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PROVIDE ABSTRACT

KEY WORDS

gene therapy, dendritic cells, transplantation, immunosuppression, tolerance

ABBREVIATIONS

DC dendritic cells
APC antigen-presenting cells

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Dendritic cells (DCs) are uniquely well-equipped antigen-presenting cells (APCs), now regarded both as instigators and critical regulators of immune reactivity.[1-4] This functional dichotomy is governed by various factors, the most important of which appears to be the stage of DC differentiation/maturation. The potent immunostimulatory properties of mature myeloid DCs are ascribed to high surface expression of major histocompatibility complex (MHC) and costimulatory molecules, in particular CD40, CD80, CD86, and more recently, OX40 ligand (L). The capacity to secrete bioactive IL-12 p70 heterodimer is also crucial for T cell activation/differentiation. Adoptive transfer of mature, donor-derived DCs accelerates alloantigen (Ag)-specific graft rejection. By contrast, immature donor DCs, deficient in costimulators, and resembling DCs freshly isolated from nonlymphoid tissues, can inhibit alloAg-specific T cell responses and prolong graft survival, sometimes indefinitely. The unstable or inconsistent nature of this effect may, at least in part, reflect the eventual in vivo maturation of the donor-derived DCs following their systemic administration, a process likely to be influenced by the nature and extent of immunosuppressive therapy. Genetic engineering of DCs offers potential to enhance and optimize the tolerogenicity of these cells, while minimizing systemic effects of immunosuppressive transgene products. Early results show that genetically engineered donor or recipient-derived DCs can prolong organ allograft survival. The challenge now is to overcome existing limitations to maximize the therapeutic efficacy of this approach to promoting tolerance induction (Table 1).

DCs and Tolerance Induction

DC tolerogenicity, acknowledged 12 to 15 years ago in the context of intrathymic self-tolerance,[5,6] is now well recognized. Tolerance induced following intrathymic inoculation of alloAg appears to be dependent on thymic DCs,[7] and indeed, bone marrow–derived host DCs pulsed with donor allopeptide and injected intrathymically can induce organ or pancreatic islet transplant tolerance in antilymphocyte serum-conditioned hosts.[8,9] The presentation of peripherally derived Ag by DCs within secondary lymphoid tissue is effective not only for T cell priming but also for the induction of T cell tolerance to self-Ag expressed exclusively by peripheral (extralymphoid) tissues.[10,11] Cross-presentation of Ag, including alloAg, by DCs may be markedly enhanced by the uptake of apoptotic bodies derived from peripheral tissues. Immature DCs that have captured apoptotic bodies are potentially tolerogenic.[11]

The capacity of DCs to down-regulate immune reactivity has been demonstrated by their ability to suppress T cell responses in models of T cell ontogeny, allo-, tumor-, and autoimmunity (reviewed in Ref. 12). Various agents, including ultraviolet B radiation, the cytokines IL-10 and transforming growth factor (TGF)β1, immunosuppressive drugs (corticosteroids, cyclosporine, mycophenolate mofetil, and deoxyspergualin), vitamin D3, prostaglandin (PG) E2, the chimeric fusion protein cytotoxic T lymphocyte Ag (CTLA)4Ig (that blocks the B7-CD28 costimulatory pathway), and anti-CD40L mAb, can inhibit DC maturation and/or enhance their tolerogenic potential in vitro or in vivo.

Table 1 | **Dendritic Cells (DCs), Genetic Engineering, and Transplant Tolerance**

DCs initiate and regulate immune responses
DCs of donor or recipient origin can promote tolerance induction by various mechanisms
Transgenic expression of immunosuppressive molecules (IL-10, CTLA4Ig, TGFβ, FasL) confers tolerogenic properties on DCs in vitro
Genetically engineered DCs can prolong murine organ or pancreatic islet allograft survival
Several limitations need to be overcome to optimize their tolerogenicity
"Novel" genes (e.g., galectin 1, HLA-G, Serrate–1) and strategies to inhibit DC maturation (e.g., NFKB ODN*) may enhance therapeutic efficacy

ODNs = oligodeoxynucleotides.

Mechanisms by which DCs Regulate Immune Reactivity

DCs appear able to regulate immune reactivity by various mechanisms. Further details are available in recent reviews.[2,13] Many groups are now pursuing the role of DCs in immune deviation (i.e., skewing of T cells to the Th2 phenotype), in immune regulation (i.e., induction of T regulatory [reg] cells, such as Tr1 cells that make IL-10), and in bona fide tolerance, that is, deletion or anergy.

Induction of T Cell Anergy or Apoptosis

DCs whose allostimulatory function is impaired, either as a result of their incomplete maturation, selective blockade of costimulatory molecules, the influence of specific cytokines (e.g., IL-10 or TGFβ), or genetic engineering (to express mammalian or viral [v]IL-10, CTLA4Ig, or FasL [CD95L]), can induce alloAg-specific T cell hyporesponsiveness (anergy) or apoptosis in vitro, and suppress immune reactivity (reviewed in Ref. 14).

Our work and others' have shown that expression of the death-inducing ligand FasL[15,16] may render DCs capable of subverting T cell responses by promoting activation-induced cell death. Blockade of the B7/CD28 pathway by CTLA4Ig significantly increases myeloid DC-induced apoptosis of alloactivated T cells. This appears to be mediated, at least in part, via the Fas pathway.

Selective Activation of Th2 Cells (Immune Deviation)

There are numerous examples of DC-inducing immune deviation. Thus, Ag-specific suppression of cell-mediated immunity, achieved by intravenous (i.v.) administration of Ag-pulsed Langerhans cells or splenic DCs, has been attributed to selective activation of Th2 cells.[17] DCs grown in the presence of PGE_2 and unable to secrete IL-12 p70 promote the development of Th2 cells.[18] IL-10 skews the Th1/Th2 balance to Th2 cells by blocking IL-12 p70 synthesis by DCs.[19] Notably, in transplantation, prolongation of skin graft survival by portal venous (p.v.) immunization with donor strain DCs is associated with polarization of host T cells to produce IL-4 and IL-10 upon restimulation in vitro.[20] Conceivably, the capacity of DC subsets to skew selectively toward different types of Th cell response in vivo may reflect differential sensitivity of Th subsets to apoptosis mediated by DC subsets.

Induction of T Regulatory (T reg) Cells

Evidence has emerged that DCs can promote the induction of T reg cells. Thus, an IL-10-secreting, nonproliferating human $CD4^+$ T cell population with regulatory properties can be induced by repeated stimulation with allogeneic immature DCs.[21] We have reported recently that a novel, murine liver-derived B cell-like DC ($CD205^+B220^+CD19^-$) can induce allogeneic T cells with a cytokine profile resembling Tr1 cells.[22] Moreover, immature, Ag-pulsed autologous myeloid DCs induce Ag-specific IL-10-producing $CD8^+$ T cells in humans.[23]

The Tolerogenic Potential of Genetically Engineered DCs

An attractive conceptual approach to enhancement/stabilization of the tolerogenic potential of DCs is their genetic modification to express "immunosuppressive" molecules (identified below in parentheses) that

DENDRITIC CELLS

Inducers and regulators of alloimmunity

1. inhibit or block cell surface costimulatory molecule expression (e.g., IL-10, TGFβ, or CTLA4–Ig) and skew the alloAg-specific T cell response toward Th2 predominance or
2. promote the deletion (apoptosis) of alloAg-specific T cell clones (e.g., FasL or tumor necrosis factor [TNF]-related apoptosis-inducing ligand [TRAIL]).

Other more novel transgenes with potential to inhibit T cell responses if expressed by DCs, include soluble(s) CD40, single chain anti-CTLA4 Ab, HLA-G, galectin-1, CD31, and the *Serrate 1* (*Jagged 1*) ligand of the *Notch 1* signaling pathway.[24] In principle, ectopic expression of the above molecule(s) by donor-derived DC trafficking to the precise microenvironment where Ag presentation and allospecific T cell responses are initiated minimizes systemic delivery of the immunosuppressive gene product, with diminution of potential undesired side effects.

Over the past several years, a variety of delivery vehicles have been employed to demonstrate that genetic engineering of DCs to express immunosuppressive molecules can markedly inhibit their T cell allostimulatory capacity. Thus, retroviral delivery of vIL-10 or TGFβ1 to replicating DC progenitors, or adenoviral (Ad) transduction of DCs to express CTLA4Ig, promotes alloAg-specific T cell hyporesponsiveness in vitro. These gene-modified DCs traffic in vivo to T cell areas of normal allogeneic secondary lymphoid tissue, where in at least one report, they have been shown to express the transgene product. It appears that these DCs survive in enhanced numbers and for longer periods than control gene–transduced DCs. In the case of DCs expressing specific antiinflammatory cytokines (vIL-10 or TGFβ1) or CTLA4Ig, their administration is associated with skewing of ex vivo antidonor responses toward Th2 predominance.

There is also evidence that particle-mediated delivery of cDNA encoding FasL into a mature DC line confers the capacity to induce Ag-specific immunosuppression. Moreover, transfer of cDNA encoding FasL to murine DCs by lipofection renders the DCs capable of inducing apoptosis in Fas^+ T cell targets and of promoting alloAg-specific hyporesponsiveness in vivo. Collectively, these studies underscore the potential of genetically engineered DCs for regulation of alloimmune responses, especially since the in vivo effects have been observed in recipients given no immunosuppressive therapy.

Early Progress in Transplant Models

These findings have prompted early evaluation of genetically engineered DCs in experimental organ transplantation (Table 2). Moderate success has been achieved in murine models. The best results with single gene transfer to DCs have been obtained by Min et al.[25] who found that multiple intraperitoneal injections of FasL-transduced DCs markedly extended murine vascularized heart allograft survival. Electroporation of cDNA encoding CTLA4Ig into a mouse DC line renders the cells capable of prolonging pancreatic islet allograft survival.[26] Coates et al. showed that in NOD-SCID mice engrafted with human skin and reconstituted with allogeneic human PBMC mixed with AdIL-10-transduced DCs autologous to the skin donor, the grafts exhibit reduced evidence of rejection.[27] To date, there are no reports of donor-specific tolerance being achieved across MHC barriers using genetically modified donor-derived DCs alone. Studies in large animals have been limited to preliminary work on AdIL-10-transduced DCs in sheep.[28]

What Limits Success?

This limited efficacy is most likely due to a number of nonexclusive factors (summarized in Table 3) that constitute important issues for further investigation. These include

1. *un*sustained immaturity of the potentially tolerogenic DCs;
2. inappropriate/inadequate numbers of injected DCs;
3. suboptimal route/frequency of cell injection;
4. administration of a suboptimal/inadequate DC subset (myeloid versus "lymphoid-related DCs");
5. absence of persistent (long-term) transgene expression;
6. failure to (virally) transduce the entire population of administered DCs (especially with retrovirus);
7. adverse (immunostimulatory) effects of (Ad) vectors; and

Table 2 | **Evidence That Genetically Engineered Myeloid Dendritic Cells (DCs) Can Prolong Allograft Survival**

Origin of DC	Gene Transduced (method)	Regimen	Graft	Reference
Donor	FasL (lipofection)	Six postoperative i.p. injections of 5.10^6 DC	Heart	Min et al. (2000)
Donor	CTLA4Ig[a] (electroporation)	Twenty-five million DC i.v. on days 0 and 6	Pancreatic islets	O'Rourke et al. (2000)
Donor	TGFβ and IL-10[b] (adenovirus)	Portal venous injection of 5.10^6 DC 36 h before transplant	Kidney	Gorczynski et al. (2000)
Donor	CTLA4-Ig[c] (adenovirus)	Two $\times 10^6$ DC i.v. 7 days before transplant	Heart	Bonham et al. (2001)
Donor	L-10 (adenovirus)	i.p. injection of 10^6 DC autologous to the skin donor + allogeneic human PBMC	Human skin in NOD-SCID mice	Coates et al. (2001)
Donor	TGFβ (retrovirus)	Two $\times 10^6$ DC i.v. 7 days before transplant	Heart	Takayama et al. (2001)
Recipient	Donor MHC class I (adenovirus)	One month before transplant + anti-CD4 mAb	Heart	Billing et al. (2001)

i.p. = intraperitoneal. i.v. = intravenous
a. DC cell line
b. 1:1 mixture of DC transduced with either Ad IL-10 or Ad TGFβ
c. adenoviral transduction of DCs treated with NFκB anti-sense oligodeoxynucleotides

8. failure of manipulation of donor DCs to inhibit the indirect pathway of allorecognition.

Interestingly, we have shown that blocking of costimulation in vitro (by CTLA4Ig) or in vivo (with anti-CD40L mAb) markedly enhances the ability of DCs to promote apoptotic death of alloactivated T cells and to induce long-term allograft survival. It remains to be seen whether prolongation of graft survival by donor-derived DCs overexpressing CTLA4Ig is mediated by enhanced apoptosis of alloreactive T cells, a mechanism that may be crucial to the induction of transplantation tolerance.[29]

The Question of DC Subsets

Virtually all work reported to date on genetic engineering of DCs to promote their tolerogenicity has focused on murine myeloid DCs. Distinct DC subsets, that exhibit tolerogenic properties, both in the mouse and the human, offer potential for gene delivery to enhance these activities. In mice, $CD8\alpha^+$ DCs (that now appear to be derived from lymphoid or myeloid progenitors)[30] and $CD8\alpha^-$ DCs (myeloid DCs) can differentially regulate Th cell responses.[31,32] It has been suggested that $CD8\alpha^+$ DCs are involved in the maintenance of peripheral tolerance.[33] They express high levels of MHC class II/self peptide in T cell areas of lymph nodes, lack the myeloid marker CD11b, and express high levels of the multilectin receptor CD205. They kill $CD4^+$ T cells via Fas (CD95)-mediated apoptosis[16] and exhibit tolerogenic activity in vivo. Based on these properties, speculation has arisen that $CD8\alpha^+$ DCs and classic myeloid ($CD8\alpha^-$) DCs are specialized for induction of tolerance and immunity, respectively.[34] Indeed, tolerogenic properties of $CD8\alpha^+$ DCs can be detected when they represent as small as 3% of the total DC population ($CD8\alpha^+$ plus $CD8\alpha^-$).[35] Our recent findings indicate that, in a heart transplant model, i.v. infusion of highly purified immature $CD8\alpha^-$ or $CD8\alpha^+$ of donor origin, 7 days before transplant, prolongs MHC-mismatched allograft survival, but that only mature $CD8\alpha^+$ DCs retain their ability.[36] The mechanistic basis of the capacity of mature $CD8\alpha^+$ DCs to retain tolerogenic activity in this model has not been established. In mice, adoptive transfer of Ag (eg KLH)-pulsed $CD8\alpha^+$ DCs or $CD8\alpha^-$ DCs induces a predominant Th1 or Th2 response, respectively.[31,32] Thus, a complex and rather confusing picture of the comparative function of DC subsets in vivo has emerged.[37] Despite these difficulties, comparative functional studies of DC subsets genetically en-

Table 3 | **FACTORS THAT MAY LIMIT THE THERAPEUTIC EFFICACY OF GENETICALLY ENGINEERED DC* IN TRANSPLANT MODELS**

Unsustained immaturity
Inappropriate/inadequate numbers of "tolerogenic" DCs
Suboptimal route/frequency of administration
Administration of an inadequate DC subset (myeloid versus "lymphoid-related" DCs)
Absence of persistent, long-term transgene expression
Influence of the transgene product on DC migration
Failure to transduce the entire DC population
Immunostimulatory potential of Ad vectors
Failure to inhibit the indirect pathway of allorecognition

*DC of donor origin.

gineered to express genes such as vIL-10 or CTLA4Ig, should prove of considerable interest.

Two main populations of human DCs, so-called DC1 (monocytoid) and DC2 (plasmacytoid), have been described. Plasmacytoid T cells, that are immediate precursors of DC2 and develop into DCs after stimulation with IL-3 and CD40L, are located in T cell–dependent areas of secondary lymphoid tissues, or in blood.[38] Human DC1s induce Th1 cell differentiation, and human DC2s, that make high levels of interferon (IFNα) after microbial challenge, selectively induce Th2 cells.[39,40] Human DC2s can be mobilized selectively in vivo with granulocyte colony-stimulating factor. Their potential for therapy of human allogeneic stem cell or organ transplantation has been recognized.[41] Whether their genetic modification to express gene(s) encoding immunosuppressive/tolerogenic molecules could further enhance this potential remains to be determined.

Other Novel Strategies

We have reported[42] that retroviral transduction of mouse bone marrow–derived myeloid DCs to produce vIL-10 substantially impairs their allostimulatory activity. Nevertheless, retroviral transduction of DCs is comparatively inefficient. Production of a "sorting" retroviral vector, encoding both vIL-10 and enhanced green fluorescent protein, permits selection (purification by flow cytometry) of positive transfectants with substantially reduced T cell stimulatory activity (potentially tolerogenic, vIL-10-secreting DCs).[43] The limitation of the strategy is that flow sorting diminishes the yield of cells available for in vivo testing.

In a recent report, significantly prolonged organ (renal) allograft survival was seen in mice given a mixture (1:1) of donor-derived myeloid DCs transduced with Ad constructs encoding either TGFβ or IL-10, via the p.v. route, 36 h before transplantation. This effect was correlated with inhibition of cytotoxic T cell induction and enhancement of Th2 responses.[44]

Use of recombinant (r) Ad vectors (to deliver CTLA4Ig, TGFβ1, or FasL) has achieved the highest efficiency of gene transfer, but the capacity of the virus to promote DC maturation may limit the therapeutic efficacy of rAd-transduced DCs. An important novel finding is that NFκB-specific "decoy" oligodeoxynucleotides (ODNs) stably inhibit DC maturation. They also markedly suppress DC maturation induced by various stimuli, including rAd vectors. Importantly, immunosuppressive transgene expression is not inhibited. Of even greater significance is the recent observation that a single, pretransplant injection of NFκB ODN-treated donor myeloid DCs, rAd-transduced to express CTLA4Ig, can markedly prolong vascularized heart allograft survival. Forty percent of the animals exhibit long-term graft survival (>100 days) and demonstrate donor-specific skin graft tolerance.[45]

Cross-presentation of alloAg by recipient DCs may play an important role in allotolerance. Billing et al. reported recently that administration of trans-

genic CBK (H-2^k+K^b as a transgene) DCs to CBA (H-2^k) recipient mice, 27 days before transplant, in conjunction with 2 doses of anti-CD4 mAb on days –28 and –27, induces long-term survival in 75% of fully allogeneic C57BL/10 (H-2^b) heart graft recipients. This group has further demonstrated that infusion of immature, recipient-derived DCs transduced with an Ad vector encoding donor type MHC class I gene (H-$2K^b$) 1 month before transplant, together with anti-CD4 mAb, prolongs the survival of fully allogeneic cardiac grafts.[43] These findings suggest that genetically modified autologous DCs may provide a useful vehicle for the delivery of donor alloAg in the induction of transplant tolerance.

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References

1. Steinman RM. The dendritic cell system and its role in immunogenicity. **Annu Rev Immunol 1991;9:271**.
2. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. **Nature 1998;392(6673):245**.
3. Thomson AW, Lu L, Murase N, Demetris AJ, Rao AS, Starzl TE. Microchimerism, dendritic cell progenitors and transplantation tolerance. **Stem Cells 1995;13(6):622**.
4. Thomson AW, Lu L. Strategies to promote tolerance induction using dendritic cells. In: Thomson AW, editor. **PROVIDE BOOK TITLEDordrecht: Kluwer Academic; 2001. p. 215**.
5. Jenkinson EJ, Jhittay P, Kingston R, Owen JJ. Studies of the role of the thymic environment in the induction of tolerance to MHC antigens. **Transplantation 1985;39(3):331**.
6. Matzinger P, Guerder S. Does T-cell tolerance require a dedicated antigen-presenting cell? **Nature 1989;338(6210):74**.
7. Oluwole SF, Jin MX, Chowdhury NC, Engelstad K, Ohajekwe OA, James T. Induction of peripheral tolerance by intrathymic inoculation of soluble alloantigens: evidence for the role of host antigen-presenting cells and suppressor cell mechanism. **Cell Immunol 1995;162:33**.
8. Garrovillo M, Ali A, Oluwole SF. Indirect allorecognition in acquired thymic tolerance: induction of donor-specific tolerance to rat cardiac allografts by allopeptide-pulsed host dendritic cells. **Transplantation 1999;68:1827**.
9. Ali A, Garrovillo M, Jin M-X, Hardy MA, Oluwole SF. Major histocompatibility complex class I peptide-pulsed host dendritic cells induce antigen-specific acquired thymic tolerance to islet cells. **Transplantation 2000;69:221**.
10. Adler AJ, Marsh DW, Yochum GS, et al. $CD4^+$ T cell tolerance to parenchymal self-antigens requires presentation by bone marrow-derived antigen-presenting cells. **J Exp Med 1998;187(10):1555**.
11. Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. **J Exp Med 2000;191(3):411**.
12. Lu L, Thomson AW. Dendritic cell tolerogenicity and prospects for dendritiecell-based therapy of allograft rejection and autoimmune disease. In: Lotze MT, Thomson AW, editors. Dendritic cells: biology and clinical applications. 2d ed. **San Diego: Academic; 2001. p. 587**.
13. Thomson AW, Lu L. Dendritic cells as regulators of immune reactivity: implications for transplantation. **Transplantation 1999;68:1**.
14. Lu L, Khoury SJ, Sayegh MH, Thomson AW. Dendritic cell tolerogenicity and prospects for cell-based therapy of allograft rejection and autoimmunity. In: Lotze MT, Thomson AW, editors. Dendritic cells. Biology and clinical applications. **San Diego: Academic Press; 1999. p. 487**.
15. Lu L, Qian S, Hershberger PA, Rudert WA, Lynch DH, Thomson AW. Fas ligand (CD95L) and B7 expression on dendritic cells provide counter-regulatory signals for T cell survival and proliferation. **J Immunol 1997;158(12):5676**.
16. Süss G, Shortman K. A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. **J Exp Med 1996;183(4):1789**.
17. Morikawa Y, Tohya K, Ishida H, Matsuura N, Kakudo K. Different migration patterns of antigen-presenting cells correlate with Th1/Th2-type responses in mice. **Immunology 1995;85(4):575**.
18. Kalinski P, Hilkens CM, Snijders A, Snijdewint FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. **J Immunol 1997;159(1):28**.
19. De Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M. Effect of interleukin-10 on dendritic cell maturation and function. **Eur J Immunol 1997;27(5):1229**.
20. Gorczynski RM, Cohen Z, Fu XM, Hua Z, Sun Y, Chen Z. Interleukin-13, in combination with anti-interleukin-12, increases graft prolongation after portal venous immunization with cultured allogeneic bone marrow-derived dendritic cells. **Transplantation 1996;62(11):1592**.
21. Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. **J Exp Med 2001;193(11):1285**.
22. Lu L, Bonham CA, Liang X, et al. Liver-derived DEC205(+)B220(+)CD19(-) dendritic cells regulate T cell responses. **J Immunol 2001;166(12):7042**.
23. 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(2):233**.
24. Hackstein H, Morelli AE, Thomson AW. Designer dendritic cells for tolerance induction - guided not misguided missiles. **Trends Immunol 2001;22:437**.
25. Min W-P, Gorczynski R, Huang X-Y, et al. Dendritic cells genetically engineered to express Fas Ligand induce donor-specific hyporesponsiveness and prolong allograft survival. **J Immunol 2000;164:161**.
26. O'Rourke RW, Kang SM, Lower JA, et al. A dendritic cell line genetically modified to express CTLA4-Ig as a means to prolong islet allograft survival. **Transplantation 2000;69(7):1440**.
27. Coates PT, Krishnan R, Kireta S, Johnston J, Russ GR. Human myeloid dendritic cells transduced with an adenoviral interleukin-10 gene construct inhibit human skin graft rejection in humanized NOD-scid chimeric mice. **Gene Ther 2001;8(16):1224**.
28. Coates T, Krishnan R, Chew G, et al. Dendritic cell Th2 cytokine gene therapy in sheep. In: Cortesini R, Suciu-Foca Cortesini N, editors. Transplantation proceedings. vol. 33. **Rome, Italy: Elsevier; 2001. p. 180**.
29. Wells AD, Li XC, Li Y, et al. Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. **Nature Med 1999;5(11):1303**.
30. Traver D, Akashi K, Manz M, et al. Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. **Science 2000;290(5499):2152**.
31. Pulendran B, Smith JL, Caspary G, et al. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. **Proc Natl Acad Sci U S A 1999;96(3):1036**.

32. Maldonado-Lopez R, De Smedt T, Michel P, et al. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. **J Exp Med 1999;189(3):587**.
33. Shortman K, Caux C. Dendritic cell development: multiple pathways to nature's adjuvants. **Stem Cells 1997;15(6):409**.
34. Fazekas de St Groth B. The evolution of self-tolerance: a new cell arises to meet the challenge of self-reactivity. **Immunol Today 1998;19(10):448**.
35. Grohmann U, Fallarino F, Silla S, et al. CD40 ligation ablates the tolerogenic potential of lymphoid dendritic cells. **J Immunol 2001;166(1):277**.
36. O'Connell PJ, Li W, Takayama T, Logar AJ, Qian S, Thomson AW. CD8a+ (lymphoid-related) and CD8a- (myeloid) dendritic cells differentially regulate vascularized organ allograft survival. In: Cortesini R, Suciu-Foca Cortesini N, editors. XVIII International Congress of the Transplantation Society. vol. 33. **Rome, Italy: Elsevier; 2000. p. 94**.
37. Liu YJ, Kanzler H, Soumelis V, Gilliet M. Dendritic cell lineage, plasticity and cross-regulation. **Nat Immunol 2001;2(7):585**.
38. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. **J Exp Med 1997;185(6):1101**.
39. Rissoan MC, Soumelis V, Kadowaki N, et al. Reciprocal control of T helper cell and dendritic cell differentiation. **Science 1999;283(5405):1183**.
40. Siegal FP, Kadowaki N, Shodell M, et al. The nature of the principal type 1 interferon-producing cells in human blood. **Science 1999;284(5421):1835**.
41. Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. **Blood 2000;95(8):2484**.
42. Takayama T, Nishioka Y, Lu I, 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**.
43. Billing J, Fry JW, Wheeler PR, Morris PJ, Wood KJ. Donor-specific dendritic cells modulate cardiac allograft rejection. **British Transplantation Society, 4th Annual Congress, 2001: Abstract L28**.
44. Gorczynski RM, Bransom J, Cattral M, et al. Synergy in induction of increased renal allograft survival after portal vein infusion of dendritic cells transduced to express TGFbeta and IL-10, along with administration of CHO cells expressing the regulatory molecule OX-2. **Clin Immunol 2000;95(3):182**.
45. Bonham AC, Peng L, Chen Z, et al. Immunomodulation by dendritic cells transfected with CTLA4-Ig is enhanced by NF-kB decoy oligodeoxyribonucleotides. **Am J Transplant 2001;1(1):394**.