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CROSS TOLERANCE OF RECIPIENT-DERIVED TGF-β DENDRITIC CELLS

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Abstract

Administration of donor-derived immature dendritic cells (DC) treated with transforming growth factor-beta (TGF-β) to prevent allograft rejection is not applicable for clinical use. We therefore attempted to explore the use of recipient-derived DC pulsed with donor antigens via the indirect pathway (cross-priming). DC were propagated from C3H ($H2^k$) bone marrow (BM) with GM-CSF and IL-4. TGF-β (0.2ng/ml) was added at the initiation of culture and the resultant (TGF-β DC) were pulsed with B10 ($H2^b$) splenocyte lysate. Expression of MHC class I and II were not affected, while CD40, CD80 and CD86 co-stimulatory molecules on DC was significantly inhibited by treatment with TGF-β. C3H DC pulsed with B10 antigens stimulated proliferate responses in C3H T cells was inhibited when DC were treated with TGF-β, and the CTL activity was also inhibited. This correlated with reduced IFN-γ and increased IL-10 production. A single injection of TGF-β DC prolonged allograft survival (MST 18 days vs. 10 days in no-DC treatment control, $p<0.05$). These data indicate that an approach utilizing recipient DC as a "vaccine" strategy is possible.

Keywords

dendritic cell; Antigen presentation; TGF-β; transplantation

INTRODUCTION

The induction of immunity or tolerance by dendritic cells appears to be related to their state of functional maturation. Transforming growth factor-beta (TGF-β) has been implicated as a key factor in the regulation of tolerance. [1] Administration of TGF-β -modified donor-type DC resulted in significant prolongation of organ allograft survival in the absence of immunosuppression. [1,2] Prevention rather than treatment will be the best approach to this problem; induction of cross-tolerance is one resolution. We have explored the use of recipient-derived DC pulsed with donor spleen cell lysate, in which the donor antigens were presented

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to host T cells via an indirect pathway (cross-priming).[3] A single injection of NF-κB ODN DC pulsed with donor antigens significantly prolonged allograft survival in an antigen-specific manner. [3] However, the effect of recipient TGF-β DC pulsed with donor antigens on allograft survival is still unknown.

We demonstrated that a single injection of bone-marrow derived DC treated with TGF-β pulsed with alloantigen increased cross tolerance after heart transplantation in mice and induced antigen-specific T cell hyporesponsiveness in this study.

METHODS AND MATERIALS

DC were isolated from femurs of C3H mice and propagated in GM-CSF plus IL-4 cells (both from Schering-Plough, Kenilworth, NJ). To generate immature (i) DC, TGF-β (0.2ng/ml, R & D Systems Inc., Minneapolis, MN) was added at the initiation of culture of DC (BM TGF-β iDC). These BM TGF-β DC were pulsed with B10 spleen cell lysates at 1/1 to 1/10 of DC/spleen ratio for 48 hours.[3] DC phenotype was examined by flow cytometry. The spleen T cell response was determined by one-way MLR and generation of CTL assay. We analyzed for the presence of cytokines (IL-10, IFN-γ levels) using commercial ELISA kits (R&D Systems) and performed RNase protection assay to determine levels of mRNA. Fully allogeneic intraabdominal vascularized heart transplantation was performed. Graft survival was assessed by daily transabdominal palpation of heart. Cessation of heartbeat indicated rejection of the allograft.

RESULTS

As demonstrated previously, the most effective cross-presentation of T cells is achieved by pulsing DC with B10 spleen lysate at a concentration 5 times the number of dendritic cells. [3] While, MHC class I/II expressions were not impaired after treatment with TGF-β plus alloantigens, there was significant inhibition of CD40, CD80 and CD86 expression. TGF-β DC-pulsed with alloantigens induced allogeneic donor-specific hyporesponsiveness in mixed leukocyte reaction, and significantly inhibited CTL activity against allospecific EL4 ($H\text{-}2^b$) targets. Mature (m) DC pulsed with B10 antigens elicited high IFN-γ but low IL-10 production in syngeneic T cells, while T cells after stimulation by TGF-β iDC produced low IFN-γ but high levels of IL-10. Compared with the control group receiving no DC pretreatment (median survival time [MST] 10 days), pretreatment with mDCs significantly accelerated B10 heart allograft rejection (MST 7 days, $p < 0.05$). In contrast, administration of TGF-β iDCs prolonged survival of cardiac allografts (MST 18, $p < 0.05$). The immunosuppressive effect of TGF-β DC is donor-specific, as they failed to prolong survival of alloantigen from third party (BALB/c, $H\text{-}2^d$ MST 13 days) strain.

DISCUSSION

The immune responses associated with the capture and presentation of cell-derived antigens by host APC play an important role in chronic rejection. [4] TGF-β is a potent immunosuppressive cytokine which has been shown to block the maturation of GM-CSF-stimulated mouse BM-derived DC.[5,6] It has also been shown to arrest DC maturation and promote the growth of immature, costimulatory molecules signaling B7 which have been demonstrated to be involved in the induction of peripheral tolerance. [5,6] In this study, pulsing with alloantigens did not elicit up-regulation of costimulatory molecule expression on TGF-β DC, indicating that TGF-β arrested DC maturation.

In vitro, we have maintained DC of an immature phenotype which is associated with significantly reduced allostimulatory capacity. [1] *In vivo*, it has been previously reported that

TGF-β prolongs rodent cardiac allograft survival.[7] In our study, with one single injection of TGF-β-treated recipient DC pulsed with alloantigen, we have achieved prolongation of allogeneic heart survival. Tolerance that results from MHC-mismatched recipients in the absence of any immunosuppression, has been called cross-tolerance. In our study, alloantigen pulsed TGF-β DC induced antigen-specific hyporesponsiveness of T cells in MLR, with increased levels of IL-10 and reduced levels of IFN-γ production. We propose that the immature TGF-β DC may be beneficial for graft acceptance, with inhibiting Th1 type cytokine production and progression of Th0 cells to Th2.

Immunosuppressants have led to considerable improvement in survival rates although patients must continue to receive these drugs for life. [8] Using our TGF-β approach clearly results in significant suppression of immune function, at the level of cytokine production and the concomitant induction of immunologic tolerance. These data indicate that an approach to use recipient DC as a “vaccine” strategy provides a feasible approach for establishing long-term survival in organ transplantation. As was illustrated in our studies, cross tolerance plays a significant role in the long-term induction of graft survival. But, recipient-derived iDCs treated with TGF-β did not induce longer graft survival than those treated with NF-κB ODN.[3,9] In the future, we propose treating recipient-derived DC with both NF-κB ODN and TGF-β.

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Table

C3H BM-derived mDCs or TGFβ iDCs With or Without Pulsing With B10 Antigens Cultured Were Stained With CD40, CD80, CD86 by Flow Cytometry. Numbers Indicate The Percentage of Positive Cells in The $CD11c^+$ Cell Population. The DCs With C3H Spleen T cells Culture Supernatants Were Examined for Levels of IFN-γ and IL-10 by ELISA.

	CD40	CD80	CD86	IL10 (pg/ml)	IFN-γ (pg/ml)
mDC	72.9%	68.6%	60.2%	100.5 ± 16.7	665.0 ± 107.1
mDC + Ag	72.2%	65.4%	53.4%	70.8 ± 10.8	1096.1 ± 305.6
TGFβ iDC	37.7%	37.6%	26.9%	201.0 ± 80.7	569.0 ± 53.3
TGFβ iDC + Ag	56.6%	52.3%	27.7%	1185.0 ± 379.7	441.0 ± 28.2

DC, dendritic cell; Ag, antigen