCs re-suspended with PBS (containing 2% BSA) were added to a flow tube (5×10^4 cells/100 μl/tube) and added with FITC or PE labeled fluorescent antibody (or PE-IgG Isotype Control, 2-3 μl) against the superficial marker. The mixture was incubated at 4°C for 45 min, rinsed with PBS twice and centrifuged (1500 rpm, 5 min). After removal of the supernatant, the cells were re-suspended with 400 μl PBS and analyzed with FACS caliber (Becton Dickinson, USA). The labeled antibodies included: MHC-II (I-Ab)-FITC, PD-L1-PE, PD-1-PE (all from BD Biosciences, USA), CD86-PE and PIR-B-PE (Biolegend, USA).

Isolation, purification and proliferation of T-cells

Spleen tissues taken at different time points were ground and rinsed with serum-free RPMI1640 to collect cells. After addition of lymphocyte isolation solution, the cells were centrifuged (18°C, 2500 rpm, 20 min). The cells of low density at middle layer were taken out and rinsed with PBS twice for cell accounting and activity measurement. The T-cells were purified with technique of magnetic activated cell sorting (MACS), according to the manual (Miltenyi Biotec, Auburn, CA, USA).

The cell proliferation was measured with MTT. T-cells after isolation and purification were added to 96-well plate (5×10^5/well) with ConA (5 μg/ml) and incubated at 37°C for 68 h. After removal of 100 μl culture medium and addition of MTT (5 mg/ml), the cells were incubated for 4 h. Finally, the cells were added with Triton-ISOP (100 μl/well) and incubated for 4 h at 37°C till the crystal was completely resolved for measurement of OD560 value.

Co-culture of DCs and T-cells and PD-L1 antibody intervening

In vitro co-culture of DCs and T-cells was used to observe the effect of septic spleen DCs on the activities of T-cells and the intervening effect of PD-L1 antibody. The purified DCs of septic mice were added to 96-well plate (2×10^4 cells/well), 3 wells for each sample. Fresh-isolated spleen T-cells (2×10^5 cells/well) from normal C57BL/6 mice were inoculated in the wells pre-plated with DCs. PD-L1 antibody (final concentration of 20 μl/ml) or control medium was added for incubation of 24 h with exchange of the culture medium. After 68 h, the supernatant was collected and measured of the concentration of IL-2, IL-10 and IL-12 using ELISA. MTT was added to the 96-well plated for continuous incubation of 4 h to measure the proliferation of T-cells.

Statistic analysis

The data were expressed as mean ± SD. SPSS13.0 was used for statistic analysis with ONEWAY-ANOVA. Intra-group comparison was performed with LSD-test and intergroup comparison was performed with t-test. $P<0.05$ was set as significant level.

Results

Developmental features of MODS in mice induced by zymosan

According to report by Jansen [23], we induced MODS model of mice by zymosan. At 3 h after injection of zymosan, the mice showed depression, less activities and feeding; these signs gradually aggravated and the death peak occurred at 24-48 h (mortality of 30.1%). After 48 h, the animals gradually recovered to normal activities, having no death (mortality of 0). After 5 d, the animals showed systemic signs again and aggravated at 10-12 d, displaying somnolence, apastia and dyspnea. The second peak of mortality was 21.9%. The two death

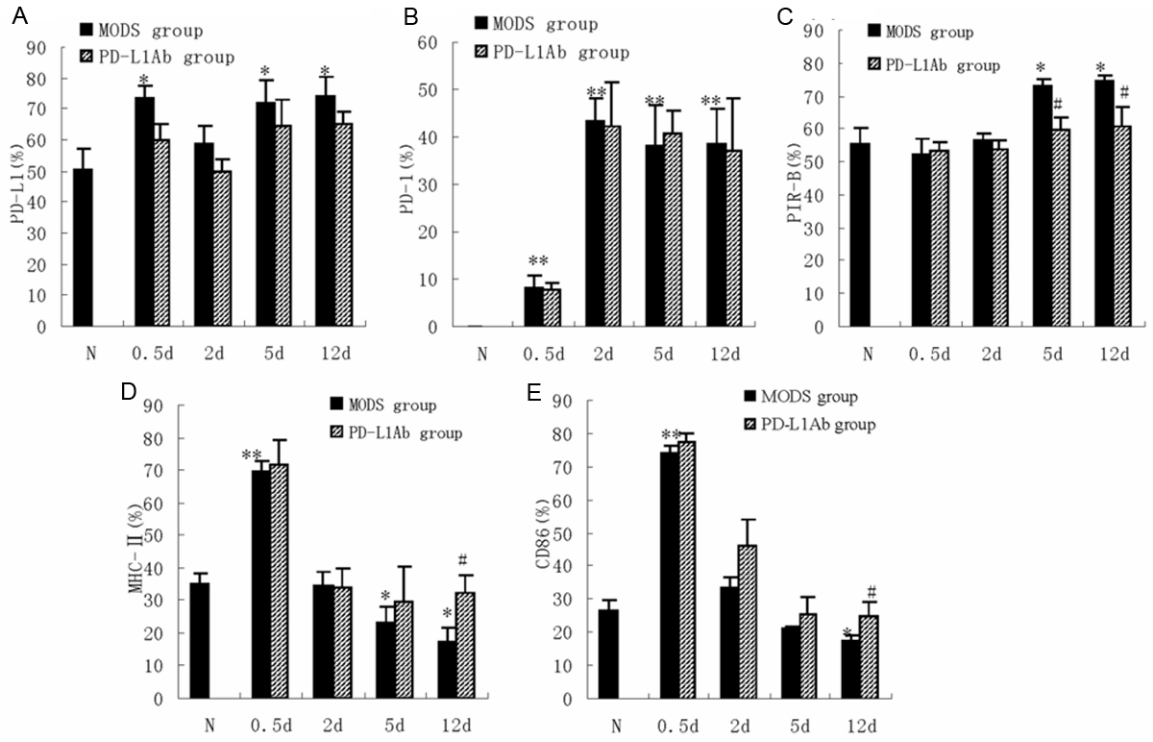

Figure 1. Expression of co-stimulator and co-inhibitor on spleen DC. PD-L1 (A), PD-1 (B), PIR-B (C), CD86 (E) and MHC-II (D) expressions on DC were measured at day 0.5 d-12 d in mice after injection with zymosan and zymosan plus PD-L1 antibody. Flow cytometry data were expressed as percentages of positive cells out of total DCs. Results were presented as box-plots as well as individual values. The LSD-test was performed.

peaks during the development of the disease model were equivalent to the excessive inflammatory response at early stage and the dominant immune inhibition at late stage.

Expression changes of PD-1, PD-L1 and PIR-B on spleen DCs of MODS mice

There were many studies about the co-inhibitor pathway of PD-1 and PD-L1 in tumor and autoimmune diseases [19], suggesting its involvement in the establishment of immune tolerance of antigen presenting cells [21]. The expression rate of PD-L1 was over 50% on DCs of normal mice spleen, upregulated at 0.5 d after injection of zymosan (*vs* control group, $P<0.05$), recovered to nearly normal at 2 d, and increased again at 5 d and 12 d (*vs* control group, $P<0.05$) (**Figure 1A**). Different from PD-L1, PD-1 expression was undetectable on normal DCs, increased at 0.5 d after injury and maintained stable till 12 d (*vs* control group, $P<0.01$) (**Figure 1B**). PD-L1 antibody had no effect on the expression of PD-L1 and PD-1.

PIR-B is one inhibitory receptor expressed on DCs surface and its expression increase is the symbol of formation of tolerant DCs [24, 25]. The expression of PIR-B on spleen DCs was not changed at the early stage of zymosan-induced injury (2 d), increased at 5 d and 12 d (*vs* control group, $P<0.05$). PD-L1 antibody inhibited the upregulation of PIR-B on DCs at late stage of MODS induced by zymosan (*vs* corresponding timepoints of MODS group, $P<0.05$) (**Figure 1C**).

Expression change of MHC-II and CD86 on spleen DCs

MHC-II and CD86 are the 1st and 2nd signaling molecules, respectively, for the antigen presenting function of DCs; their expression levels on DCs directly affect the immune activities of DCs [26]. The expression of MHC-II on spleen DCs was greatly increased at 0.5 d after zymosan injection (*vs* control group, $P<0.01$), gradually decreased and was lower than normal level at 5 d and 12 d after zymosan injection. PD-L1 antibody inhibited the downregulation of MHC-II

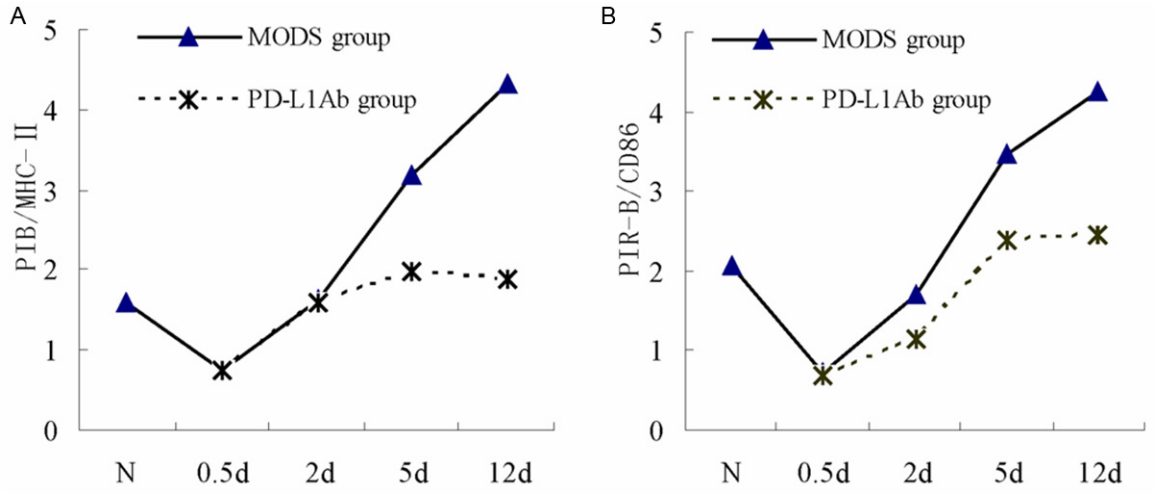

Figure 2. The ratios of PIR-B expression to MHC-II (A) and to CD86 (B) expression on spleen DCs. By flow cytometry PIR-B, CD86 and MHC-expression was calculated with percentages of positive cells out of total DCs.

on DCs at 12 d (*vs* MODS 12 d, P<0.05) to normal level (**Figure 1D**). Similar to MHC-II, the expression of CD86 was rescued by PD-L1 antibody at 5 d and 12 d (**Figure 1E**).

In order to analyze the relative intensity change of immune inhibition and immune stimulation of DCs, we compared the relative values of PIR-B expression with MHC-II expression or CD expression. The result indicated that ratios of PIB-B/MHC-II and PIB-B/CD86 were greatly increased while PD-L1 antibody could recover the ratios of PIB-B/MHC-II and PIB-B/CD86 (**Figure 2**).

Changes of IL-12 and IL-10 in spleen and serum

IL-12(IL-12p70), a heterodimer composed of p35 and p40 subunits, is mainly derived from mononuclear macrophages and DCs having ability of antigen presenting. IL-12p70 binds to IL-12 receptor of T-cells promoting immune response while IL-12p40, in contrary, is the natural antagonist of IL-12 [27]. Under condition of physiology or pathology, IL-12p70/p40 induces T-helper cell (Th1/Th2) shift and modulates immune response through modulating Th cells. Therefore, spleen change of IL-12p70/p40 reflects the immune states of antigen presenting cells including DCs. After zymosan injection, the content of IL-12p70 in spleen gradually decreases while IL-12p40 gradually increases. At 5 d and 12 d, the IL-12p70 reached the bottom while IL-12p40 reached the peak (*vs* control group, P<0.05; **Figure 3A-E**). Spleen inhibitory cytokine IL-10 was increased at 5 d and 12 d after zymosan injury (*vs* control group, P<0.05; **Figure 3G**, **3H**). PD-L1 antibody blocked the imbalance between superficial DC co-stimulator and co-inhibitor induced by zymosan and affected the secretion of IL-12 and IL-10, resulting in significant difference between PD-L1 antibody group and MODS group at 12 d (P<0.05, **Figure 3**).

The serum concentration of IL-12p70 was only 50% of control group at 12 d after injury (P<0.05) while IL-12p40 was greatly increased at late stage of MODS. The serum concentration of IL-10 was largely increased at 5 d and 12 d (*vs* control group, P<0.05; **Figure 3C**, **3F**, **3I**). In PD-L1 antibody group, the serum content of IL-12p70 was kept at control level while IL-12p40 was lower than MODS group at 12 d. The serum IL-10 was mild lower than MODS group at 5 d and 12 d (P>0.05, **Figure 3C**, **3F**, **3I**).

Protection of PD-L1 antibody on T-cell proliferation induced by zymosan

At 2 d after zymosan injection, the proliferative activity of spleen T-cells was largely decreased from 2 d to 12 d (P<0.05). PD-L1 antibody recovered the proliferation of T-cells at 5 d and 12 d, significantly higher than MODS group (P<0.05) (**Figure 4A**). Simultaneously, the

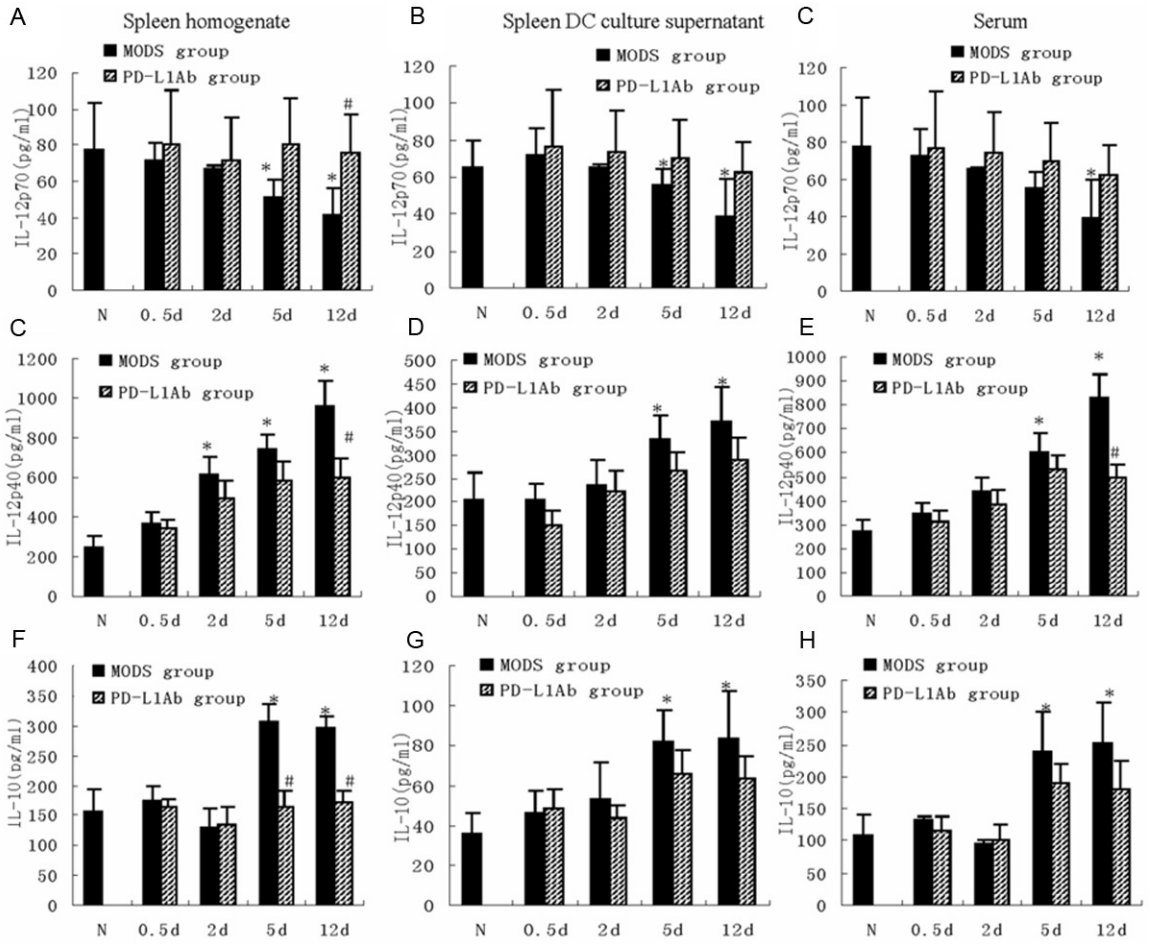

Figure 3. Expression changes of IL-12p70, IL-12p40 and IL-10 in spleen homogenate (A-C), spleen DC (D-F) and serum (G-I) *P<0.05 vs control group; #P<0.05 vs same time point of zymosan alone group (MODS).

secretion of IL-2 by T-cells in vitro culture was significantly decreased at 5 d and 12 d (P<0.05), PD-L1 antibody increased the secretion of IL-2 but without significant difference (P>0.05) (**Figure 4B**). The serum concentration of IL-2 showed same trend with the secretion of IL-2 by T-cells in culture, suggesting that the immunity of T-cells at 5 d and 12 d was significantly decreased while PD-L1 antibody had protective effect on T-cells activity.

Improvement of DC and T-cells by PD-L1 antibody in vitro

In order to investigate if the inhibition T-cell in MODS was correlated with the formation of tolerant DC through PD-L1/PD-1 pathway, we isolated spleen DCs at different stage of MODS mice and cultured the DCs with normal spleen T-cells. The result indicated that the spleen DCs of MODS mice had strong inhibitory effect on the proliferation of T-cells of normal spleen at 5 d and 12 d, and inhibited the secretion of IL-2 (P<0.05); PD-L1 antibody could improve the proliferation of T-cells and increase the secretion of IL-2 (P<0.05) (**Figure 4C**, **4D**).

In addition, PD-L1 antibody increased the concentration of IL-12p70 and decreased the concentrations of IL1-2p40 and IL-10 by DC in the medium co-culture of spleen DC from MODS mice and normal T-cell (**Figure 5**).

Discussion

The present study indicated that, at the late stage of MODS mice induced by zymosan, MHC-II and CD86 of spleen DCs were downregulated while PD-L1/PD-1 and PIR-B were upregulated, the immunity of DCs and T-cells was

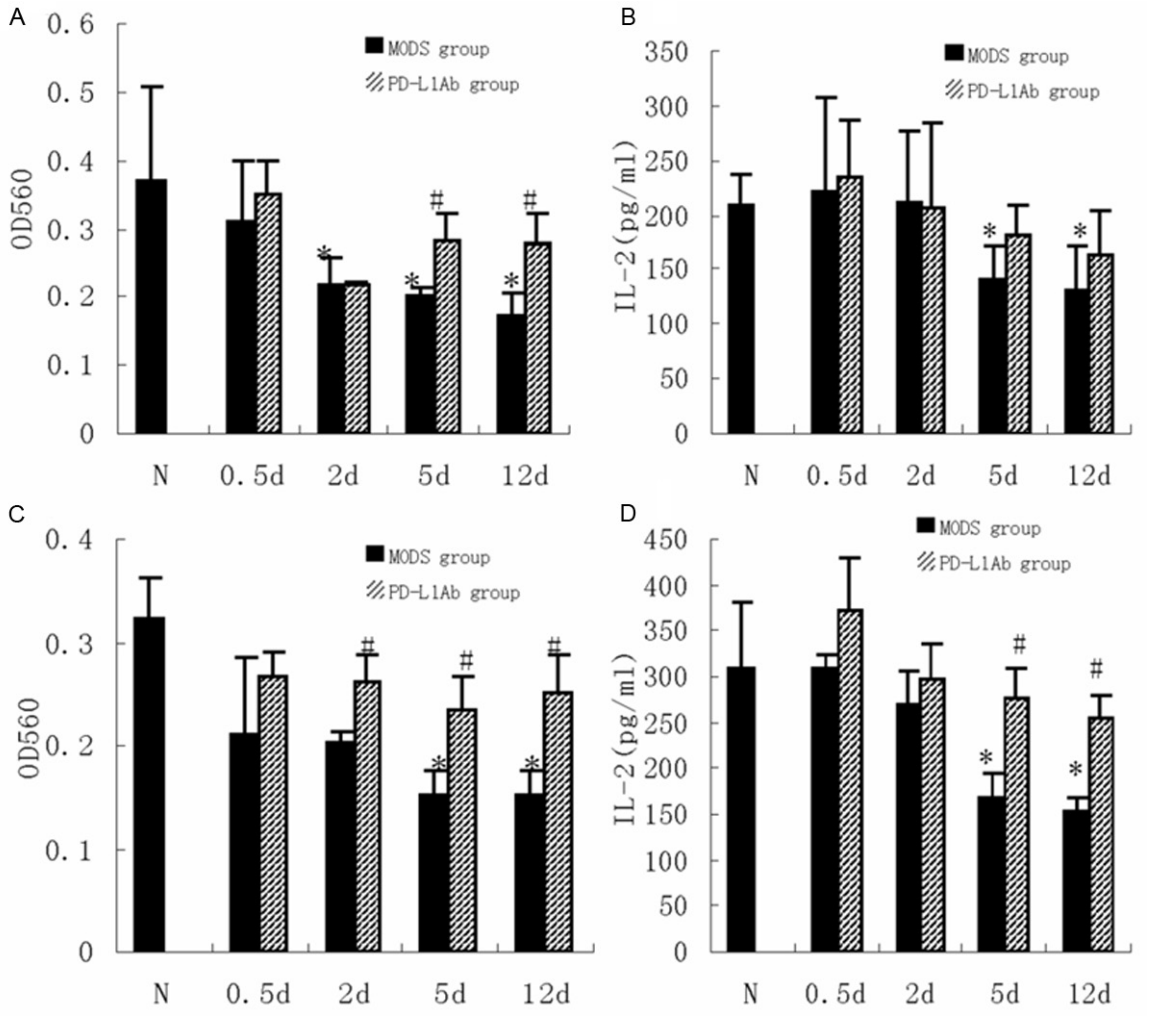

Figure 4. Changes of activities of spleen T-cells. A, B: single culture of T-cells from different groups; C, D: co-culture of spleen DC from different groups with T-cells from normal spleen. *P<0.05 vs control group; #P<0.05 vs corresponding time point of zymosan group.

decreased. The tolerant DC of MODS mice had inhibitory effects on T-cells. These changes in late stage of MODS mice were blocked by PD-L1 antibody.

As one pro-inflammatory agent, zymosan can induce systemic inflammatory reaction, typical clinic signs of sepsis and MODS, demonstrated as excessive inflammatory response at early stage and immune inhibition at late stage, and corresponding 2 death peaks [23, 28]. The development stages of this animal model are easily to be identified with clear different immune features at different stages, and therefore is ideal model for study of pathogenic mechanism and prevention strategy of MODS. The present study showed similar developmental stages of MODS mice induced by zymosan with previous studies [23, 28], suggesting successful establishment of MODS model.

Spleen is the largest peripheral immune organ having multiple types of immune cells including monocytes, DC, T-cells and B-cells. DCs can modulate the proliferation, differentiation and apoptosis of T-cells through multiple receptor-ligand pathways [29], and induce immune response and immune tolerance [4, 30]. Consistently, in the present study, we found that the function of spleen DC was inhibited. These studies suggest important role of spleen DC in MODS induced by sepsis. The immune state of DC is modulated by the co-stimulator and co-inhibitor expressed on DC. During sepsis, the appearance of massive apoptosis of spleen cells induces massive release of

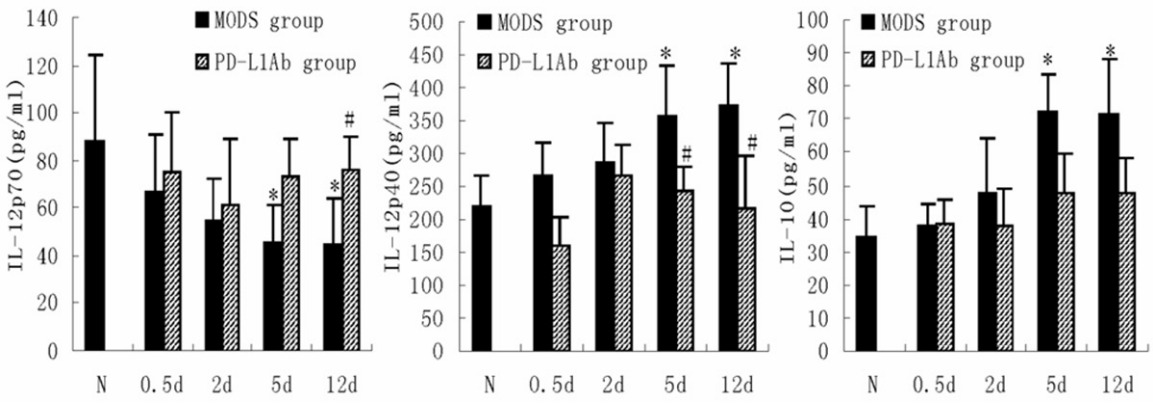

Figure 5. Changes of IL-12p70, IL-12p40 and IL-10 expression in supernatants of co-culture of spleen DC of zymosan induced mice with normal spleen T-cells. *P<0.05 vs control group; #P<0.05 vs the corresponding of zymosan alone group (MODS).

HMGB1, which is the important reason for immune inhibition at late stage of sepsis, MODS onset and death [31]. Splenectomy or apoptosis inhibitor can reduce the release of HMGB1 and decrease the mortality [32]. Consistently, in the present study, we found downregualtion of IL-12p70, IL-2 and T-cell function, and upregulation of IL-10 and IL-12p40 at late stage of MODS mice induced by zymosan, accompanied with the second death peak. Under the condition of insufficient positive signals, the negative signal provided by PD-L1/PD-1 pathway took dominant advantage, controlled the stimulation intensity of T-cells through limiting the release of IL-2 and IFN-γ, and inhibited activities of T-cell [14, 33]. Our study indicated that the positive immune molecule CD86 on spleen DCs at late stage of MODS were down-regulated while negative co-stimulator PD-L1/PD-1 was up-regulated. These results suggested a correlation between the immune reduction of DCs and the imbalance of co-stimulator and co-inhibitor expression. This is also consistent with our previous study that administration of immune enhancer Flt3 improved the mature of tolerant DC to recover immunity and reduce mortality [34].

Previous studies have proved that PD-L1 was the major inhibitory modulation receptor in PD-1 knockout mice suffering autoimmune diseases [19] and that blockade of the interaction between PD-L1 and PD-1 improved the immune condition [22, 35]. Consistently, the present result indicated that the functions of spleen DCs and T-cells were greatly improved during the stage of immune tolerance at 5 d and 12 d after zymosan injury by blockade of PD-L1/PD-1 pathway. These results suggested that PD-L1 antibody could attenuate the inhibition of DCs induced by zymosan. At the same time, we noticed PD-L1 antibody increased serum IL-2, proliferation of T-cells and secretion of IL-2 by T-cells. In vitro co-culture of DC with T-cells showed PD-L1 antibody attenuated the inhibitory effects of DC on the proliferation of T-cells and secretion of IL-2 during the late stage of MODS, suggesting PD-L1 antibody could recover the immunity of tolerant DCs and reduce the inhibitor of DC on T-cells through blockade of the interaction between PD-L1 and PD-1.

In addition, we found PD-L1 antibody increased the expression of MHC-II and co-stimulator CD86. This result was similar with our previous study [36] and other study about increased expression of MHC-II by PD-L1 blockade in fungal sepsis [37]. Previous study indicated that MHC-II can interact with TCR expressed on T-cells to modulate the immunity of T-cells [38]. These results suggested that PD-L1 antibody can upregulate the expression of positive immune molecules of MHC-II and CD86 on DCs to recover the immunity of tolerant DCs.

As corresponding receptor molecule of immunoglobin-like transcription 3, 4 (ILT3, LT4) [39] and inhibitory receptor expressed on DC and macrophages [24, 25, 40], PIR-B can downregulate the transcription and translation of many downstream gens including CD80, CD86, IL-12p70 and MHC-II [39]. Upregulation of PIR-B

expression has important effect on the formation of tolerant DCs and is specific marker judging formation of tolerant DCs [24, 41]. Consistently, we found that PD-L1 antibody had inhibitory effect on the expression of PIR-B on spleen DCs, resulting in upregulation of transcription and translation of CD86, IL-12p70 and MHC-II on DCs. Analysis of the relative ratio of PIR-B to MHC-II and CD86 showed that PD-L1 antibody decreased the ratio of PIR-B/MHC-II and PIR-B/CD86 at late stage after zymosan injury. These studies suggested that PD-L1 blockade could improve DC activities through downregulation of PIR-B, which may be one mechanism for PD-L1 antibody to recover the activation of tolerant DCs.

In summary, our study indicated that the imbalance between co-stimulator and co-inhibitor of spleen DCs at late stage of MODS mice induced by zymosan mediated the formation of tolerant DCs and decreased the activities of T-cells. Blockade of PD-L1/PD-1 pathway improved the immune disorder at late stage of MODS induced by sepsis. These results suggested that blockade of PD-L1/PD-1 pathway may be valuable strategy in preventing and treating immune tolerance at late stage of sepsis.

Acknowledgements

The study was supported by the Capital Medical Development Foundation, 2011-5002-02.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jiangyang Lu, Department of Pathology, The First Affiliated Hospital of General Hospital of PLA, Beijing 100048, China. Tel: +86-010-68689159; Fax: +86-010-68689159; E-mail: jiangyanglu123@yeah.net

References

[1] Deans KJ, Haley M, Natanson C, Eichacker PQ and Minneci PC. Novel therapies for sepsis: a review. J Trauma 2005; 58: 867-874.

[2] Riedemann NC, Guo RF and Ward PA. The enigma of sepsis. J Clin Invest 2003; 112: 460-467.

[3] Hotchkiss RS and Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348: 138-150.

[4] Rittirsch D, Flierl MA and Ward PA. Harmful molecular mechanisms in sepsis. Nat Rev Immunol 2008; 8: 776-787.

[5] Moraes TJ and Downey GP. Death of the septic monocyte: is more better? Crit Care 2006; 10: 146.

[6] Laudanski K and Wyczechowska D. Monocyte-related immunopathologies in trauma patients. Arch Immunol Ther Exp (Warsz) 2005; 53: 321-328.

[7] Carlet J, Cohen J, Calandra T, Opal SM and Masur H. Sepsis: time to reconsider the concept. Crit Care Med 2008; 36: 964-966.

[8] dib-Conquy M and Cavaillon JM. Compensatory anti-inflammatory response syndrome. Thromb Haemost 2009; 101: 36-47.

[9] Remick DG. Pathophysiology of sepsis. Am J Pathol 2007; 170: 1435-1444.

[10] Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD, Kreisel D, Krupnick AS, Srivastava A, Swanson PE, Green JM and Hotchkiss RS. Immunosuppression in patients who die of sepsis and multiple organ failure. JAMA 2011; 306: 2594-2605.

[11] Mebius RE and Kraal G. Structure and function of the spleen. Nat Rev Immunol 2005; 5: 606-616.

[12] Wu L and Dakic A. Development of dendritic cell system. Cell Mol Immunol 2004; 1: 112-118.

[13] Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B and Palucka K. Immunobiology of dendritic cells. Annu Rev Immunol 2000; 18: 767-811.

[14] Sato K, Yamashita N, Baba M and Matsuyama T. Modified myeloid dendritic cells act as regulatory dendritic cells to induce anergic and regulatory T cells. Blood 2003; 101: 3581-3589.

[15] Scumpia PO, McAuliffe PF, O'Malley KA, Ungaro R, Uchida T, Matsumoto T, Remick DG, Clare-Salzler MJ, Moldawer LL and Efron PA. CD11c+ dendritic cells are required for survival in murine polymicrobial sepsis. J Immunol 2005; 175: 3282-3286.

[16] Flohe SB, Agrawal H, Schmitz D, Gertz M, Flohe S and Schade FU. Dendritic cells during polymicrobial sepsis rapidly mature but fail to initiate a protective Th1-type immune response. J Leukoc Biol 2006; 79: 473-481.

[17] Dong H and Chen X. Immunoregulatory role of B7-H1 in chronicity of inflammatory responses. Cell Mol Immunol 2006; 3: 179-187.

[18] Poirier N, Blancho G and Vanhove B. A more selective costimulatory blockade of the CD28-B7 pathway. Transpl Int 2011; 24: 2-11.

[19] Okazaki T and Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007; 19: 813-824.

[20] Dong H, Zhu G, Tamada K, Flies DB, van Deursen JM and Chen L. B7-H1 determines accumulation and deletion of intrahepatic CD8 (+) T lymphocytes. Immunity 2004; 20: 327-336.

[21] Keir ME, Butte MJ, Freeman GJ and Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26: 677-704.
[22] Zhang Y, Zhou Y, Lou J, Li J, Bo L, Zhu K, Wan X, Deng X and Cai Z. PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction. Crit Care 2010; 14: R220.
[23] Jansen MJ, Hendriks T, Verhofstad AA, Lange W, Geeraedts LM Jr and Goris RJ. Gradual development of organ damage in the murine zymosan-induced multiple organ dysfunction syndrome. Shock 1997; 8: 261-267.
[24] Liu Z, Li W, Zhang M, Zhou H, Han H and Zou P. Paired immunoglobin-like receptors A and B are new targets for inducing dendritic cells tolerance in mice. J Huazhong Univ Sci Technolog Med Sci 2007; 27: 252-256.
[25] Mitsuhashi Y, Nakamura A, Endo S, Takeda K, Yabe-Wada T, Nukiwa T and Takai T. Regulation of plasmacytoid dendritic cell responses by PIR-B. Blood 2012; 120: 3256-3259.
[26] Selenko-Gebauer N, Majdic O, Szekeres A, Hofler G, Guthann E, Korthauer U, Zlabinger G, Steinberger P, Pickl WF, Stockinger H, Knapp W and Stockl J. B7-H1 (programmed death-1 ligand) on dendritic cells is involved in the induction and maintenance of T cell anergy. J Immunol 2003; 170: 3637-3644.
[27] Gillessen S, Carvajal D, Ling P, Podlaski FJ, Stremlo DL, Familletti PC, Gubler U, Presky DH, Stern AS and Gately MK. Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. Eur J Immunol 1995; 25: 200-206.
[28] Volman TJ, Hendriks T and Goris RJ. Zymosan-induced generalized inflammation: experimental studies into mechanisms leading to multiple organ dysfunction syndrome. Shock 2005; 23: 291-297.
[29] de HM, Oldenhove G, Urbain J, Thielemans K, Maliszewski C, Leo O and Moser M. Depending on their maturation state, splenic dendritic cells induce the differentiation of CD4 (+) T lymphocytes into memory and/or effector cells in vivo. Eur J Immunol 2004; 34: 1861-1869.
[30] Belz GT, Heath WR and Carbone FR. The role of dendritic cell subsets in selection between tolerance and immunity. Immunol Cell Biol 2002; 80: 463-468.
[31] Huston JM, Wang H, Ochani M, Ochani K, Rosas-Ballina M, Gallowitsch-Puerta M, Ashok M, Yang L, Tracey KJ and Yang H. Splenectomy protects against sepsis lethality and reduces serum HMGB1 levels. J Immunol 2008; 181: 3535-3539.
[32] Qin S, Wang H, Yuan R, Li H, Ochani M, Ochani K, Rosas-Ballina M, Czura CJ, Huston JM, Miller E, Lin X, Sherry B, Kumar A, Larosa G, Newman W, Tracey KJ and Yang H. Role of HMGB1 in apoptosis-mediated sepsis lethality. J Exp Med 2006; 203: 1637-1642.
[33] Riley JL. PD-1 signaling in primary T cells. Immunol Rev 2009; 229: 114-125.
[34] Tian G, Lu JY, Wang HW, Liu Q and Yang Y. Flt3 ligand in recovering the function of the splenic dendritic cells and the immune system in mice with multiple organ dysfunction syndrome. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2008; 20: 45-48.
[35] Karwacz K, Bricogne C, MacDonald D, Arce F, Bennett CL, Collins M and Escors D. PD-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on CD8+ T cells. EMBO Mol Med 2011; 3: 581-592.
[36] Li ZH, Lu JY, Wang HW, Yang Y and Tong X. The expression of IL-2 in mice of multiple organ dysfunctional syndromes and its role in imbalance of immunity. Medical Journal of Chinese People's Liberation Army 2003; 28: 887-889.
[37] Chang KC, Burnham CA, Compton SM, Rasche DP, Mazuski R, Smcdonough J, Unsinger J, Korman AJ, Green JM and Hotchkiss RS. Blockade ofthe negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. Crit Care 2013; 17: R85.
[38] de Jong EC, Smits HH and Kapsenberg ML. Dendritic cell-mediated T cell polarization. Springer Semin Immunopathol 2005; 26: 289-307.
[39] Chang CC, Ciubotariu R, Manavalan JS, Yuan J, Colovai AI, Piazza F, Lederman S, Colonna M, Cortesini R, la-Favera R and Suciu-Foca N. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat Immunol 2002; 3: 237-243.
[40] Munitz A, Cole ET, Beichler A, Groschwitz K, Ahrens R, Steinbrecher K, Willson T, Han X, Denson L, Rothenberg ME and Hogan SP. Paired immunoglobulin-like receptor B (PIR-B) negatively regulates macrophage activation in experimental colitis. Gastroenterology 2010; 139: 530-541.
[41] Liu ZR, Zhang M, Li WM, Zhou H and Zou P. Study of paired immunoglobin-like receptor B expression on dendritic cells and its relationship with immune tolerance in mouse. Zhonghua Xue Ye Xue Za Zhi 2007; 28: 689-693.