Co-transfection with IL-10 and TGF-β1 into immature dendritic cells enhances immune tolerance in a rat liver transplantation model

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**ABSTRACT**

**Background** Dendritic cells (DC) transfected with interleukin-10 (IL-10) and transforming growth factor-β1 (TGF-β1) enhance T cell immunity and tolerance. However, no quantitative studies have investigated the suppressive functions of immature dendritic cells (imDC) co-transfected with IL-10 and TGF-β1. **Methods:** Effects of imDC co-transfected with IL-10 and TGF-β1 (IL-10-TGF-β1-imDC) on immune tolerance induction in a rat transplantation model were investigated. In addition, effects of IL-10-TGF-β1-imDC relative to IL-10 transfected imDC (IL-10-imDC) and TGF-β1-transfected imDC (TGF-β1-imDC) were compared. **Results:** The infusion of IL-10-TGF-β1-imDC into recipients prolonged liver graft survival, which were sustained for more than 90 days. IL-12 serum levels decreased, whereas alanine transaminase (ALT) and total bilirubin (TBIL) slightly increased in rats infused with IL-10-TGF-β1-imDC when compared to the IL-10-imDC and TGF-β1-imDC groups. Furthermore, a higher percentage of TUNEL positive cells were observed and histological analysis of the allografts indicated a rejection activity index (RAI) of mild acute rejection. **Conclusion:** Our results suggest infusion of IL-10 and TGF-β1 co-transfected imDC induces alloantigen-specific T cell hypo-responsiveness, inhibits antigen-specific immunological responses to liver allografts, prolongs liver allograft survival, and enhances the immune tolerance. This
approach may provide a promising and alternative for enhancing donor-specific tolerance during liver transplantation.

INTRODUCTION

Transplantation is an established therapy for end-stage organ failure. Although immunosuppressive therapy has greatly elevated the chance of success in short-term grafts, it is often accompanied by severe side effects (6). Dendritic cells (DCs) are the most potent antigen presenting cells (APCs) and play a critical role in the initiation of primary immune responses. The functional activity of DCs depends mainly on their state of activation and differentiation. Mature DCs (mDCs) induce development of T effector cells, whereas immature DCs (imDCs) maintain peripheral tolerance (11). mDCs express elevated levels of MHC and co-stimulatory molecules, whereas imDCs are deficient in co-stimulatory molecules. Therefore, imDCs present antigen to T cells in the absence of co-stimulation, which lead to T-cell anergy or the generation of regulatory T cells (Tregs) (5). Tolerogenic dendritic cells (tDCs) are generally characterized by an immature or semi-mature phenotype, with reduced expression of costimulatory molecules. tDCs are therefore a promising tool for specific cellular therapy to induce immunological tolerance in transplantation and autoimmunity (1).

IL-10 is an immunomodulatory cytokine that modulates the immune response. The main role of IL-10 is to inhibit antigen-specific T cells from undergoing proliferation and to keep DCs from secreting inflammatory cytokines. Some studies have indicated that IL-10-producing DCs exhibit powerful tolerogenic characteristics
with elevated IL-10-production and low T cell activation, as well as the potential to
induce Tregs. Moreover, in a functional suppression assay only IL-10 DC induced
Tregs, which strongly suppressed T cell reactivity (1, 2, 10). Other experiments have
shown that IL-10 inhibits expression of co-stimulatory molecules in phagocytes. It
also inhibits mononuclear phagocytes from presenting antigens and reduces T cell-
and B cell-mediated immune responses. In addition to its effect on T cells, IL-10
inhibits the immune activity of other cell-types, such as NK cells, mononuclear cells,
and macrophages (17). Taken together these findings suggest that IL-10 acts as a
potential negative regulator of cellular proliferation during the immune response and
inflammation, which plays an important role in immune tolerance. Therefore, IL-10
has emerged as a promising therapeutic factor for the treatment of autoimmune
reactions during transplantation.

In general, transforming growth factor (TGF)-β1 plays a crucial role in immune
tolerance. Studies have shown that TGF-β1 has multiple inhibitory effects on the
proliferation, differentiation, and survival of T cells, B cells, macrophages and other
immune cells (16, 22). It is also an essential cytokine for the differentiation of Th1
cells and inhibits proliferation of allogeneic lymphocytes in vitro. TGF-β1-DCs also
decrease IL-12 expression, which plays an important role in Th1 differentiation (20).
They inhibits Thl secretion of TNF-alpha, IL-2, and IFN-γ, and prevents Th2 cells
from secreting IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. TGF-β1-DCs induce
CD41Foxp31 Tregs, and TGF-β1-imDCs inhibit imDCs function (3). They are known
to exert immunosuppressive effects upon various target cells in the immune system in
vitro and have been shown to mediate immunosuppressive effects in vivo (26). Taken together, these findings indicate TGF-β1 plays a major role in maintaining immunologic self-tolerance.

Both IL-10 and TGF-β1 are critical immune modulatory genes, and their modulation alters DC function. Previously, imDC transfected with recombinant plasmid containing both IL-10 and TGF-β1 resulted in the down-regulation of MHC class II, CD80, and CD86 (4). IL-10-TGF-β1-imDC induced T cell hyporesponsiveness, limiting proliferation. However, IL-10-TGF-β1-imDCs were more effective than IL-10-imDC and TGF-β1-imDC, respectively. DC co-expressing IL-10 and TGF-β1 enhanced the tolerogenicity of imDC. However, thus far, we are unaware of quantitative studies investigating the suppressive function of immature dendritic cells (imDC) co-transfected with IL-10 and TGF-β1 in donor-specific tolerance following organ transplantation. The aim of this study was to use a rat liver transplantation model to examine the immune tolerance effects of IL-10-TGF-β1–imDC, and to compare the effect of IL-10-TGF-β1–imDC with IL-10-imDC and TGF-β1-imDC, respectively. Interestingly, IL-10-imDC and TGF-β1-imDC were found to inhibit imDCs immunity and enhanced their tolerogenicity. Injection of donor imDC co-transfected with IL-10 and TGF-β1 prolonged liver allograft survival. Our study describes a potentially promising way to enhance donor-specific tolerance during organ transplantation.

MATERIALS AND METHODS
Animals

Male DA and Lewis rats (8-10 weeks old and weighing 230±20 g) were purchased from the laboratory animal center of the 2nd affiliated hospital at Harbin Medical University (Heilongjiang, China) and Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). A total of 161 pairs of DA/Lewis rats were divided into 7 groups: control group (n=23), mDC group (n=23), imDC group (n=23), Vector-imDC group (n=23), IL-10-imDC group (n=23), TGF-β1-imDC group (n=23), and IL-10-TGF-β1-imDC group (n=23). Five rats in each group were sacrificed at day 3, 7, and 10 post-transplantation, respectively, for harvest of liver specimens. Eight rats post-transplantation in each group were used for survival observation. Two rats in the control group, imDC group, and TGF-β1-imDC group, and 1 rat in the other groups died after surgical operation within 30 minutes, respectively. Experimental conditions were kept the same in all groups. All rats were housed in microisolator cages in the barrier facility of Fujian Medical University (Fuzhou, China). All experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of Fujian Medical University.

Preparation of DCs

Rat bone marrow cells (RBMCs) were collected from the tibia and femur of DA rats by flushing with a 25-gauge needle. RBMCs were cultured in RPMI 1640 medium supplemented with 15% v/v fetal bovine serum medium (FBS) (Life Technologies, San Diego, CA, USA) and 10 ng/ml recombinant rat granulocyte-macrophage
colony-stimulating factor (rat recombinant GM-CSF, PeproTech, Rocky Hill, NJ, USA) at 37°C plus 5% CO₂ for 8 days. Cells were plated in 6-well plates at a density of 1-2×10⁶/ml cells in RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum, 10.0 ng/ml rat rIL-4 (PeproTech), and 20.0 ng/ml of rat recombinant GM-CSF. Old medium with floating cells was gently removed after 3 days and replaced with fresh medium. After 2-3 days, non-adherent cells and loosely adherent aggregated proliferating DC were harvested. mDCs were prepared in a similar manner except for the use of 10ng/ml rat recombinant GM-CSF and 10 ng/ml rat recombinant TNF-alpha (PeproTech) at day 6 as opposed to day 8.

**Identification of imDC and mDC**

ImDCs and mDCs were confirmed morphologically using an inverted microscope (Nikon, Melville, NY, Japan) and Scanning Electron (SEM) and Transmission Electron Microscopy (TEM) (Zeiss, Berlin, Germany). Both types of DCs were stained with anti-OX62-FITC, anti-CD80-FITC, and anti-CD86-FITC. Mouse IgG was used as a negative control. Cells were then incubated with appropriate secondary antibodies for 30 min at 4°C, after which the cells were washed twice in PBS with 1% w/v BSA. FACS analysis was performed using FACScan flow cytometry (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).

**Plasmids**

Plasmids encoding pIRES2-EGFP-hIL-10 and pIRES2-EGFP-hTGF-β1 were kindly
provided by Dr. Huang G (Fujian Medical University). Plasmid inserts were confirmed by double digestion with restriction enzymes XhoI+EcoRI and BglII+HindIII, respectively. IL-10 and TGF-β1 genes were also PCR amplified and confirmed by sequencing. The following primers were used: IL-10F (forward): 5’-CCGCTCGAG CCACC ATGCACAGCTCAGCTCT-3’; IL-10R (reverse): 5’-CCGGAATTC TCAGTTTCGTATCTTCATTGTC-3’; TGF-β1F: 5’-GCCCTGGA CACCAACTATTGCT-3’; TGF-β1R: 5’-TCAGCTGCACCTTGAGGAGCGC-3’.

Transfection and identification

DCs were transfected on day 7 with pIRES2-EGFP- hIL-10, pIRES2-EGFP-hTGF-β1, or empty control vector using Lipofectamine 2000TM (Invitrogen) following the manufacturer’s instructions. The resulting DCs were termed IL-10-TGF-β1-imDC, IL-10-imDC, TGF-β1-imDC and vector-imDC, respectively. imDC protein was detected by ELISA (Jingmei Biotech, Shenzhen, China) and Western blot analysis.

Establishment of a rat heterotopic liver transplantation model

A heterotopic liver transplantation model was established using DA rats as donors and Lewis rats as recipients, as described previously (14, 15, 19). Syngeneic transplants were used as controls. In treatment groups, 1 ml normal saline containing 2×10⁶ DA-derived imDC, mDC, vector-imDC, IL-10-imDC, TGF-β1-imDC, and IL-10-TGF-β1-imDC DCs were injected intravenously into Lewis rats 5 days prior to
liver transplantation. Examination of recipients was used to determine allograft survival and rejection was confirmed histologically. To test the capacity of IL-10 and TGF-β1 modified imDC for prolonging liver allograft survival, DA donor-derived TGF-β1 modified imDC (2×10^6 cells in 100 μl PBS) was infused intravenously into Lewis recipients on day 7.

**Liver function measurement**

Total bilirubin (TBIL) and alanine transaminase (ALT) serum levels were quantitated to assess liver function on days 3, 7, and 10 following liver transplantation.

**Cytokine measurement by ELISA**

IL-12 serum concentration was determined by ELISA (450 nm) using Quantikine M kits (Jingmei Biotech, Shenzhen, China), following the manufacturer’s instructions.

**TUNEL method**

Animals were sacrificed on days 3 and 7 following liver transplantation and tissue was harvested. Grafted liver was isolated, fixed in formalin, and embedded in paraffin. Four-μm sections were then cut, deparaffinized, and digested for 15min by protease K. T cell apoptosis was detected by terminal transferase-mediated UTP nick end-labeling (TUNEL) using an in situ apoptosis detection kit (Boehringer Mannheim, Germany). Paraffin sections were deparaffinized in xylene, rehydrated and incubated with 20 mg/ml of proteinase K for 10 min and rinsed in distilled water. Endogenous
peroxidase activity was inhibited with 3% hydrogen peroxide. Sections were then incubated with equilibration buffer for at 10°C for 15 s, followed by TdT enzyme in a humidified atmosphere at 37°C for 60 min. Sections were subsequently pre-warmed in working strength stop wash buffer at room temperature for 10 min, followed by anti-streptavidin-peroxidase for 45 min. PBS washes were used after each step. DAB staining and counter staining was performed in hematoxylin and eosin. Two individuals examined slides independently. Approximately 100 TUNEL-positive cells were counted in randomly chosen fields per each case and the percentage of apoptotic cells stained brown was determined.

**Histological analysis**

Histological assessments play an important role in the diagnosis and management of liver allograft rejection. The allografts were examined on days 3, 7, and 10 following transplantation. The overall rejection grade was assessed and the rejection activity index (RAI) was calculated according to the Banff schema. For statistical analysis, the RAI score was subsequently grouped as follows: RAI below 2 was classified as absent, 2 as indeterminate rejection, 3-4 as mild, 5-6 as moderate, and greater than 6 as severe (13). All histological evaluations were done in a double blinded manner by two experimenters.

**Statistical analysis**

SPSS v12 (IBM, Chicago, IL, USA) Analytical Software was used for analysis and
the values shown as mean ± standard deviation (SD). Differences between groups were analyzed using analysis of variance (ANOVA) or Student's t test. Graft survival data were analyzed statistically by life table methods, and differences in survival time were tested with the Log-rank method. A p-value of 0.05 was considered statistically significant.

RESULTS

Liver function evaluation

The normal ALT and TBIL levels were 26.3±7.3 U/L and 11.36±4.35 μmol/L, respectively, in the Lewis rats. After 7 and 10 days of transplantation, the ALT and TBIL levels were significantly increased in the control and mDC groups (P<0.001), but were significantly decreased in the imDC, vector imDC, IL-10 imDC, and TGF-β1 imDC groups when compared with values obtained on the 3rd day (P<0.05). Meanwhile, the ALT and TBIL levels were markedly decreased after 7 and 10 days of transplantation in the imDC, vector imDC, IL-10 imDC, and TGF-β1 imDC groups when compared with those of the control and mDC groups. ALT and TBIL levels in the IL-10 and TGF-β1 imDC groups were significantly lower than those in the imDC, vector imDC, IL-10 imDC, and TGF-β1 imDC groups (P<0.05) (Fig.1A-1B). These results indicate that liver function in the IL-10- and TGF-β1 imDC-treated groups was continuously being restored after transplantation. Our data suggest that the functional reserve of the liver is maintained in the presence of elevated levels of IL-10 and TGF-β1.
Detection of apoptosis by TUNEL

TUNEL-positive lymphocytes were detected in the portal area in liver sections of every group (Fig. 2A-2F). The percentage of TUNEL positive cells was significantly higher in the IL-10 and/or TGF-β1-imDCs groups than that of controls (p<0.05), particularly in those treated with IL-10-TGF-β1-imDC (Fig. 2G). In summary, the percentage of TUNEL positive cells increased in the presence of elevated IL-10 and TGF-β1. The stimulation was highest in the IL-10-TGF-β1-imDC group.

IL-10 and TGF-β1 overexpression downregulates IL-12 following liver transplantation

IL-12 serum levels in rats that had received treatment with IL-10 and/or TGF-β1-imDCs treatment compared with the mDC, imDC, and vector-imDC control groups on days 3 and 7 are shown in Table 1. The IL-10-TGF-β1-imDC recipients exhibited lower serum IL-12 than that of controls and those receiving mDCs, imDCs, and vector-imDCs (*: P<0.05). Animals treated with imDCs, vector-imDCs, TGF-β1-imDCs, IL-10-imDCs, and TGF-β1-IL-10-imDCs treated animals exhibited lower IL-12 levels than those of controls and mDC groups at day 3 post transplantation (▲: P<0.05). In addition, TGF-β1-imDCs, IL-10-imDCs, and TGF-β1-IL-10-imDCs were all lower relative to the control, mDCs, imDCs, and vector-imDCs groups at day 7 post transplantation (**: P<0.05). Finally, imDCs, vector-imDCs, TGF-β1-imDCs, IL-10-imDCs, and TGF-β1-IL-10-imDCs all...
exhibited lower IL-12 levels than control and mDC treated rats at day 7 post transplantation (\(\Delta \Delta\): P<0.05). These results indicate IL-10 and/or TGF-\(\beta\)1 overexpression down regulates IL-12 levels following liver transplantation.

**Histological assessment of donor liver grafts**

Control RAI values progressively increased for indeterminate, mild, moderate, and severe acute rejection (Fig 3A-3F and Table 2). After 3, 7, and 10 days of transplantation, RAI values in the imDC, vector imDC, IL-10 imDC, and TGF-\(\beta\)1 imDC groups were significantly lower when compared with those of the control and mDC groups (P<0.001). Meanwhile, RAI values in the IL-10 and/or TGF-\(\beta\)1 imDC groups were significantly lower than those of the imDC and vector imDC groups ((P<0.01), except for that in the IL-10 imDC group 3 days after transplantation. Moreover, RAI values in the IL-10 and TGF-\(\beta\)1 imDC group were significantly lower than those in the IL-10 imDC or TGF-\(\beta\)1 imDC groups (P<0.001), except for that in the TGF-\(\beta\)1 imDC group 7 days after transplantation. The higher the total RAI scores, the more likely it was that the graft would fail due to rejection. Our data indicated that the RAI values in IL-10 and/or TGF-\(\beta\)1-imDC showed mild acute rejection, especially in IL-10 and TGF-\(\beta\)1-imDC, where the total RAI value was less than 4.

**Prolonged survival of liver allografts in recipients infused with IL-10 and TGF-\(\beta\)1-imDC**
The survival of liver grafts following infusion of IL-10 and/or TGF-β1-imDC was more than 40, 58, or 90 days, respectively (Fig. 4). Thus, survival time was prolonged, especially in IL-10-TGF-β1-imDC. The survival time in rats treated with IL-10 and/or TGF-β1-imDCs was significantly prolonged when compared with that in the mDC, imDCs, vector-imDC, and control groups (all P<0.001). However, no significant differences were observed between the imDC and vector-imDC groups (P>0.05). Additionally, no significant differences were detected when comparing the control group with the mDC group (P>0.05). These results indicate that infusion of IL-10 and/or TGF-β1-imDC induces alloantigen tolerance and prolongs liver allograft survival. IL-10-TGF-β1-imDC was more effective than IL-10-imDC or TGF-β1-imDC individually.

DISCUSSION

In the present study, we investigated the effects of imDC co-transfected with IL-10 and TGF-β1 genes (IL-10-TGF-β1-imDC) in inducing immune tolerance following liver transplantation in rats. Infusion of IL-10 and/or TGF-β1-imDC, particularly IL-10-TGF-β1-imDC, prolonged liver graft survival. Liver grafts survived more than 40, 58, or 90 days, depending on the treatment, indicating that of IL-10 or/and TGF-β1-imDC induces alloantigen tolerance and prolongs liver allograft survival. IL-10-TGF-β1-imDC was more effective than IL-10-imDC and TGF-β1-imDC. These results suggest injection of donor imDC co-transfected with IL-10 and TGF-β1 genes prolongs survival of liver allografts.
We have previously examined the function of imDC co-transfected with IL-10 and TGF-β1 genes in vitro (4), and found that IL-10-TGF-β1-imDC down-regulates MHC class II, CD80, and CD86 in imDCs. IL-10-TGF-β1-imDC induces T cell hyporesponsiveness, and inhibits proliferation. Interestingly, IL-10-TGF-β1-imDC was more effective than either IL-10-imDC or TGF-β1-imDC. IL-10 and TGF-β1 genes co-expressed in DC affected the immunity of imDCs and enhanced their tolerogenicity.

We overexpressed two cytokines, IL-10 and TGF-β1, in bone marrow-derived imDCs and investigated their immunity and tolerogenicity in vitro. By using DA rats as donors and Lewis rats as recipients, a heterotopic liver transplantation model was established. In treated groups, DA-derived imDC, mDC, vector-imDC, IL-10-imDC, TGF-β1-imDC, or IL-10-TGF-β1-imDC DCs was injected intravenously into Lewis rats 5 days prior to the liver transplantation respectively. We observed imDCs co-transfected with IL-10 and TGF-β1 genes inhibit DC differentiation and maturation. Histological assessment of donor liver grafts revealed RAI values in IL-10- and/or TGF-β1-imDC-treated rats consistent with mild acute rejection. For example, in IL-10 and TGF-β1-imDCs, the total RAI value was less than 4. However, the RAI values in controls increased progressively for moderate, and severe acute rejection. In our liver allograft model, graft survival in recipients infused with IL-10 and/or TGF-β1-imDC were prolonged, especially in the IL-10-TGF-β1-imDC group. Liver grafts survived more than 40, 58, and 90 days, with IL-10-TGF-β1-imDC more effective than IL-10-imDC or TGF-β1-imDC alone. These results strongly suggest
that infusion of IL-10-imDC and TGF-β1-modified imDC into recipients induces
alloantigen-specific T cell hyporesponsiveness and prolongs liver allograft survival.
ELISA data indicated lower levels of serum IL-12 in rats that had received IL-10
and/or TGF-β1-imDCs compared with those of controls. This suggests that IL-10
and/or TGF-β1 inhibit IL-12 expression in DCs and Th1 differentiation (9, 21), and
that they influence DC function in an autocrine manner. Alternatively, they may affect
T cells in a paracrine way and suppress antigen-induced T cell proliferation.

IL-10 and TGF-β1 are important multifunctional regulatory molecules in the
immune response. Additional studies show that they are secreted proteins that regulate
cellular proliferation, differentiation, and cell death in several immune cells (3, 18). In
our study, the percentage of TUNEL positive cells was significantly increased in the
IL-10 and/or TGF-β1-imDCs when compared with that of controls (p<0.05),
particularly in IL-10-TGF-β1-imDC. The percentage of TUNEL positive cells was
significantly increased in the presence of elevated levels of IL-10 and TGF-β1.

Treatment of monocyte-derived DC with vitamin D3 (24), TGF-β1 (8, 16, 23),
rapamycin (21), dexamethasone (7, 12) or IL-10 (1, 9) induces inhibitory DC.
However, the suppressive capacity of imDC co-transfected with IL-10 and TGF-β1
has been examined in just a few studies. IL-10 and TGF-β1 are important
multifunctional immune regulatory molecules. TGF-β1 is a key factor during Th
differentiation, which up-regulates Foxp3 expression in Th0 cells, driving them to
differentiate into CD4⁺Foxp3⁺ Tregs (25). Cai et al reported (3) that imDCs
transfected by TGF-β1 induce higher percentages of CD4⁺Foxp3⁺ Tregs in vitro.
Tregs play a crucial role in immune tolerance, which inhibit the proliferation of autoreactive T lymphocytes (25).

In summary, we found that IL-10-TGF-β1-imDC induce allergen-specific T cell immune tolerance more effectively than IL-10-imDC and TGF-β1-imDC. This suggests that infusion of IL-10-imDC and TGF-β1 modified imDCs into recipients induces alloantigen-specific T cell hyporesponsiveness and inhibits antigen-specific immunological responses to liver allografts. It also prolongs survival of liver allografts. Our study provides a potentially promising and alternative approach to enhance donor-specific tolerance during organ transplantation.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

L.C., L.Z., W.H., M.Q., and L.G. performed experiments; L.C. drafted manuscript; J.L. and A.H. edited and revised manuscript; J.L. and A.H. conception and design of research; L.C. analyzed data; L.C. interpreted results of experiments; W.H. prepared
FIGURE LEGENDS

Figure 1A. Serum ALT levels on days 3, 7, and 10 post-liver transplantation time points. ALT levels increased significantly 7 and 10 days after transplantation in the control and mDC groups when compared with those measured on the 3rd day (P<0.001). ALT levels in IL-10- and TGF-β1 imDC groups were lower significantly than those of the imDC, vector imDC, IL-10 imDC, and TGF-β1 imDC groups (P<0.05).

Figure 1B. Serum TBIL levels on days 3, 7, and 10 post-liver transplantation time points. TBIL levels significantly increased 7 and 10 days after transplantation in the control and mDC groups when compared with those measured on the 3rd day (P<0.001). TBIL levels in the IL-10- and TGF-β1 imDC group were lower significantly than those of the imDC, vector imDC, IL-10 imDC, and TGF-β1 imDC groups (P<0.05).

Figure 2. Detection of apoptotic lymphocytes in liver using TUNEL assay. Apoptotic lymphocytes were observed in the portal area in every group (Fig. 2A-2F). All images are at ×400 and AEC. A: mDC. B: imDC. C: Empty vector imDC. D: IL-10 imDC. E: TGF-β1 imDC. F: IL-10-TGF-β1 imDC. G: Apoptotic lymphocytes
significantly increased in IL-10 and TGF-β1-imDCs compared with levels found in IL-10 or TGF-β1-imDCs (P<0.05)

Figure 3. Histological assessment of donor liver grafts on day 10 post transplantation. All images (×400, H&E). The liver grafts in IL-10 and TGF-β1-imDCs showed no rejection, while the liver grafts in IL-10 or TGF-β1-imDCs showed mild acute rejection, and the liver grafts in controls showed moderate to severe acute rejection. A: mDC. B: imDC. C: Empty vector imDC. D: IL-10 imDC. E: TGF-β1 imDC. F: IL-10-TGF-β1 imDC.

Figure 4. Prolonged liver allograft survival by intravenous infusion with IL-10 and/or TGF-β1-DC 7 days before transplantation. The survival time with IL-10 and TGF-β1 (more than 90 days) was significantly prolonged compared with those of IL-10 or TGF-β1 alone (P=0.0000).

REFERENCES


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Table 1  Detection of serum IL-12 in transfected DCs recipients with ELISA

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 7</th>
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<tbody>
<tr>
<td>Control</td>
<td>102.01±14.32</td>
<td>169.02±10.14</td>
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<tr>
<td>mDC</td>
<td>107.8±1.43</td>
<td>182.24±11.93</td>
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<td>79.51±6.87</td>
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<tr>
<td>IL-10-TGF-β1-imDC</td>
<td>66.48±9.25</td>
<td>69.13±11.63</td>
</tr>
</tbody>
</table>

Unpaired 2-tailed Student's t test, The IL-10-TGF-β1-imDC recipients exhibited lower serum IL-12 than that of controls and those receiving mDCs, imDCs, and vector-imDCs (*: P<0.05). imDCs, vector-imDCs, TGF-β1-imDCs, IL-10-imDCs, and TGF- β1-IL-10-imDC-treated animals exhibited lower IL-12 levels than those of controls and mDC groups at day 3 post-transplantation (**: P<0.05). In addition, TGF-β1-imDCs, IL-10-imDCs, and TGF- β1-IL-10-imDCs were all lower relative to those of control, mDCs, imDCs, and vector-imDCs groups at day 7 post-transplantation (***: P<0.05). Finally, imDCs, vector-imDCs, TGF-β1-imDCs, IL-10-imDCs, and TGF-β1-IL-10-imDCs all exhibited lower IL-12 levels than those of control and mDC-treated rats at day 7 post-transplantation (**: P<0.05).

Table 2  Assessment of rejection activity index (RAI) after liver transplantation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>3.50±0.18</td>
<td>7.12±0.39</td>
<td>8.87±0.20</td>
</tr>
<tr>
<td>mDC</td>
<td>4.14±0.28</td>
<td>7.57±0.37</td>
<td>8.57±0.13</td>
</tr>
<tr>
<td>imDC</td>
<td>2.50±0.43*</td>
<td>4.33±0.34*</td>
<td>5.58±0.34*</td>
</tr>
<tr>
<td>Vector-imDC</td>
<td>2.57±0.36*</td>
<td>4.71±0.42*</td>
<td>5.5±0.22*</td>
</tr>
<tr>
<td>IL-10-imDC</td>
<td>2.20±0.28*</td>
<td>3.14±0.26*</td>
<td>4.71±0.28*</td>
</tr>
<tr>
<td>TGF-β1-imDC</td>
<td>1.5±0.22*</td>
<td>2.33±0.21*</td>
<td>4.33±0.21*</td>
</tr>
<tr>
<td>IL-10-TGF-β1-imDC</td>
<td>1.14±0.14*</td>
<td>2.28±0.21*</td>
<td>3.28±0.18*</td>
</tr>
</tbody>
</table>
\* P<0.01 imDC vs. control and mDC

\* P<0.01 IL-10- and/or TGF-1-imDC vs. imDC and Vector-imDC

\* P<0.01 IL-10- and TGF-β1-imDC vs. IL-10- or TGF-β1-imDC