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• BASIC RESEARCH •

Prolongation of liver allograft survival by dendritic cells modified with NF-κB decoy oligodeoxynucleotides

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Abstract

AIM: To induce the tolerance of rat liver allograft by dendritic cells (DCs) modified with NF-κB decoy oligodeoxynucleotides (ODNs).

METHODS: Bone marrow (BM)-derived DCs from SD rats were propagated in the presence of GM-CSF or GM-CSF+IL-4 to obtain immature DCs or mature DCs. GM-CSF+IL-4-propagated DCs were treated with double-strand NF-κB decoy ODNs containing two NF-κB binding sites or scrambled ODNs to ascertain whether NF-κB decoy ODNs might prevent DC maturation. GM-CSF-propagated DCs, GM-CSF+NF-κB decoy ODNs or scrambled ODNs-propagated DCs were treated with LPS for 18 h to determine whether NF-κB decoy ODNs could prevent LPS-induced IL-12 production in DCs. NF-κB binding activities, costimulatory molecule (CD40, CD80, CD86) surface expression, IL-12 protein expression and allostimulatory capacity of DCs were measured with electrophoretic mobility shift assay (EMSA), flow cytometry, Western blotting, and mixed lymphocyte reaction (MLR), respectively. GM-CSF-propagated DCs, GM-CSF+IL-4 -propagated DCs, and GM-CSF+NF-κB decoy ODNs or scrambled ODNs-propagated DCs were injected intravenously into recipient LEW rats 7 d prior to liver transplantation and immediately after liver transplantation. Histological grading of liver graft rejection was determined 7 d after liver transplantation. Expression of IL-2, IL-4 and IFN-γ mRNA in liver graft and in recipient spleen was analyzed by semiquantitative RT-PCR. Apoptosis of liver allograft-infiltrating cells was measured with TUNEL staining.

RESULTS: GM-CSF-propagated DCs, GM-CSF+NF-κB decoy ODNs-propagated DCs and GM-CSF+ scrambled ODNs-propagated DCs exhibited features of immature DCs, with similar low level of costimulatory molecule(CD40, CD80, CD86) surface expression, absence of NF-κB activation, and few allocostimulatory activities. GM-CSF+IL-4-propagated DCs displayed features of mature DCs, with high levels of costimulatory molecule (CD40, CD80, CD86) surface expression, marked NF-κB activation, and significant allocostimulatory activity. NF-κB decoy ODNs completely abrogated IL-4-induced DC maturation and allocostimulatory activity as well as LPS-induced NF-κB activation and IL-12 protein expression in DCs. GM-CSF+NF-κB decoy ODNs-propagated DCs promoted apoptosis of liver allograft-infiltrating cells within portal areas, and significantly decreased the expression of IL-2 and IFN-γ mRNA but markedly elevated IL-4 mRNA expression both in liver allograft and in recipient spleen, and consequently suppressed liver allograft rejection, and promoted liver allograft survival.

CONCLUSION: NF-κB decoy ODNs-modified DCs can prolong liver allograft survival by promoting apoptosis of graft-infiltrating cells within portal areas as well as down-regulating IL-2 and IFN-γ mRNA and up-regulating IL-4 mRNA expression both in liver graft and in recipient spleen.

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INTRODUCTION

Dendritic cells (DC) play a critical role in the initiation and regulation of immune response and are instrumental in the induction and maintenance of tolerance[1-7]. The function of DCs is regulated by their state of maturation. Immature DCs resident in nonlymphoid tissues such as normal liver are deficient at antigen capture and progressing[8,9], whereas mature DCs, resident in secondary lymphoid tissues, are potent antigen-presenting cells (APC), which can induce naive T-cell activation and proliferation[9-13]. The ability of DCs to initiate immune responses is determined by their surface expression of major histocompatibility complex (MHC) gene products and costimulatory molecules (CD40, CD80, CD86), and the secretion of the immune regulator, interleukin (IL)-12[9-18]. Immature DCs that express surface MHC class II, but deficient in surface costimulatory molecules and few expressions of IL-12, can induce T-cell anergy[8,19,20], and inhibit immune reactivity[21,22].

Immature donor-derived DCs that are deficient in surface costimulatory molecules freshly isolated from commonly transplanted organs, can induce alloAg-specific T cell anergy *in vitro*[23]. These DCs prolong survival of fully allogeneic grafts in rodents, in same cases, indefinitely[24,25]. In addition, pharmacologic inhibition of DC maturation in nonhuman primates is associated with the induction of organ transplant tolerance[26]. Moreover, immature human DCs have been shown to induce T regulatory cells *in vitro*[27] and to promote Ag-specific T cell tolerance in healthy volunteers[28]. Thus, DCs offer potential both for therapy of allograft rejection and promotion of transplant tolerance.

The inherent ability of DCs to traffic exquisitely to T cell areas of secondary lymphoid tissues[8,29] and to regulate immune responses makes them attractive targets for manipulation with genes encoding immunosuppressive molecules, such as IL-4, IL-10, CTLA4Ig, Fas ligand (CD95L), or transforming growth factor (TGF)-β1, that suppress T cell response by various

mechanisms. A potential obstacle to the successful use of genetically engineered DCs for therapeutic immunosuppression is their maturation/activation *in vivo* following interactions with proinflammatory factors that may overcome the desired effect of transgene products. Recent studies showed that both DC maturation and immunostimulatory ability depended on NF-κB-dependent gene transcription[29-34], inhibition of NF-κB activation could suppress DC maturation/activation induced by IL-4 or LPS stimulation[29,34,35], and DCs treated with NF-κB decoy oligodeoxynucleotides(ODNs) containing specific NF-κB binding sites could induce tolerance of cardiac allograft[29,34].

Although genetically engineered DCs have been used in tolerance induction of cardiac allograft, there are few evidences that genetically engineered DCs can be used to induce tolerance of liver allograft. In the present study, whether NF-κB decoy ODNs-treated DCs could prolong liver allograft survival in rats was studied.

MATERIALS AND METHODS

NF-κB decoy ODNs

Double-stranded NF-κB decoy ODNs or scrambled ODNs (as a control for NF-κB decoy ODNs) were generated using equimolar amounts of single-stranded sense and antisense phosphorothioate-modified oligonucleotide containing two NF-κB binding sites (sense sequence 5'-AGGGACTTTCCGCTG-GGGACTTTCC-3', NF-κB binding sites bold lines and underlined)[34] and scrambled oligonucleotide (sense sequence 5'-TTGCCGTACCTGACTTAGCC-3')[36]. Sense and antisense strands of each oligonucleotide were mixed in the presence of 150 mmol/L PBS, heated to 100 °C, and allowed to cool to room temperature to obtain double-stranded DNA.

Propagation of bone marrow-derived DC populations

Bone marrow cells harvested from femurs of normal SD rats were cultured in 24-well plates (2×10^6 per well) in 2 mL of RPMI 1640 complete medium supplemented with antibiotics,10 mL/L fetal calf serum (FCS) and 4.0 ng/mL recombinant rat GM-CSF to obtain immature DCs. In addition to GM-CSF, 10 ng/mL recombinant rat IL-4 was added to cultures to obtained mature DCs. To select plates, 10 μmol/L NF-κB decoy or scrambled ODNs was added at the initiation of culture of DCs[34] to test the ability of NF-κB decoy ODNs to inhibit IL-4-induced DC maturation. Cytokine-enriched medium was refreshed every 2 d, after gentle swirling of the plates, half of the old medium was aspirated and an equivalent volume of fresh, cytokine-supplemented medium was added. Thus, nonadherent granulocytes were depleted without dislodging clusters of developing DC attached loosely to a monolayer of plastic-adherent macrophages. Nonadherent cells released spontaneously from the clusters were harvested after 7 d. In certain experiments, after propagation for 7 d, GM-CSF-propagated DCs, GM-CSF+ NF-κB decoy ODNs - propagated DCs and GM-CSF + scrambled ODNs - propagated DCs were cultured with 10 μg/mL LPS for 18 h to test the ability of NF-κB decoy ODNs to prevent LPS-induced IL-12 production in DCs.

Phenotypical features of DCs

Expression of cell surface molecules was quantitated by flow cytometry as described in our previous study. Aliquots of 2×10^5 DCs propagated for 7 d *in vitro* were incubated with the following primary mouse anti-rat mAbs against CD40, CD80, CD86, or rat IgG as an isotype control for 60 min on ice [1 μg/mL diluted in PBS/(10 mL/L FCS)]. Cells were washed with PBS/(10 mL/L FCS) and labeled with FITC-conjugated goat anti-mouse IgG, diluted 1/50 in PBS/(10 mL/L FCS) for 30 min on ice. At the end of this incubation, the cells were washed, propidium iodide/PBS were added, and the cells were subsequently analyzed by a FACS-4 200 flow cytometer.

DCs allostimulatory capacity

One-way mixed leukocyte reactions (MLR) were performed in 96-well, round-bottomed microculture plates. Graded doses of γ-irradiated (20Gy) allogeneic(SD) stimulator cells (DCs) were added to 2×10^5 nylon wool-eluted LEW rat splenic T cells (responders) and maintained in complete medium for 72 h in 50 mL/L CO_2 in air at 37 °C. [^{3}H]thymidine(1 μCi/well) was added for the last 18 h of culture. Cells were harvested onto glass fiber mats using an automatic system, and [^{3}H]thymidine incorporation was determined by a liquid scintillation counter. Results were expressed as mean±SD.

Isolation of nuclear proteins

Nuclear proteins were isolated from DC extract by placing the sample in 0.9 mL of ice-cold hypotonic buffer [10 mmol/L HEPES (pH7.9), 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L ethylene glycol tetraacetic acid, 1 mmol/L DTT, protease inhibitors (aprotinin, pepstatin, and leupeptin, 10 mg.mol/L each)]. Homogenates were incubated on ice for 20 min, vortexed for 20 s after adding 50 μL of 100 g/L Nonidet P-40, and then centrifuged for 1 min at 4 °C in an Eppendorf centrifuge. Supernatants were decanted, nuclear pellets after a single wash with hypotonic buffer without Nonidet-P40 were suspended in an ice-cold hypertonic buffer [20 mmol/L HEPES (pH7.9), 0.4 mol/LNaCl , 1 mmol/LEDTA, 1 mol/L DTT, protease inhibitors], incubated on ice for 30 min at 4 °C, mixed frequently, and centrifuged for 15 min at 4 °C. 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 of double-stranded poly (dI-dC), 10 mmol/L Tris-HCl (pH7.5), 50 mmol/LNaCl, 0.5 mmol/L EDTA, 0.5 mmol/LDTT, 1 mmol/L $MgCl_2$, and 100 mL/L glycerol], 5 μg of nuclear protein , and 35 fmol of double-stranded NF-κB consensus oligonucleotide (5'-AGT TGAGGGGACTTT CCCAGGC -3') that was endly labeled with γ-^{32}P (111TBq /mmol at 370 GBq^{-1}) using T4 polynucleotide kinase. The binding reaction mixture was incubated at room temperature for 20 min and analyzed by electrophoresis on 70 g/L nondenaturing polyacrylamide gels. After electrophoresis, the gels were dried by a gel-drier and exposed to Kodak X-ray films at -70 °C.

Western blotting for IL-12 protein expression in DCs stimulated with LPS

GM-CSF-propagated DCs, GM-CSF+ NF-κB decoy ODNs-propagated DCs and GM-CSF + scrambled ODNs-propagated DCs were cultured with 10 μg/mL LPS for 18 h. DCs were starved in serum-free medium for 4 h at 37 °C. These cells were washed twice with cold PBS, resuspended in 100 μL lysis buffer (1 mL/L Nonidet P-40, 20 mmol/L Tris-HCl, pH8.0, 137 mmol/L NaCl, 100 mL/L glycerol, 2 mmol/L EDTA, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 1 mmol/L PMSF, and 1 mmol/L sodium orthovanadate), and total cell lysates were obtained. Homogenates were centrifuged at 10 000 *g* for 10 min 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 h at room temperature, then incubated for 2 h with Abs against IL-12 p35 and IL-12 p40 and IL-12 p70. Membranes were washed and incubated for 1 h with HRP-labeled horse anti-goat or goat anti-rabbit IgG. Immunoreactive bands were visualized by ECL detection reagents. The binding bands were quantified by a

scanning the densitometer of a bio-image analysis system. The results were expressed as a relative optical density.

Liver transplantation

Sixty male LEW rats and sixty male SD rats weighing 250 -300 g were used in all experiments. Allogeneic liver transplantation model was established using a combination of SD rats with LEW rats. All operations were performed under ether anesthesia in sterile conditions. Orthotopic liver transplantation was performed according to the method described in our previous study. Normal saline (group A), 1×10^7 GM-CSF-propagated DCs (group B), 1×10^7 GM-CSF+IL-4-propagated DCs (group C), and 1×10^7 GM-CSF+ NF-κB decoy ODNs or scrambled ODNs-propagated DCs (group D or group E) were injected intravenously through the penile vein into recipient LEW rats 7 d prior to liver transplantation and immediately after liver transplantation, respectively. Liver graft tissues and recipient spleen samples ($n = 8$) were harvested 7 d after liver transplantation and immediately frozen in liquid nitrogen and kept at -80 °C until use. Part of the liver graft tissues was sectioned and preserved in 40 g/L formaldehyde.

Histology

Part of liver tissues was sectioned and preserved in 40 g/L formaldehyde, embeded in paraffin, and stained with hematoxylin and eosin. Histological grading of rejection was determined according to the criteria described by Williams.

Apoptosis of liver graft-infiltrating cells (GIC)

Apoptotic cells in tissue sections were detected with the *in situ* cell death detection kit. Liver graft tissue sections were dewaxed and rehydrated according to standard protocols. Tissue sections were incubated with proteinase K(20 μg/mL in 10 mmol/L Tris/HCl, pH 7.4-8.0) for 15 to 30 min at 21-37 °C. Endogenous peroxidase activity was quenched with blocking solution (3 mL/LH_2O_2 in methanol) for 30 min at room temperature before exposure to TUNEL reaction mixture at 37 °C for 60 min. After washed in stop wash buffer, peroxidase(POD) was added to react for 30 min at 37 °C. DAB-substrate was used for color development, and the sections were counterstained with Harrs' hematoxylin. TUNEL staining was mounted under glass coverslip and analysed under a light microscope.

Semiquantitative RT -PCR assay for expression of IL-2, IL-4 and IFN-γmRNA in liver graft and spleen

IL-2, IL-4 and IFN-γ mRNA expression was determined by semiquantitative RT-PCR amplification in contrast with housekeeping gene β-actin, respectively. Total RNA from 10 mg liver allograft and recipient spleen tissue was extracted using Tripure™ reagent. First-strand cDNA was transcribed from 1 μg RNA using AMV and an Oligo(dT_{15}) primer. PCR was performed in a 25 μL reaction system containing 10 μL cDNA, 2 μL 10 mmol/L dNTP, 2.5 μL 10×buffer, 2.5 μL 25 mmol/L $MgCl_2$, 2 μL specific primer, 5 μL water and 1 μL Taq (35 cycles: at 95 °C for 60 s, at 59 °C for 90 s, and at 72 °C for 10 s). Primers[37-39] used in PCR reactions were as follows: IL-2, 5' primer 5'-CAT GTA CAGCA TGCAGCTCGCATCC-3', 3' primer 5'-CCACCACAGTTGCTG GCTCATCATC-3', to give a 410-bp PCR product; IL-4, 5' primer 5'-TGATGGGTCTCAGCCCCCACCTTGC-3', 3' primer 5'-CTT TCAGTGTTGTGAGCGTGGACTC-3', to give a 378-bp PCR product; IFN-γ, 5' primer 5'-AAGACA ACCAGGCCATCAGCA-3', 3' primer 5'-AGCCACAGTGTGAGTTCAGTC-3', to give a 547-bp product; β-actin, 5' primer 5'-ATGCCATCC TGCGT CTGGACCTGGC-3', 3' primer 5'-AGCATTTGCGGTGCAC GATGGAGGG-3', to give a 607-bp product. PCR products of each sample were subjected to electrophoresis in a 15 g/L agarose gel containing 0.5 mg/L ethidium bromide. Densitometrical analysis using NIH image software was performed for semiquantification of PCR products, and mRNA expression was evaluated by the band-intensity ratio of IL-2, IL-4 and IFN-γ to β-actin, and presented as percent of β-actin (%).

Statistical analysis

Statistic analysis of data was performed using the *t*-test and rank sum test, $P<0.05$ was considered statistically significant.

RESULTS

NF-κB decoy ODNs inhibited IL-4 or LPS - induced NF-κB activation in DCs

To confirm whether NF-κB decoy ODNs might specifically bind to NF-κB, analysis of NF-κB activity was performed with nuclear extracts obtained from GM-CSF-propagated DCs, GM-CSF+IL-4-propagated DCs, GM-CSF+LPS-propagated DCs, GM-CSF+IL-4+ODNs-propagated DCs and GM-CSF+LPS +ODNs - propagated DCs by EMSA. As shown in Figure 1, EMSA analysis showed no NF-κB activation in GM-CSF - propagated DCs but significant NF-κB activation in IL-4 or LPS -stimulated DCs. NF-κB decoy ODNs completely inhibited IL-4 or LPS-induced NF-κB activation in DCs, whereas scrambled ODNs had little effect on inhibition of IL-4 or LPS - induced NF-κB activation in DCs.

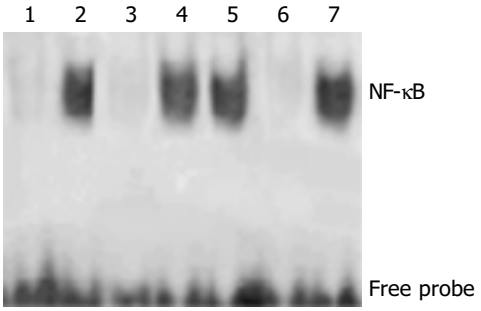

Figure 1 Inhibition of IL-4 or LPS -induced NF-κB activation in DCs by NF-κB decoy ODNs. Nuclear proteins of GM-CSF DCs, GM-CSF + IL-4 DCs, GM-CSF+IL-4 + NF-κB decoy ODNs DCs, GM-CSF+IL-4 + scrambled ODNs DCs, GM-CSF + LPS DCs, GM-CSF+LPS+ NF-κB decoy ODNs DCs and GM-CSF+LPS+ scrambled ODNs DCs were measured by EMSA (lanes 1-7).

NF-κB decoy ODNs inhibited IL-4 - induced costimulatory molecule surface expression in DCs

Functional maturation of DCs was associated with up-regulation of costimulatory molecules (CD40, CD80, and CD86). To test the ability to inhibit DC maturation, NF-κB decoy ODNs or scrambled ODNs were added at the initiation of culture of GM-CSF+IL-4 - stimulated SD rat BM-derived DCs. After culture for 7 d, surface expression of CD40, CD80, and CD86 was analyzed by flow cytometry. Figure 2 shows the effects of ODNs on phenotype of the cultured DCs in the presence of GM-CSF+IL-4. Flow cytometric analysis showed GM-CSF-propagated DCs exhibited immature phenotypical features with very low levels of CD40, CD80 and CD86 surface expression, GM-CSF+IL-4-propagated DCs displayed mature phenotypical features with high level of CD40, CD80, and CD86 surface expression. NF-κB decoy ODNs prevented IL-4-induced DCs maturation, and maintained DCs in the immature state, with low levels of surface costimulatory molecule expression. Whereas the scrambled ODNs could not prevent IL-4-induced DCs maturation, and maintained DCs in the mature state, with similar high levels of surface costimulatory molecule expression compared with GM-CSF+IL-4-propagated DCs (data not shown) .

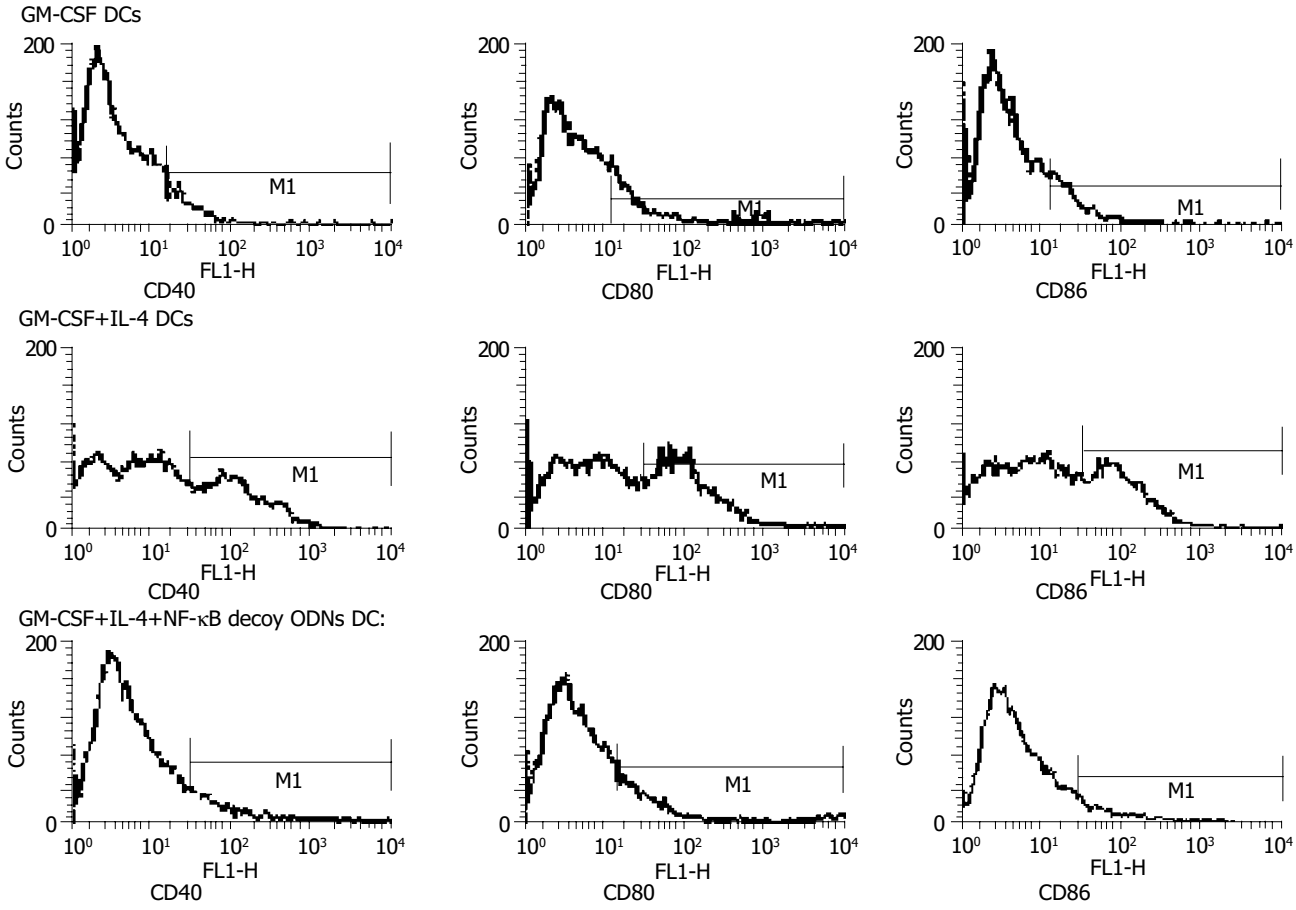

Figure 2 Suppression of IL-4-induced costimulatory molecule expression in DCs by NF-κB decoy ODNs.

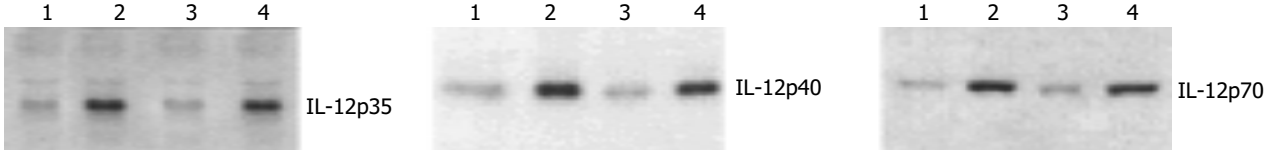

Figure 3 Suppression of IL-12 protein expression in LPS-stimulated DCs by NF-κB decoy ODNs. Protein extracts from GM-CSF DCs, GM-CSF+LPS DCs, GM-CSF+LPS +NF-κB decoy ODNs DCs and GM-CSF+LPS +scrambled ODNs DCs were measured by Western blot (lane 1-4).

NF-κB decoy ODNs abrogated LPS- induced IL-12 protein expression in DCs

Previous studies showed that IL-12 protein expression was significantly up-regulated in mature DCs[10,17,19,40]. To further confirm whether NF-κB decoy ODNs might prevent DC maturation, LPS-induced IL-12 protein (p35, p40 and p70) expression in DCs was measured. As shown in Figure 3, very low level of IL-12 protein was detected in GM-CSF DCs, and markedly high level of IL-12 protein was detected in LPS-stimulated DCs (P<0.001). NF-κB decoy ODNs completely abrogated the LPS-induced production of IL-12 protein in DCs, whereas scrambled ODNs had almost no effect on down-regulation of LPS - induced IL-12 protein expression in DCs.

DCs allostimulatory capacity was inhibited by NF-κB decoy ODNs

The effect of NF-κB decoy ODNs on DCs immunostimulatory activity was evaluated by *in vitro* MLR. Graded number of γ-irradiated DCs (SD) was cultivated for 72 h with a fixed number of allogeneic (LEW) splenic T cells in MLR. The results of a representative experiment are shown in Figure 4. In comparison with IL-4 stimulated mature DCs or IL-4 + scrambled ODNs-propagated DCs, which were potent inducers of DNA synthesis and consistent with their mature surface phenotype, IL-4+NF-κB decoy ODNs- propagated DCs induced only a minimal T cell proliferation. The poor stimulatory capacity of IL-4 +NF-κB decoy ODNs DCs remained unchanged after a longer incubation with allogeneic T cells (4- or 5-d MLR, data not shown). The results suggested that allostimulatory capacity of DCs was inhibited by NF-κB decoy ODNs.

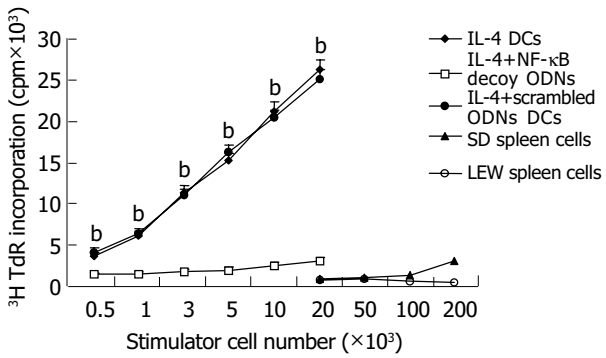

Figure 4 Suppression of allostimulatory function of IL-4-stimulated DCs by NF-κB decoy ODNs. ^bP<0.001 *vs* IL-4+NF-κB decoy ODNs -propagated DCs.

NF-κB decoy ODNs- treated DCs prolonged donor-specific liver allograft survival

To examine the effect of NF-κB decoy ODNs-treated DCs on liver allograft survival *in vivo*, 1×10^7 unmodified immature bone marrow-derived DCs from SD rats (GM-CSF DCs), GM-CSF+IL-4-propagated mature DCs, and GM-CSF+NF-κB decoy ODNs or scrambled ODNs-propagated immature DCs were injected intravenously through the penile vein into recipient LEW rats 7 d prior to liver transplantation and immediately after liver transplantation, respectively. Table 1 and Figure 5 show the effect of DCs on liver allograft rejection and recipient survival. GM-CSF+IL-4-propagated mature DCs accelerated the liver allograft rejection and shorted the survival time of recipient animals. Immature donor DCs (GM-CSF or GM-CSF+scrambled ODNs-propagated DCs) significantly suppressed liver allograft rejection and prolonged graft survival compared with untreated controls. In comparison with GM-CSF or GM-CSF +scrambled ODNs-propagated DCs, NF-κB decoy ODNs-treated DCs exerted a marked effect on liver allograft rejection and recipient survival, and significantly suppressed the liver allograft rejection and prolonged survival time of recipient animals.

Table 1 Rejection stages of liver allografts

Rejection stages	Group A (*n*)	Group B (*n*)	Group C (*n*)	Group D (*n*)	Group E (*n*)
0	0	1	0	6	2
1	2	6	0	2	6
2	6	1	2	0	0
3	0	0	6	0	0

Group B *vs* group A, $u = 2.475$, $P<0.05$; Group C *vs* group A, $u = 2.951$, $P<0.01$; Group D *vs* group A, $u = 3.298$, $P<0.01$; Group D *vs* group B, $u = 2.416$, $P<0.05$; Group E *vs* group A, $u = 0.901$, $P>0.05$.

NF-κB decoy ODNs-treated DCs promoted apoptosis of liver allograft-infiltrating cells in portals

Although the precise mechanisms remain unclear, spontaneous acceptance of liver grafts in mice has been associated with high levels of apoptosis in GIC population[41]. In contrast, FL liver gllografts that were rejected acutely showed reduced apoptotic activity in GIC within portal areas and enhanced apoptosis of hepatocytes[42]. These data suggested a critical immunoregulatory role of apoptosis in determining the outcome of hepatic allografts. To determine whether the prolongation of liver allografts survival induced by NF-κB decoy ODNs-treated DCs was associated with enhanced apoptosis of GIC, apoptotic activity in the graft was examined by TUNEL staining with immunohistochemistry analysis. *In situ* TUNEL staining of liver graft sections revealed that a certain level of apoptosis of GIC was induced by immature DCs (GM-CSF or GM-CSF+scrambled ODNs-propagated DCs), and mature DCs appeared to protect GIC from apoptosis. The greatest degree of apoptosis of GIC within portal areas of liver grafts was induced by NF-κB decoy ODNs-treated DCs (Figure 6). These data strongly suggested that augmentation of apoptosis of activated GIC within portal areas of liver grafts might be critical in promoting the tolerance of liver allografts.

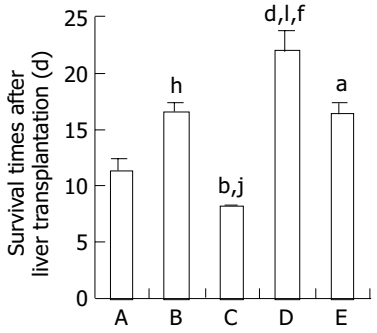

Figure 5 Prolongation of survival of liver allograft recipient by NF-κB decoy ODNs-treated DCs. $^bP<0.001$ *vs* group A, $^dP<0.001$ *vs* group A, $^fP<0.001$ *vs* group C, $^hP<0.001$ *vs* group A, $^jP<0.001$ *vs* group B, $^lP<0.001$ *vs* group B, $^aP>0.05$ *vs* group B.

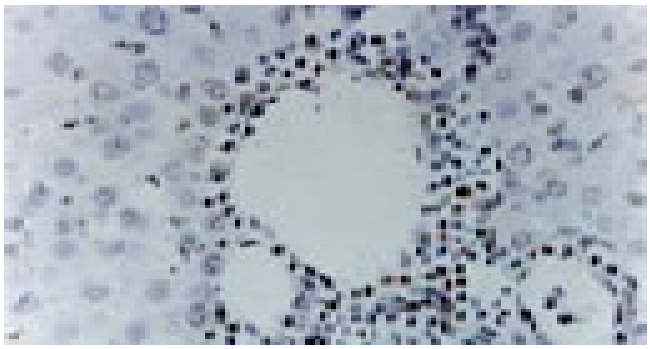

Figure 6 Augmentation of apoptosis of liver allograft-infiltrating cells in portals. by NF-κB decoy ODNs-treated DCs (×400).

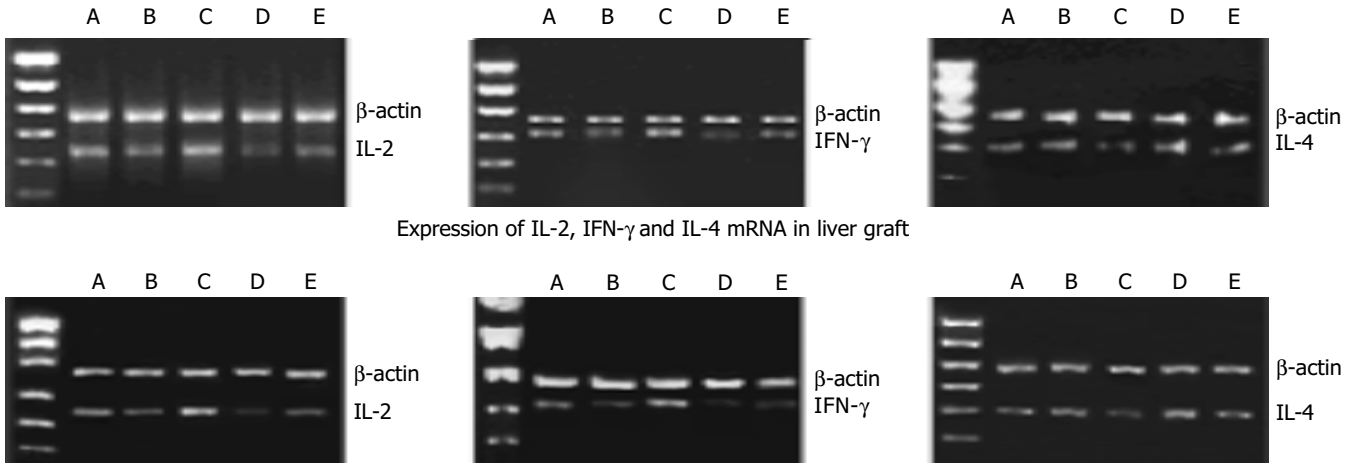

Figure 7 Suppression of IL-2 and IFN-γ mRNA expression and up-regulation of IL-4 mRNA expression both in liver graft and in recipient spleen by NF-κB decoy ODNs-modified DCs.

NF-κB decoy ODNs-treated DCs suppressed IL-2 and IFN-γ mRNA but elevated IL-4 mRNA expression both in liver graft and in recipient spleen

To determine the relationship of specific cytokine production to the outcome of liver transplantation, cytokine mRNA expression in liver graft and recipient spleen 7 d after liver transplantation was examined by RT-PCR assay. IL-2, IL-4 and IFN-γ mRNA were readily detected both in liver graft and in recipient spleen 7 d after liver transplantation in animals from group A. Administration of immature DCs (group B and group E) partially down-regulated IL-2 mRNA and INF-γ mRNA expression and partially up-regulated IL-4 mRNA expression in liver graft and recipient spleen. Administration of IL-4 stimulated mature DCs (Group C) significantly up-regulated expression of IL-2 and IFN-γ mRNA but markedly down-regulated IL-4 mRNA expression in liver graft and recipient spleen ($P<0.001$ *vs* group A, B, D, E. Statistic data not shown). However, administration of NF-κB decoy ODNs-modified DCs (groupD) significantly suppressed expression of IL-2 and IFN-γ mRNA and significantly elevated expression of IL-4 mRNA both in liver grafts and in recipient spleen ($P<0.001$ *vs* group A, B, C, E. Statistic data not shown). Taken together, these results suggested that prolongation of liver allograft survival induced by NF-κB decoy ODNs-modified DCs might be associated with down-regulation of IL-2 and IFN-γ production and up-regulation of IL-4 production in liver graft and recipient lymphoid tissue.

DISCUSSION

It is accepted that both donor and recipient DCs mediate the rejection of graft in organ transplantation. Thus, conversion of these two sets of DCs to specifically inactivate recipient alloreactive T cells should allow the long-term acceptance of graft in the absence of continuous immunosuppression. In the present study, we showed that stably immature DCs modified with NF-κB decoy ODNs markedly suppressed the rejection of liver allograft and prolonged liver allograft survival.

The ability of DCs to traffic to T cell areas of secondary lymphoid tissues and subsequently direct immune responses makes them ideal candidates for cell-based therapies of allograft rejection. Several studies showed that immature donor DCs, deficient in surface costimulatory molecules, could induce T-cell hyporesponsiveness[20-22] and prolong graft survival in unmodified hosts[43,44].

Several strategies have been used to arrest the maturation of DCs and to potentiate their tolerogenicity. While some have shown their promising, all have significant disadvantages. CsA could inhibit DC function, but its effect was weak and temporary, easily to be overcome by cytokines such as IL-4[45]. CsA may inhibit DC maturation *in vivo*, but also interferes with signal transduction through the T cell receptor complex, thus impairing the development of antigen-specific tolerance. Indeed, CsA interferes with T cell maturation and selection and can lead to generation of autoreactive T cells. Additionally, CsA is associated with significant toxicity. Antibodies to fusion proteins specific for cell-surface molecules (anti-CD40, CTLA4Ig) could prevent DC costimulation of T cells[46]. However, antibodies and fusion proteins have a limited half-life. Late up-regulation of DC costimulatory molecules upon encountering the host microenvironment requires treatment of multiple antibodies. Expression of immunosuppressive gene products such as IL-10, TGF-β and CTLA4-Ig by DCs could result in further inhibition of alloimmune responses *in vitro*[20,29,47,48]. However, adenoviral vectors used to efficiently deliver transgene expression could simultaneously activate DCs[20,29]. Thus, transgene expression was overcome by the vigorous upregulation of costimulatory molecules on the transfected DC surface. Antisence oligodeoxynucleotides to molecules such as ICAM-1 could prolong kidney and heart allograft survival[49]. However, effects were not donor specific, nor did grafts survive indefinitely. To date, strategies using genetically DCs alone in experimental organ transplantation have failed to induce tolerance. In the setting of transplantation, proinflammatory cytokines and other factors were capable of promoting DC maturation abound within recipient tissues. Thus, late maturation and inherent T cell stimulatory potential of genetically engineered DCs may overcome the effects of localized immunosuppressive transgene expression. However, our and other data indicated that preconditioning donor DCs *in vitro* with ODN to block NF-κB nuclear translocation were sufficient to stably suppress the up-regulation of costimulatory molecules and IL-12 production in response to potent activating stimuli, such as LPS and IL-4.

NF-κB is an important transcriptional regulator of the immune response in a variety of cell types, but its precise function in DCs has not been extensively evaluated. Nonetheless, interference with its actions, either by CsA or using ODN decoy approach, could result in significant suppression of immune function at the level of cytokine production, effector function, and costimulation capacity.

Although long-term allograft survival has been achieved after infusion of costimulatory molecule-deficient DCs in a few specific mouse strain combinations, the ability of immature DCs to prolong allograft survival in most models is still not satisfactory.

In this study, by targeting the NF-κB pathway in DCs with short NF-κB decoy ODNs, DCs were maintained in an immature phenotype associated with significantly reduced allostimulatory capacity *in vitro*. More importantly, with administration of NF-κB decoy ODNs-treated donor DCs, significant suppression of liver allograft rejection and marked prolongation of recipient survival were achieved in the absence of immunosuppression. *In vivo*, only the effect of NF-κB decoy ODNs-treated donor DCs on liver allograft survival was maximal, although some suppression of liver allograft rejection and survival prolongation were observed in recipients injected with immature DCs without NF-κB decoy ODNs modification.

The mechanisms by which NF-κB decoy ODNs-treated DCs prolong survival of liver allografts are unclear. We found *in vitro* and *in vivo* evidence that stably immature NF-κB decoy ODNs-treated DCs could suppress T cell allostimulatory ability, promote apoptosis of graft-infiltrating cells, and inhibit Th1 immunostimulatory cytokines such as IL-2 and IFN-γ mRNA expression and increase Th2 cytokine (IL-4) mRNA expression both in liver graft and in recipient spleen. Apoptosis of alloreactive T cells appears to be an important mechanism underlying the survival prolongation of organ graft, apoptosis of immunoreactive T cells within the graft and host secondary lymphoid tissue plays a pivotal role in determining the balance between liver transplant tolerance and rejection. Previous studies showed that blockade of costimulation by donor-derived DCs markedly promoted apoptosis of alloreactive T cells in host lymphoid tissue and prolonged organ graft survival[50,51]. However, prevention of apoptosis of alloreactive T cells could block the induction of peripheral transplant tolerance[42,52]. In the present study, *in situ* TUNEL staining of liver grafts from NF-κB decoy ODNs-modified DCs-treated recipients showed the greatest degree of apoptosis of lymphocytes within portal areas of liver grafts. The result strongly suggested that the enhanced apoptosis of liver allograft-infiltrating lymphocytes might be an important mechanism for survival prolongation of liver allograft induced by NF-κB decoy ODNs-treated DCs.

Another important mechanism by which NF-κB decoy ODNs-treated DCs prolong survival of liver allografts may be the alteration of immunoregulatory cytokines (such as IL-2, IL-4 and IFN-γ) mRNA expression both in liver graft and in recipient spleen. In the present study, high level expression of IL-2, IL-4 and IFN-γ mRNA was observed in grafts and recipient spleen

of untreated recipient animals, which was consistent with immune activation. Administration of immature DCs (GM-CSF DCs or GM-CSF + scrambled ODNs DCs) partially down-regulated IL-2 mRNA and IFN-γ mRNA expression and partially up-regulated IL-4 mRNA expression both in liver graft and in recipient spleen. NF-κB decoy ODNs-treated DCs appeared to skew cytokine expression toward Th2 cytokines (IL-4), and significantly suppressed Th1 cytokines (INF-γ and IL-2) mRNA expression both in liver graft and in recipient spleen. The suppressed expression of IFN-γ and IL-2 mRNA both in liver graft and in spleen might be associated with the enhanced apoptosis of T cells and the skewing toward Th2. Although there is evidence that IL-10 could exacerbate organ allograft rejection and its neutralization could modestly prolong transplant survival[53,54], the predominant expression of Th2 cytokines, such as IL-4 and IL-10, was implicated in long term survival of the allograft in general. In contrast to Th2 cytokines, expression of Th1 cytokines, especially IFN-γ, in graft models has been shown to be associated with acute rejection[29,50,55]. Thus, significantly decreased expression of Th1 cytokines such as IL-2 and IFN-γ mRNA, as well as the relatively high level of IL-4 mRNA both in liver allograft and in recipient spleen may be an important mechanism underlying the tolerance of liver allograft induced by NF-κB decoy ODNs-treated DCs.

REFERENCES

1 **Zhang JK**, Chen HB, Sun JL, Zhou YQ. Effect of dendritic cells on LPAK cells induced at different times in killing hepatoma cells. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 673-675
2 **Li MS**, Yuan AL, Zhang WD, Chen XQ, Tian XH, Piao YT. Immune response induced by dendritic cells induce apoptosis and inhibit proliferation of tumor cells. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 56-58
3 **Luo ZB**, Luo YH, Lu R, Jin HY, Zhang PB, Xu CP. Immunohistochemical study on dendritic cells in gastric mucosa of patients with gastric cancer and precancerous lesions. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 400-402
4 **Li MS**, Yuan AL, Zhang WD, Liu SD, Lu AM, Zhou DY. Dendritic cells *in vitro* induce efficient and specific anti-tumor immune response. *Shijie Huaren Xiaohua Zazhi* 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 **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
7 **Banchereau J**, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; **392**: 245-252
8 **Khanna A**, Morelli AE, Zhong C, Takayama T, Lu L, 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
9 **Morelli AE,** O'Connell PJ, Khanna A, Logar AJ, Lu L, Thomson AW. Preferential induction of Th1 responses by functionally mature hepatic (CD8alpha- and CD8alpha+) dendritic cells: association with conversion from liver transplant tolerance to acute rejection. *Transplantation* 2000; **69**: 2647-2657
10 **Mehling A**, Grabbe S, Voskort M, Schwarz T, Luger TA, Beissert S. Mycophenolate mofetil impairs the maturation and function of murine dendritic cells. *J Immunol* 2000; **165**: 2374-2381
11 **Stuart LM**, Lucas M, Simpson C, Lamb J, Savill J, Lacy-Hulbert A. Inhibitory effects of apoptotic cell ingestion upon endotoxin-driven myeloid dendritic cell maturation. *J Immunol* 2002; **168**: 1627-1635
12 **Ismaili J**, Rennesson J, Aksoy E, Vekemans J, Vincart B, Amraoui Z, Van Laethem F, Goldman M, Dubois PM. Monophosphoryl lipid A activates both human dendritic cells and T cells. *J Immunol* 2002; **168**: 926-932
13 **Sato K**, Nagayama H, Enomoto M, Tadokoro K, Juji T, Takahashi TA. Autocrine activation-induced cell death of T cells by human peripheral blood monocyte-derived $CD4^+$ dendritic cells. *Cell Immunol* 2000; **199**: 115-125
14 **Zheng H**, Dai J, Stoilova D, Li Z. Cell surface targeting of heat shock protein gp96 induces dendritic cell maturation and anti-tumor immunity. *J Immunol* 2001; **167**: 6731-6735
15 **Morel Y**, Truneh A, Sweet RW, Olive D, Costello RT. The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. *J Immunol* 2001; **167**: 2479-2486
16 **Kobayashi M**, Azuma E, Ido M, Hirayama M, Jiang Q, Iwamoto S, Kumamoto T, Yamamoto H, Sakurai M, Komada Y. A pivotal role of Rho GTPase in the regulation of morphology and function of dendritic cells. *J Immunol* 2001; **167**: 3585-3591
17 **Hertz CJ**, Kiertscher SM, Godowski PJ, Bouis DA, Norgard MV, Roth MD, Modlin RL. Microbial lipopeptides stimulate dendritic cell maturation via Toll-like receptor 2. *J Immunol* 2001; **166**: 2444-2450
18 **Thoma-Uszynski S**, Kiertscher 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
19 **Hirano A**, Luke PP, Specht SM, Fraser MO, Takayama T, Lu L, Hoffman R, Thomson AW, Jordan ML. Graft hyporeactivity induced by immature donor-derived dendritic cells. *Transpl-Immunol* 2000; **8**: 161-168
20 **Lee WC**, Zhong C, Qian S, Wan Y, Gauldie J, Mi Z, Robbins PD, Thomson AW, Lu L. Phenotype, function, and *in vivo* migration and survival of allogeneic dendritic cell progenitors genetically engineered to express TGF-beta. *Transplantation* 1998; **66**: 1810-1817
21 **Hayamizu K**, Huie P, Sibley RK, Strober S. Monocyte-derived dendritic cell precursors facilitate tolerance to heart allografts after total lymphoid irradiation. *Transplantation* 1998; **66**: 1285-1291
22 **Khanna A**, Steptoe RJ, Antonysamy 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
23 **Lu L**, McCaslin D, Starzl TE, Thomson AW. Bone marrow-derived dendritic cell progenitors (NLDC 145+, MHC class II+, B7-1dim, B7-2-) induce alloantigen-specific hyporesponsiveness in murine T lymphocytes. *Transplantation* 1995; **60**: 1539-1545
24 **Fu F**, Li Y, Qian S, Lu L, Chambers F, Starzl TE, Fung JJ, Thomson AW. Costimulatory molecule-deficient dendritic cell progenitors (MHC class II+, CD80dim, CD86-) prolong cardiac allograft survival in nonimmunosuppressed recipients. *Transplantation* 1996; **62**: 659-665
25 **Lutz MB**, Suri RM, Niimi M, Ogilvie AL, Kukutsch NA, Rossner S, Schuler G, Austyn JM. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival *in vivo*. *Eur J Immunol* 2000; **30**: 1813-1822
26 **Thomas JM**, Contreras JL, Jiang XL, Eckhoff DE, Wang PX, Hubbard WJ, Lobashevsky AL, Wang W, Asiedu C, Stavrou S, Cook WJ, Robbin ML, Thomas FT, Neville DM Jr. Peritransplant tolerance induction in macaques: early events reflecting the unique synergy between immunotoxin and deoxyspergualin. *Transplantation* 1999; **68**: 1660-1673
27 **Jonuleit H**, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 2000; **192**: 1213-1222
28 **Dhodapkar MV**, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J Exp Med* 2001; **193**: 233-238
29 **Bonham CA**, Peng L, Liang X, Chen Z, Wang L, Ma L, Hackstein H, Robbins PD, Thomson AW, Fung JJ, Qian S, Lu L. Marked prolongation of cardiac allograft survival by dendritic cells genetically engineered with NF-kappa B oligodeoxyribonucleotide decoys and adenoviral vectors encoding CTLA4-Ig. *J Immunol* 2002; **169**: 3382-3391
30 **Ouaaz F**, Arron J, Zheng Y, Choi Y, Beg AA. Dendritic cell

development and survival require distinct NF-κB subunits. *Immunity* 2002; **16**: 257-270

31 **Rescigno M**, Martino M, Sutherland CL, Gold MR, Ricciardi-Castagnoli P. Dendritic cell survival and maturation are regulated by different signaling pathways. *J Exp Med* 1998; **188**: 2175-2180

32 **Verhasselt V**, Vanden Berghe W, Vanderheyde N, Willems F, Haegeman G, Goldman M. N-acetyl-L-cysteine inhibits primary human T cell responses at the dendritic cell level: association with NF-κB inhibition. *J Immunol* 1999; **162**: 2569-2574

33 **Mann J**, Oakley F, Johnson PW, Mann DA. CD40 induces interleukin-6 gene transcription in dendritic cells: regulation by TRAF2, AP-1, NF-kappa B, AND CBF1. *J Biol Chem* 2002; **277**: 17125-17138

34 **Giannoukakis N**, Bonham CA, Qian S, Chen Z, Peng L, Harnaha J, Li W, Thomson AW, Fung JJ, Robbins PD, Lu L. Prolongation of cardiac allograft survival using dendritic cells treated with NF-kB decoy oligodeoxyribonucleotides. *Mol Ther* 2000; **1**(5 Pt 1): 430-437

35 **Nouri-Shirazi M**, Guinet E. Direct and indirect cross-tolerance of alloreactive T cells by dendritic cells retained in the immature stage. *Transplantation* 2002; **74**: 1035-1044

36 **Abeyama K**, Eng W, Jester JV, Vink AA, Edelbaum D, Cockerell CJ, Bergstresser PR, Takashima A. A role for NF-kappaB-dependent gene transactivation in sunburn. *J Clin Invest* 2000; **105**: 1751-1759

37 **Sun J**, McCaughan GW, Matsumoto Y, Sheil AG, Gallagher ND, Bishop GA. Tolerance to rat liver allografts. I. Differences between tolerance and rejection are more marked in the B cell compared with the T cell or cytokine response. *Transplantation* 1994; **57**: 1394-1357

38 **McKnight AJ**, Barclay AN, Mason DW. Molecular cloning of rat interleukin 4 cDNA and analysis of the cytokine repertoire of subsets of CD4+ T cells. *Eur J Immunol* 1991; **21**: 1187-1194

39 **Ikejima K**, Enomoto N, Iimuro Y, Ikejima A, Fang D, Xu J, Forman DT, Brenner DA, Thurman RG. Estrogen increases sensitivity of hepatic Kupffer cells to endotoxin. *Am J Physiol* 1998; **274**(4 Pt 1): G669-G676

40 **Stober D**, Schirmbeck R, Reimann J. IL-12/IL-18-dependent IFN-gamma release by murine dendritic cells. *J Immunol* 2001; **167**: 957-965

41 **Qian S**, Lu L, Fu F, Li Y, Li W, Starzl TE, Fung JJ, Thomson AW. Apoptosis within spontaneously accepted mouse liver allografts: evidence for deletion of cytotoxic T cells and implications for tolerance induction. *J Immunol* 1997; **158**: 4654-4661

42 **Qian S**, Lu L, Fu F, Li W, Pan F, Steptoe RJ, Chambers FG, Starzl TE, Fung JJ, Thomson AW. Donor pretreatment with Flt-3 ligand augments antidonor cytotoxic T lymphocyte, natural killer, and lymphokine-activated killer cell activities within liver allografts and alters the pattern of intragraft apoptotic activity. *Transplantation* 1998; **65**: 1590-1598

43 **Lu L**, Li W, Zhong C, Qian S, Fung JJ, Thomson AW, Starzl TE. Increased apoptosis of immunoreactive host cells and augmented donor leukocyte chimerism, not sustained inhibition of B7 molecule expression are associated with prolonged cardiac allograft survival in mice preconditioned with immature donor dendritic cells plus anti-CD40L mAb. *Transplantation* 1999; **68**: 747-757

44 **Lutz MB**, Suri RM, Niimi M, Ogilvie AL, Kukutsch NA, Rossner S, Schuler G, Austyn JM. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival *in vivo*. *Eur J Immunol* 2000; **30**: 1813-1822

45 **Lee JI**, Ganster RW, Geller DA, Burckart GJ, Thomson AW, Lu L. Cyclosporine A inhibits the expression of costimulatory molecules on *in vitro*-generated dendritic cells: association with reduced nuclear translocation of nuclear factor kappa B. *Transplantation* 1999; **68**: 1255-1263

46 **Yin D**, Ma L, Zeng H, Shen J, Chong AS. Allograft tolerance induced by intact active bone co-transplantation and anti-CD40L monoclonal antibody therapy. *Transplantation* 2002; **74**: 345-354

47 **Takayama T**, Kaneko K, Morelli AE, Li W, Tahara H, Thomson AW. Retroviral delivery of transforming growth factor-beta1 to myeloid dendritic cells: inhibition of T-cell priming ability and influence on allograft survival. *Transplantation* 2002; **74**: 112-119

48 **Takayama T**, Nishioka Y, Lu L, Lotze MT, Tahara H, Thomson AW. Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness. *Transplantation* 1998; **66**: 1567-1574

49 **Stepkowski SM**, Wang ME, Condon TP, Cheng-Flournoy S, Stecker K, Graham M, Qu X, Tian L, Chen W, Kahan BD, Bennett CF. Protection against allograft rejection with intercellular adhesion molecule-1 antisense oligodeoxynucleotides. *Transplantation* 1998; **66**: 699-707

50 **Li W**, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, Qian S. IL-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3 ligand-treated donors and promotes graft acceptance. *J Immunol* 2001; **166**: 5619-5628

51 **Li W**, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW, Qian S. Costimulation blockade promotes the apoptotic death of graft-infiltrating T cells and prolongs survival of hepatic allografts from FLT3L-treated donors. *Transplantation* 2001; **72**: 1423-1432

52 **Li Y**, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nat Med* 1999; **5**: 1298-1302

53 **Li W**, Fu F, Lu L, Narula SK, Fung JJ, Thomson AW, Qian S. Differential effects of exogenous interleukin-10 on cardiac allograft survival: inhibition of rejection by recipient pretreatment reflects impaired host accessory cell function. *Transplantation* 1999; **68**: 1402-1409

54 **Li W**, Fu F, Lu L, Narula SK, Fung JJ, Thomson AW, Qian S. Systemic administration of anti-interleukin-10 antibody prolongs organ allograft survival in normal and presensitized recipients. *Transplantation* 1998; **66**: 1587-1596

55 **Shirwan H**, Barwari L, Khan NS. Predominant expression of T helper 2 cytokines and altered expression of T helper 1 cytokines in long-term allograft survival induced by intrathymic immune modulation with donor class I major histocompatibility complex peptides. *Transplantation* 1998; **66**: 1802-1809

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