NF-κB activation and zinc finger protein A20 expression in mature dendritic cells derived from liver allografts undergoing acute rejection

Ming-Qing Xu, Wei Wang, Lan Xue, Lv-Nan Yan

Abstract

AIM: To investigate the role of NF-κB activation and zinc finger protein A20 expression in the regulation of maturation of dendritic cells (DCs) derived from liver allografts undergoing acute rejection.

METHODS: Sixty donor male SD rats and sixty recipient male LEW rats weighing 220-300 g were randomly divided into whole liver transplantation group and partial liver transplantation group. Allogeneic (SD rat to LEW rat) whole and partial liver transplantation were performed. DCs from liver grafts were isolated and propagated in the presence of GM-CSF in vitro. Morphological characteristics and phenotypical features of DCs propagated for 10 days were analyzed by electron microscopy and flow cytometry, respectively. NF-κB binding activity, IL-12p70 protein and zinc finger protein A20 expression in these DCs were measured by EMSA and Western blotting, respectively. Histological grading of rejection was determined.

RESULTS: Allogeneic whole liver grafts showed no signs of rejection on day 4 after the transplantation. In contrast, allogeneic partial liver grafts demonstrated moderate to severe rejection on day 4 after the transplantation. After propagation for 10 days in the presence of GM-CSF in vitro, DCs from allogeneic whole liver grafts exhibited features of immature DC with absence of CD40 surface expression, these DCs were found to exhibit detectable but very low level of NF-κB activity. IL-12 p70 protein and zinc finger protein A20 expression. Whereas, DCs from allogeneic partial liver graft 4 days after transplantation displayed features of mature DC, with high level of CD40 surface expression, and as a consequence, higher expression of IL-12p70 protein, higher activities of NF-κB and higher expression of zinc finger protein A20 compared with those of DCs from whole liver grafts (P<0.001).

CONCLUSION: These results suggest that A20 expression is up-regulated in response to NF-κB activation in mature DCs derived from allogeneic liver grafts undergoing acute rejection. Given the NF-κB inhibition function of this gene, it is suggested that their expression survives to limit NF-κB activation and maturation of DCs, and consequently inhibits the acute rejection and induces acceptance of liver graft.

INTRODUCTION

Dendritic cells (DCs) are specialized antigen-presenting cells (APCs) that play an essential role in the activation of lymphocytes. Among APCs, which also include macrophages and B cells, only DCs are believed to be capable of activating naive T cells. DC function is regulated by their state of maturation. Immature DCs resident in nonlymphoid tissues such as normal liver are deficient at antigen capture and processing. Mature DCs are capable of mimicking in vivo DCs and consequently inhibits the acute rejection and induces acceptance of liver graft. NF-κB and higher expression of zinc finger protein A20 compared with those of DCs from whole liver grafts (P<0.001).

CONCLUSION: These results suggest that A20 expression is up-regulated in response to NF-κB activation in mature DCs derived from allogeneic liver grafts undergoing acute rejection. Given the NF-κB inhibition function of this gene, it is suggested that their expression survives to limit NF-κB activation and maturation of DCs, and consequently inhibits the acute rejection and induces acceptance of liver graft.

Xu MQ, Wang W, Xue L, Yan LN. NF-κB activation and zinc finger protein A20 expression in mature dendritic cells derived from liver allografts undergoing acute rejection. World J Gastroenterol 2003; 9(6): 1296-1301. http://www.wjgnet.com/1007-9327/9/1296.asp
inducible gene product in endothelial cells (EC), and has been shown to be dependent upon NF-κB for its expression. A20 is expressed in a variety of cell types including fibroblasts, B, T, and β-cells in response to different stimuli including LPS, IL-1 and CD40 cross-linking. A20 is itself a NF-κB-dependent gene and is part of a negative regulatory loop critical for modulation of cell activation. A20 serves a broad cytoprotective function in EC by protecting EC from apoptosis and down-regulating inflammatory responses via NF-κB inhibition. A20 knockout mice are born cachectic and die within 3 weeks from severe and uncontrolled inflammation that further confirms the potent anti-inflammatory function of A20. A20 is also part of the physiologic NF-κB-dependent survival response of hepatocytes to injury, limited expression of A20 in hepatocytes drastically improves the fate of mice in the D-gal/LPS model of toxic FHF where A20 protects hepatocytes from apoptosis and promotes the liver regeneration. In addition, investigation has shown that A20 expression is up-regulated in human renal allografts in response to immune injury inferred by acute rejection, and the result suggests that A20 could limit graft injury.

Although A20 is a very effective inhibitor of NF-κB activation induced by LPS, IL-1 and CD40 cross-linking, little is known about the role of A20 in the regulation of maturation of DCs derived from allogeneic liver grafts accompanied by acute rejection.

The purpose of the present study was to investigate the binding activity of NF-κB DNA and A20 expression in mature DCs derived from allogeneic partial liver grafts undergoing acute rejection in rats. Attempts were made also to correlate A20 expression in DCs derived from liver grafts with the acceptance of allogeneic liver grafts.

**MATERIALS AND METHODS**

**Animals**

Sixty donor male SD rats and sixty recipient male LEW rats weighing 220-300 g were randomly divided into whole liver transplantation group and partial liver transplantation group. Allogeneic whole and 50 % partial liver transplantation were performed using a SD to LEW combination. The animals were purchased from Chinese Academy of Sciences and Sichuan University. They were maintained with a 12-hour light/dark cycle in a conventional animal facility with water and commercial chow provided ad libitum, with no fasting before the transplantation.

**Liver transplantation**

All operations were performed under ether anesthesia in clean but not sterile conditions. All surgical procedures were performed from 8 a.m. to 5 p.m. Donors and recipients of similar weight (±10 g) were chosen. Liver reduction was achieved by removing the left lateral lobe and the two caudate lobes. All operations were performed from 8 a.m to 5 p.m. Donors and recipients of similar weight (±10 g) were chosen. Liver reduction was achieved by removing the left lateral lobe and the two caudate lobes.

**Histology**

Part of liver tissues was sectioned and preserved in 10 % formalin, embedded in paraffin, cut with microtome, and stained with hematoxylin and eosin. The histological grading of rejection was determined according to the criteria described by Williams.

**Propagation and purification of liver graft-derived DC populations**

DCs from liver graft 0 hour and 4 days after the transplantation were propagated in GM-CSF from nonparenchymal cells (NPC) isolated from collagenase-digested liver graft tissue, as described by Lu et al. Nonadherent cells, released spontaneously from proliferating cell clusters, were collected after culture for 10 days, and purified by centrifugation 500 x g, for 10 minutes at room temperature on a 16 % w/v metrizamide gradient (DC purity 80-85 %).

**Morphological and phenotypical features of DCs**

Morphological characteristics of DCs derived from liver graft were observed by electron microscopy. Expression of cell surface molecules was quantitated by flow cytometry as described in our previous study. Aliquots of 2x10^5 DCs propagated for 10 days in vitro were incubated with the following primary mouse anti - rat mAbs against OX62, CD40 (Serotec, USA), or rat IgG as an isotype control for 60 minutes on ice (1 µg/ml diluted in PBS/1.0 % FCS). The cells were washed with PBS/1.0 % FCS and labeled with FITC-conjugated goat anti-mouse IgG, diluted 1/50 in PBS/1.0 % FCS for 30 minutes on ice. At the end of this incubation, cells were washed, propidium iodide/PBS were added, and the cells were subsequently analyzed in an FACS-4200 flow cytometer (Becton-Dikinson, USA).

**Isolation of nuclear proteins**

Nuclear proteins were isolated from DCs extract by placing the sample in 0.9 ml of ice-cold hypotonic buffer [10 mM·L^-1 HEPES (pH7.9), 10 mM·L^-1 KCl, 0.1 mM·L^-1 EDTA, 0.1 mM·L^-1 ethylene glycol tetraacetic acid, 1 mM·L^-1 DTT; Protease inhibitors (aprotinin, pepstatin, and leupeptin, 10 mg·L^-1 each)]. The homogenates were incubated on ice for 20 minutes, vortexed for 20 seconds after adding 50 µl of 10 % Nonide-P40, and then centrifuged for 1 minutes at 4 °C in an Eppendorf centrifuge. Supernatants were decanted, the nuclear pellets after a single wash with hypotonic buffer without Nonide-P40 were suspended in an ice-cold hypotonic buffer [20 mM·L^-1 HEPES (pH7.9), 0.4 M·L^-1 NaCl, 1 mM·L^-1 EDTA, 1 mM·L^-1 DTT; Protease inhibitors], incubated on ice for 30 minutes at 4 °C, mixed frequently, and centrifuged for 15 minutes at 4 °C. The supernatants were collected as nuclear extracts and stored at -70 °C. Concentrations of total proteins in the samples were determined according to the method of Bradford.

**Electrophoretic mobility shift assay (EMSA) for NF-κB activation of DCs**

NF-κB binding activity was performed in a 10-µl binding reaction mixture containing 1×binding buffer [50 mg·L^-1 of double-stranded poly (dl-dC), 10 mM·L^-1 Tris-HCl (pH7.5), 50 mM·L^-1 NaCl, 0.5 mM·L^-1 EDTA, 0.1 mM·L^-1 MgCl2, and 100 mM·L^-1 glycerol], 5 µg of nuclear protein, and 35 fmol of double-stranded NF-κB consensus oligonucleotide (5’-AGT TGA GGG GAC TTT CCC AGG-3’) that was endly labeled with γ-32P (111TBq mM^-1 at 370 GBq/µl) using T4 polynucleotide kinase. The binding reaction mixture was incubated at room temperature for 20 minutes and analyzed by electrophoresis on 7 % nondenaturing polyacrylamide gels. After electrophoresis, the gels were dried by Gel-Drier (Biol-Rad Laboratories, Hercules, CA) and exposed to Kodak X-ray films at -70 °C.

**Western blotting for IL-12 p70 and zinc finger protein A20 expression in DCs**

DCs cultured for 10 days in vitro were starved in serum-free medium for 4 hours at 37 °C. These cells were washed twice in cold PBS, resuspended in 100 µl lysis buffer (1 % Nonidet P40, 20 mM Tris-HCl, pH8.0, 137 mM NaCl, 10 % glycerol), and 35 fmol of double-stranded NF-κB consensus oligonucleotide (5’-AGT TGA GGG GAC TTT CCC AGG-3’) that was endly labeled with γ-32P (111TBq mM^-1 at 370 GBq/µl) using T4 polynucleotide kinase. The binding reaction mixture was incubated at room temperature for 20 minutes and analyzed by electrophoresis on 7 % nondenaturing polyacrylamide gels. After electrophoresis, the gels were dried by Gel-Drier (Biol-Rad Laboratories, Hercules, CA) and exposed to Kodak X-ray films at -70 °C.
2 mM EDTA, 10 μg·mL⁻¹ leupeptin, 10 μg·mL⁻¹ aprotinin, 1 mM PMSF, and 1 mM sodium orthovanadate), and total cell lysates were obtained. The homogenates were centrifuged at 10 000g for 10 minutes at 4 °C. Cell lysates (20 μg) were electrophoresed on SDS-PAGE gels, and transferred to PVDC membranes for Western blot analysis. Briefly, PVDC membranes were incubated in a blocking buffer for 1 hour at room temperature, then incubated for 2 hours with Abs raised against IL-12 p70 and A20 (Santa Cruz, CA). The membranes were washed and incubated for 1 hour with HRP-labeled IgG. Immunoreactive bands were visualized by ECL detection reagent. The binding bands were quantified by scanning densitometer of a Bio-Image Analysis System. The results were expressed as relative optical density.

**Statistics analysis**

Statistical analysis of data was performed using the Student’s t-test; P<0.05 was considered statistically significant.

**RESULTS**

**Histological rejection**

Histological rejection features of allografted livers were compared between the whole and partial groups on day 4 after the transplantation, allogeneic whole liver grafts demonstrated no rejection. In contrast, partial liver grafts demonstrated moderate to severe rejection, including inflammatory cellular infiltration in the portal tract, endotheliitis, bile duct damage and hepatocytes necrosis.

**Phenotypic characteristics of liver graft-derived DCs propagated in vitro**

As shown in our previous study,[42] after cultured for 10 days in the presence of GM-CSF, DCs both from whole and partial liver grafts displayed typical morphological features of DC, including anomalous shape, bigger body, and numerous longer dendrites. Flow cytometry showed 80-85 % of these DCs strongly expressed rat DC - specific OX62 antigen molecule, which suggested that high purity DCs were obtained. Flow cytometric analysis showed that DCs from whole liver grafts and from partial liver grafts 0 hour after the transplantation were negative for the costimulatory molecule CD40 expression, which was an immature phenotype (CD40⁻), whereas DCs from partial liver graft 4 days after the transplantation showed high level of CD40 expression, which was a mature phenotype (CD40⁺). These results suggested maturation of DCs resident in allogeneic partial liver graft undergoing acute rejection.

**IL-12 p70 protein expression in DCs derived from allogeneic partial liver grafts**

Our previous study showed that IL-12 p35 and IL-12 p40 subunit expressions were significantly up-regulated in mature DCs derived from allogeneic partial liver grafts undergoing acute rejection.[42] In the present study, we evaluated IL-12 p70 protein expression in DCs from allogeneic liver grafts. As shown in Figure 1, DCs derived from both whole and partial liver grafts 0 hour after the transplantation expressed detectable but very low level of IL-12 p70, and expression level of IL-12 p70 in DCs from whole liver graft 4 days after transplantation was not elevated compared with those of DCs from whole liver graft 0 hour after the transplantation (P>0.05). However, expression of IL-12 p70 in DCs from partial liver graft 4 days after transplantation was markedly increased, and their expression levels were significantly higher than those of DCs both from partial liver graft 0 hour and whole liver graft 4 days after transplantation (P<0.001). In order to investigate the intracellular mechanisms specifically

**Figure 1** Expression of IL-12 p70 in liver graft-derived DCs by Western blotting. Lanes 1, 2: Expression of IL-12 p70 in DCs from partial liver graft and whole liver graft 0 hour after transplantation. Lanes 3, 4: Expression of IL-12 p70 in DCs from partial liver graft and whole liver graft 4 days after transplantation. *P<0.001 vs 4 d WLT group; †P<0.001 vs 0 hour PLT group.

**Electrophoretic mobility shift assay (EMSA) for NF-κB activation of DCs**

As shown in Figure 2, EMSA analysis showed detectable but very low level of NF-κB activity of DCs derived from both whole and partial liver grafts 0 hour after transplantation, and NF-κB activity of DCs from whole liver graft 4 days after transplantation was not increased compared with those of DCs from liver graft 0 hour after transplantation (P>0.05). However, NF-κB activity of DCs from partial liver graft 4 days after transplantation was significantly elevated compared with those of DCs from partial liver graft 0 hour and whole liver graft 4 days after transplantation (P<0.001).

**Figure 2** NF-κB activation of DCs derived from allogeneic liver grafts. Lanes 1, 2: NF-κB activation of DCs from partial liver graft and whole liver graft 0 hour after transplantation. Lanes 3, 4: NF-κB activation of DCs from partial liver graft and whole liver graft 4 days after transplantation. *P<0.001 vs 4 d WLT group; †P<0.001 vs 0 hour PLT group.

**A20 protein expression in DCs derived from partial liver allografts**

In order to investigate the intracellular mechanisms specifically
responsible for regulation of DC activation and maturation, we evaluated NF-κB inhibitor zinc finger protein A20 expression in DCs derived from allogeneic liver grafts. As shown in Figure 3, DCs from both whole and partial liver grafts 0 hour after the transplantation expressed detectable but very low level of A20, and expression level of A20 in DCs derived from whole liver graft 4 days after transplantation was not increased compared with those of DCs from whole liver graft 0 hour after transplantation \((P > 0.05)\). However, expression of A20 in DCs from partial liver graft 4 days after transplantation was markedly up-regulated, and its expression level was significantly higher than those of DCs both from partial liver graft 0 hour and whole liver graft 4 days after transplantation \((P < 0.001)\).

**Figure 3** Expression of A20 protein in DCs derived from allogeneic liver grafts. Lanes 1, 2: Expression of A20 protein in DCs from partial liver graft and whole liver graft 0 hour after transplantation. Lanes 3, 4: Expression of A20 protein in DCs from partial liver graft and whole liver graft 4 days after transplantation. \(P < 0.001\) vs 4 d WLT group; \(^{a,b}P < 0.001\) vs 0 hour PLT group.

**DISCUSSION**

Despite the emergence of DCs as key cellular players in the immune system, the signal transduction events that regulate DC maturation and function have been poorly understood. This study was conducted to explore whether NF-κB activation and its inducible expression gene of A20 could be detected in mature DCs derived from liver allograft undergoing acute rejection. Our aim was to determine whether A20 gene is involved in NF-κB inhibition of these mature DCs. In the present study, it has been shown that engagement of CD40 on DCs derived from allogeneic partial liver grafts undergoing acute rejection leads to a powerful NF-κB activation of these mature DCs, and as a consequence, leads to high level expression of the protective gene A20 in these DCs. Expression of this gene in mature DCs derived from liver graft undergoing acute rejection is consistent with their known potential to be induced in response to NF-κB activation.

It has been shown that DCs derived from allogeneic partial liver grafts undergoing acute rejection displayed mature phenotypic high level CD40 expression and NF-κB activation. Although resting DCs residing in normal liver tissues display only low levels of CD40, B7, and MHC class II molecule expression\([11, 12, 24]\). The ischemia/reperfusion injury which is consecutive to the transplantation procedure will rapidly activate them. In addition, partial heptectomy has been reported to induce the expression of MHC II on Kupffer cell.

Interstitial dendritic cells and sinusoidal endothelium in rats, together with the up-regulated TNF-α production after partial heptectomy would induce the expression of B7 and CD40 molecules on DCs\([16]\). These factors could stimulate maturation of DCs derived from partial liver grafts. These mature DCs may contribute to the allogeneic liver graft rejection induction. Previously other studies showed that mature DC could provide signals able to trigger T cell proliferation after TCR engagement\([43, 44]\). These accessory, or “costimulatory” signals, are consecutive to interactions between costimulatory molecules present on activated DC such as B7, CD40, and OX40-ligand and their respective counter-receptors, CD28, CD40-ligand, and OX40, on T cell membranes. Several intracellular signals follow the engagement of costimulatory molecules. Interactions of CD40L, CD28, and OX40 with their ligands on DCs activate the transcription factor NF-κB in both T cells and DCs\([29, 30]\). In turn, NF-κB initiates the transcription of numerous genes involved in immune activation, such as chemokines and cytokines\([20, 30]\), and also of costimulatory molecules themselves. For instance, CD40 ligation on the APC will up-regulate its expression of B7 molecules. This initiates positive feedback loops ultimately contributing to T cell expansion\([45]\). Among the costimulatory molecules, CD40 and B7 seem to play a crucial role in alloreactive responses. Indeed, blockade of both CD40 and B7 molecules at the time of transplantation prevents allograft rejection and induces alloreactive T cell anergy\([46, 47]\). In the present study, DCs derived from allogeneic partial liver grafts undergoing acute rejection demonstrated high level expression of CD40, which could interact with the CD40-ligand on T cells, leading to a powerful NF-κB activation and high level of IL-12p70 expression in these mature DCs. Given an essential role for NF-κB transcription in LPS- and CD40L - induced expression of IL-12 (IL-12 p35, p40 and p70) in DCs\([20]\). IL-12 is a key inducer of liver graft rejection\([49]\), together with high level of IL-12 p35 and IL-12 p40 protein expression in the mature DCs derived from allogeneic liver grafts undergoing acute rejection\([42]\). Our results suggest that NF-κB may play a key role in the maturation of DCs derived from allogeneic liver grafts undergoing acute rejection.

To provide further insights into potential intracellular mechanisms responsible for maturation regulation of DCs derived from allogeneic liver grafts undergoing acute rejection, we measured the protective A20 gene expression in these DCs. Zinc finger protein A20 is a potent inhibitor of NF-κB\([51]\), and although other studies have shown A20 is mainly expressed in endothelial and infiltrating mononuclear cells of human renal allografts undergoing acute rejection\([51]\), little is known about whether it is also involved in the regulation of DC maturation and activation. Our results first demonstrate that high level A20 expression is detected in the mature DCs (which present significant NF-κB activation) derived from acute rejecting liver allografts, but few A20 is detected in the immature DCs (which present few NF-κB activation) derived from nonrejecting liver allografts. Given A20 is itself a NF-κB - dependent gene and is part of a negative regulatory loop critical for modulation of cell activation\([37, 38]\), together with NF-κB which may play a key role in the maturation of DCs derived from liver allografts undergoing acute rejection, it is suggested that A20 expression in these mature DCs derived from liver allografts undergoing acute rejection survives to inhibit NF-κB activation and to limit maturation of these DCs, and as a consequence to limit the graft rejection.

In summary, we demonstrate for the first time an association between NF-κB activation and expression of the protective gene A20 and maturation of DCs derived from liver allografts undergoing acute rejection. NF-κB binding activity and A20 expression in these mature DCs are strongly up-regulated in
response to acute rejection. NF-xB may play a key role in the maturation of DCs derived from allogeneic liver grafts undergoing acute rejection, and A20 expression in these mature DCs derived from liver allografts undergoing acute rejection survives to inhibit NF-xB activation and to limit maturation of these DCs.

REFERENCES

1. Zhang JK, Chen HB, Sun JL, Zhou YQ. Effect of dendritic cells on LPACK cells induced at different times in killing hepatoma cells. Shijie Hua ren Xiu a zhi Z ahi 1999; 7: 673-675
2. Li MS, Yuan AL, Zhang WD, Chen QX, Tian XH, Piao YT. Immune response induced by dendritic cells induce apoptosis and inhibit proliferation of tumor cells. Shijie Hua ren Xiu a zhi Z ahi 2000; 8: 56-58
3. Luo ZB, Luo YH, Lu R, Jin HY, Zhang BP, Xu CP. Immunohistochemical study on dendritic cells in gastric mucosa of patients with gastric cancer and precancerous lesions. Shijie Hua ren Xiu a zhi Z ahi 2000; 8: 400-402
4. Li MS, Yuan AL, Zhang WD, Liu SD, Lu AM, Zhou DY. Dendritic cells in vitro induce efficient and special anti-tumor immune response. Shijie Hua ren Xiu a zhi Z ahi 1999; 7: 161-163
5. Wang FS, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. World J Gastroenterol 2001; 7: 537-541
6. Zhang JK, Li J, Chen HB, Sun JL, Qu YJ, Lu J. Antitumor activities of human dendritic cells derived from peripheral and cord blood. World J Gastroenterol 2002; 8: 87-90
7. Tang ZH, Qiu WH, Wu GS, Yang XP, Zou SQ, Qiu FZ. The immunotherapeutic effect of human dendritic cells vaccine modified with interleukin-18 gene and tumor cell lysate on mice with pancreatic carcinoma. World J Gastroenterol 2002; 8: 908-912
8. Zhang J, Zhang JK, Zhou SH, Chen HB. Effect of a cancer vaccine prepared by fusions of hepatocarcinoma cells with dendritic cells. World J Gastroenterol 2001; 7: 690-694
9. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. Annu Rev Immunol 2000; 18: 767-811
10. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392: 245-252
11. Kharra A, Morelli AE, Zhang GP, Takayama T, Lu LN, Thomson AW. Effects of liver-derived dendritic cell progenitors on Th1- and Th2-like cytokine responses in vitro and in vivo. J Immunol 2000; 164: 1346-1354
12. Morelli A, O’Connel PJ, Khanna A, Logar AJ, Lu LN, Thomson AW. Preferential induction of Th1 responses by functionally mature hepatic (CD8α- and CD8α+) dendritic cells: association with conversion from liver transplant tolerance to acute rejection. Transplantation 2000; 69: 2647-2657
13. Hertz C, Kierschmer SM, Godowski PJ, Bouis DA, Norgard MV, Roth MD, Modlin RL. Microbial lipopolysaccharide stimulate dendritic cell maturation through TLR-2 receptor. J Immunol 2001; 166: 2444-2450
14. Thoma-Uzynski S, Kierschmer SM, Ochoa MT, Bouis DA, Norgard MV, Miyake K, Godowski PJ, Roth MD, Modlin RL. Activation of toll-like receptor 2 on human dendritic cells triggers induction of IL-12, but not IL-10. J Immunol 2000; 165: 3804-3810
15. Kashi T, Takeuchi O, Kawai T, Hoshino K, Akira S. Endotoxin-induced maturation of MyD-88-deficient dendritic cells. J Immunol 2001; 166: 5688-5694
16. Kitamura H, Iwakabe K, Yahata T, Nishimura S, Ohta A, Ohni Y, Sato M, Takeda K, Okumura K, Kanaer L, Kawano T, Taniguchi M, Nishimura T. The natural killer T (NKT) cell ligand alpha-galactosylceramide mimics its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells. J Immunol 2001; 166: 1121-1128
17. Nagayama H, Sato K, Kashkawa H, Enomoto M, Morimoto C, Tadokoro K, Juji T, Asano S, Takahashi TA. IL-12 responsive and expression of IL-12 receptor in human peripheral blood monocyte-derived dendritic cells. J Immunol 2000; 165: 59-66
18. Visintin A, Mazzone A, Spitzer JH, Willi-DH, Dower SK, Sengal DM. Regulation of Toll-like receptor in human monocytes and dendritic cells. J Immunol 2001; 166: 249-255
19. Corinti S, Albanesi C, la Sala A, Pastore S, Girolomoni G. Regulatory activity of autocrine IL-10 on dendritic cell functions. J Immunol 2001; 166: 4312-4318
20. Ouaz F, Arron J, Zheng Y, Choi Y, Beg AA. Dendritic cell development and survival require distinct NF-xB subunits. Immunity 2002; 16: 257-270
21. Josser R, Li HL, Ingulli E, Sarma S, Wong BR, Vologodskaya I, Steinman RM, Choi Y, TRAN C. A tumor necrosis factor family member, enhances the longevity and adjuvant properties of dendritic cells in vivo. J Exp Med 2000; 191: 495-502
22. Miga AJ, Masters SR, Durell BG, Gonzalez M, Jenkins MK, Maliszewski C, Kikutani H, Wade WF, Nodile Rj. Dendritic cell longevity and T cell persistence is controlled by CD154-CD40 interactions. Eur J Immunol 2001; 31: 959-965
23. Cella M, Scheidegger D, Palmer-Lehmkan K, Lane P, Lanzavecchia A, Alber G. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. J Exp Med 1996; 184: 747-752
24. Lu L, Woo J, Raa AS, Li Y, Watkins SC, Qian S, Starzl TE, Demetris AJ, Thompson AW. Propagation of dendritic cell progenitors from normal mouse liver using granulocyte/macrophage colony-stimulating factor and their maturation-dependent ability to promote allograft acceptance. World J Gastroenterol 2003; 9: 5397-5400
25. Khanna A, Steptoe R, Antonyamy MA, Li W, Thomson AW. Donor bone marrow potentiates the effect of tacrolimus on nonvascularized heart allograft survival: association with microchimerism and growth of donor dendritic cell progenitors from recipient bone marrow. Transplantation 1998; 65: 479-485
26. Li W, Lu L, Wang Z, Wang L, Fung J, Thomson AW, Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts by inducing IFN-γ and their maturation-dependent ability to promote graft acceptance. J Immunol 2001; 166: 5619-5628
27. Steptoe R, Fu F, Li W, Drakes ML, Lu L, Demetris AJ, Qian S, McKenna H, Thomson AW. Augmentation of dendritic cells in murine organ donors by Flt3 ligand alters the balance between transplant tolerance and immunity. J Immunol 1997; 159: 5483-5491
28. Descimo G, Martino M, Sutherland CL, Gold MR, RicardiCastagnoli P. Dendritic cell survival and maturation are regulated by different signaling pathways. J Exp Med 1998; 188: 2175-2180
29. Verhasselt V, Vanden Bergh W, Vanderheyden N, Willems F, Haegeman G, Goldman M. N-acetyl-L-cysteine inhibits primary human T cell responses to dendritic cells at the dendritic cell level: association with NF-xB inhibition. J Immunol 1999; 162: 2569-2574
30. Mann J, Oakley F, Johnson PW, Mann DA. CD40 induces interleukin-6 gene transcription in dendritic cells. Biol Chem 2002; 277: 17125-17130
31. Zetoune FS, Murphy AR, Shao Z, Hlaiing T, Zeidler MG, Li Y, Vincenz C. A20 inhibits NF-kappa B activation downstream of multiple TLR signaling pathways and interacts with the I kappa B kinases. Cytokine 2001; 15: 282-298
32. Klinenberg VM, Van-Huffel S, Heyninck K, Beyaert R. Functional redundancy of the zinc fingers of A20 in NF-kappa B activation and protein-protein interactions. FEBS Lett 2001; 495: 121-127
33. Hoffer-Leibnitz S, Schmid JA, Stehlik C, Binder BR, Lipp J, de Martin R. Aactivation of NF-kappa B by XIAP, the X chromosome-linked inhibitor of apoptosis, in endothelial cells involves TAK1. J Biol Chem 2000; 275: 22064-22068
34. Beyaert R, Heyninck K, Van-Huffel S, A20 and A20-binding proteins as cellular inhibitors of nuclear factor-kappa B-dependent gene expression and apoptosis. Biochem Pharmacol 2000; 60: 1134-1151
35. Sarma V, Lin Z, Clark L, Rust BM, Tezwar M, Noelle RJ, Dixit VM. Activation of the B-cell surface receptor CD40 induces A20, a novel zinc finger protein that inhibits apoptosis. J Biol Chem 1995; 270: 12343-12346
36. Kupfer JG, Arcaroli J, Yum HK, Nadler SG, Yang KY, Abraham E. Role of NF-kappa B in endotoxemia-induced alterations of lung
neutrophil apoptosis. J Immunol 2001; 167: 7044-7051.

Heyninck K, De Valck D, Vanden Berghe W, Van Criekinge W, Contreras R, Fiers W, Haegeman G, Beyaert R. The zinc finger protein A20 inhibits NF-κB-dependent gene expression by interfering with an RIP- or TRAF2-mediated transactivation signal and directly binds to a novel NF-κB-inhibiting protein ABIN. J Cell Biol 1999; 145: 1471-1482.

Arvelo MB, Cooper JT, Longo C, Daniel S, Grey ST, Mahiou J, Czismadia E, Abu-Jawdeh G, Ferran C. A20 protects mice from D-galactosamine/ LPS acute toxic lethal hepatitis. Hepatology 2002; 35: 535-543.

Ferran C, Stroka DM, Badrichani AZ, Cooper JT, Wrighton CJ, Soares M, Grey ST, Bach FH. A20 inhibits NF-κB activation in endothelial cells without sensitizing to tumor necrosis factor-mediated apoptosis. Blood 1999; 91: 2249-2258.

Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. Science 2000; 289: 2350-2354.

Avihingsanon Y, Ma N, Czismadia E, Wang C, Pavlakis M, Giraldo M, Strom TB, Soares MP, Ferran C. Expression of protective genes in human renal allografts: a regulatory response to injury associated with graft rejection. Transplantation 2002; 73:1079-1085.

Xu MQ, Yao ZX. Functional changes of dendritic cells derived from allogeneic partial liver graft undergoing acute rejection in rats. World J Gastroenterol 2003; 9: 141-147.

Leclerc R, Ng WF, Steinman RM. Dendritic cells in transplantation: friend or foe? Immunity 2001; 14: 357-368.

Matzinger P. Graft tolerance: a duel of two signals. Nat Med 1999; 5: 616-617.

Stout RD, Sutlles J. The many roles of CD40 in cell-mediated inflammatory responses. Immunol Today 1996; 17: 487-492.

Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, Cho HR, Aruffo A, Hollenbaugh O, Linsley PS, Winn KJ, Pearson TC. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. Nature 1996; 381: 434-438.

Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Grag GS, Hong X, Thomas D, Fechner JH Jr, Knechtel SJ. CTLA-4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci USA 1997; 94: 8789-8794.

Edited by Xu XQ