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REVIEW

Dendritic cells, T cell tolerance and therapy of adverse immune reactions

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SUMMARY

Dendritic cells (DC) are uniquely able to either induce immune responses or to maintain the state of self tolerance. Recent evidence has shown that the ability of DC to induce tolerance in the steady state is critical to the prevention of the autoimmune response. Likewise, DC have been shown to induce several type of regulatory T cells including Th2, Tr1, Ts and NKT cells, depending on the maturation state of the DC and the local microenvironment. DC have been shown to have therapeutic value in models of allograft rejection and autoimmunity, although no success has been reported in allergy. Several strategies, including the use of specific DC subsets, genetic modification of DC and the use of DC at various maturation stages for the treatment of allograft rejection and autoimmune disease are discussed. The challenge for the future use of DC therapy in human disease is to identify the appropriate DC for the proposed therapy; a task made more daunting by the extreme plasticity of DC that has recently been demonstrated. However, the progress achieved to date suggests that these are not insurmountable obstacles and that DC may become a useful therapeutic tool in transplantation and autoimmune disease.

Keywords dendritic cells tolerance T cells transplantation autoimmunity allergy

INTRODUCTION

Dendritic cells (DC) are now regarded as critical inducers and regulators of adaptive immune responses [1–3]. This contemporary view of DC, once regarded solely as highly effective instigators of T cell-mediated immunity, reflects insights gained over the past several years into how these highly specialized antigen (Ag)-presenting cells (APC) can regulate T cell activation and function, both *in vitro* and *in vivo*. Their tolerogenic properties, first recognized within the thymus [4,5], are now well-documented in relation to peripheral immune responses in various model systems. In the healthy steady state, the principal role of DC in the periphery may be to maintain tolerance to self Ag, encountered as apoptotic cells – the result of normal tissue turnover, and that they convey to T cell areas of secondary lymphoid tissue [6]. Evidence has also accumulated, both from animal and human experiments, that DC, particularly those at an immature stage, can suppress peripheral T cell responses to foreign Ag, and induce Ag-specific tolerance [7,8]. Thus, in addition to being viewed in the traditional sense, as natural adjuvants and as potential targets for inhibition of adverse immune reactions, the regulatory functions of DC have generated considerable interest in the potential of these cells for therapy of such diverse immune responses as allograft rejection, autoimmune inflammation and allergic hypersensitivity.

Several DC subsets with distinct phenotypic and functional characteristics have been identified in mice and humans. These have been the subject of recent comprehensive reviews [9,10]. Here we review the properties of murine and human DC that have been associated with T cell tolerance *in vitro* and *in vivo*, insights into DC tolerogenicity that have been gained from disease models, and the current status of various strategies using DC for therapy of immune-mediated inflammatory disorders.

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DENDRITIC CELLS: CRITICAL REGULATORS OF IMMUNITY

Dendritic leukocytes are rare (<1% of circulating blood mononuclear cells) heterogeneous, uniquely well-equipped bone marrow

(BM)-derived Ag-presenting and processing cells present in blood and lymph and resident within virtually all tissues. DC in the periphery constantly sample the local microenvironment by uptake of self and exogenous Ag via macropinocytosis/endocytosis. Until recently, it was thought that there was a comparatively low level of migration of DC to draining secondary lymphoid tissue via afferent lymphatics, in the normal healthy steady state. Recent experiments on the kinetics of DC migration have revealed however, that, depending on their location, DC traffic rapidly to and through secondary lymphoid organs [11,12]. Using BrdU labelling, Kamath *et al*. [11] showed that DC in the spleen or mesenteric lymph node had average lifespans of between 2 and 9 days. Interestingly, the cutaneous lymph node exhibited a slower accumulation of BrdU-labelled DC, but this was related to a longer lifespan in the skin since, once the DC reached the lymph node, they had a short lifespan of 2–3 days. These data are compatible with earlier findings demonstrating a rapid turnover of DC from the airway [13] and intestinal epithelium [14]. Conveyance and presentation of epithelial cell-derived Ag captured as apoptotic cells/bodies, – the result of normal tissue turnover, may promote/maintain tolerance to self Ag, and the recent demonstration of a gastric self Ag acquired and presented by DC in the gastric lymph node supports this hypothesis [15]. Induction of $CD8^+$ T cell tolerance in mice is likely to involve the cross presentation of Ag, predominantly by $CD8\alpha^+$ DC [16,17]. The DC that, in the steady state, convey self Ags are immature and a recent study has demonstrated that these cells are capable of inducing T cell deletion in the periphery [18]. In these experiments, Ag was targeted to splenic DC by coupling to an anti-CD205 monoclonal antibody (mAb). Ag-specific T cells expanded for the first three days and then disappeared. More importantly, mice treated in this way were tolerant to a subsequent challenge with Ag in the presence of adjuvant [18]. Other mechanisms for the induction of tolerance by DC in the steady state include the targeting of Ag via the inhibitory FcγRIIb [19], and the presence of inhibitory receptors, such as immunoglobulin (Ig)-like transcript (ILT)3 and ILT4 [20].

In response to infection and microbial products (activation of DC via Toll-like receptors (TLR)/inflammation), or following organ transplantation, the rate and magnitude of DC migration from the periphery to T cell areas of lymph nodes or spleen is greatly enhanced. This translocation is associated with DC maturation, whereby Ag uptake/processing ability is down-regulated and surface expression of chemokine receptors (e.g. CCR7) that facilitate their transendothelial homing to secondary lymphoid tissue is enhanced. In addition, expression of major histocompatibility complex (MHC) and costimulatory molecules (CM) essential for T cell activation is increased. Depending on the nature of the infection, DC produce IL-12p70 – a potent T helper-1 (Th1) cell driving cytokine critical for the development of delayed-type hypersensitivity (DTH) responses such as those that underlie pathological processes in allo- and autoimmunity.

MECHANISMS BY WHICH DENDRITIC CELLS REGULATE T CELL REACTIVITY

DC can either initiate T cell activation and proliferation or promote peripheral tolerance through the deletion of autoreactive T cells depending on their state of maturation. DC whose capacity to activate T cells is impaired/modified, either as a result of incomplete maturation, or the influence of specific inhibitory cytokines (e.g. IL-10 or transforming growth factor β; TGFβ), can induce reversible Ag-specific T cell hyporesponsiveness (anergy) or apoptosis *in vitro*, and suppress immune reactivity. Inhibitory effects on T cells are not confined to immature DC. Recently, Munn *et al.* [21] described a human monocyte-derived DC subset ($CD123^+$ [IL-3Rα^+] $CCR6^+$) expressing the tryptophan-catabolizing enzyme indoleamine 2,3 dioxygenase (IDO) that could inhibit T cell proliferation *in vitro*. The IDO-mediated suppressor activity was present in both immature as well as mature $CD123^+$ DC. Although these cells have been identified *in vivo*, their role has yet to be confirmed. Specific subsets of mouse splenic DC are highly effective in mediating IDO-dependent apoptosis of T cells *in vitro* [22]. Of note, it appears that 'reverse' signalling resulting from B7 ligation by CTLA4 on T cells may contribute to tolerogenic effects via interference with tryptophan catabolism [23]. Kawahata *et al.* [24] have shown that, using DC derived from lymphoid tissue of transgenic mice, DC expressing a nuclear autoAg lead to a persistent anergic state of $CD4^+$ autoreactive T cells. The results suggest that peripheral tolerance to nuclear autoAg may be due to continuous presentation of the self-peptide by DC, and that a low level of peptide expression may also be involved in the induction of T cell hyporesponsiveness.

The rediscovery of regulatory/suppressor cells [25] has revealed that DC play an important role in the induction and maintenance of several regulatory T cell populations [20,26–30]. Thus, an IL-10-secreting, nonproliferating human $CD4^+$ T cell population with regulatory properties can be induced *in vitro* by repeated stimulation of naïve T cells with allogeneic immature DC [31]. In mice, a novel, liver-derived, B cell-like DC ($CD205^+B220^+CD19^-$) can induce allogeneic T cells with a cytokine profile resembling Tr1 cells [32]. Moreover, immature Ag-pulsed autologous myeloid DC induce Ag-specific, IL-10-producing $CD8^+$ T cells in humans [33]. Using $CD4^+$ Ag-specific T cell lines, human plasmacytoid DC (pDC) enriched from blood induce reversible T cell anergy *in vitro*. The anergic T cells produce IFNγ and IL-10 upon stimulation. Notably, these pDC can be mobilized selectively into human blood by granulocyte colony-stimulating factor (G-CSF) administration and following activation induce T cells that produce predominantly IL-10 and IL-4 [34]. It has also been shown recently that DC found either in the bronchial or intestinal mucosa induce regulatory T cell populations, and this seems to be an intrinsic property of DC in these specific locations [35–37]. In the gut, DC preferentially induce Th2/Th3 cells that secrete IL-4, IL-10 and TGFβ [35,36] which play an important role in maintaining tolerance to oral Ags. In the respiratory tract, DC produce large amounts of IL-10 following encounter with Ag and induce the production of IL-10-producing Tr1 cells [38]. Thus, depending on their location, DC have the unique capacity to stimulate specific regulatory T cells that protect those sites from potential autoimmunity.

Since many pathogenic immune responses, including allograft rejection and autoimmunity, involve Th1 responses, there has been a great deal of interest in analysing the ability of DC to deviate the immune response away from Th1 and towards Th2. Indeed, DC populations that can preferentially induce either Th1 or Th2 cells have been identified in both mouse and human [39,40] and this has been correlated to the amount of IL-12p70 that is produced by these cells. While it was thought initially that different DC lineages/subsets would stimulate either Th1 or Th2, it is now clear that DC of all lineages can be induced to stimulate either Th1 or Th2, depending on the nature of the stimulating Ag and the local environment (reviewed in [10]).

Thus, Ag-specific suppression of cell-mediated immunity, achieved by intravenous (i.v.) administration of murine Ag-pulsed Langerhans cells or splenic DC, has been attributed to selective activation of Th2 cells [41]. DC grown in the presence of prostaglandin [PG]E_2 and unable to secrete IL-12p70, promote the development of Th2 cells [42]. IL-10 skews the Th1/Th2 balance to Th2 cells by inhibiting CM expression and suppressing IL-12p70 synthesis by DC [43]. 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 DC-mediated apoptosis.

Interestingly, anergizing capacity can be acquired by DC exposed to regulatory T cells. A distinct population of human T reg (T suppressor; Ts) cells, characterized by their $CD8^+$ $CD28^-$ phenotype, suppresses Ag- specific $CD4^+$ Th cell responses by acting on the priming APC that present peptide-MHC class I complexes to which the Ts cells have been previously primed. The suppression of DC function is due to inhibition of the gene transcription regulatory protein nuclear factor (NF)-κB activation and transcription of CM in the DC. Exposure of immature DC to $CD8^+$ $CD28^-$ Ts cells increases expression of the genes encoding Ig-like inhibitory receptors ILT3 and ILT4 that render the DC capable of anergizing $CD4^+$ Th cells [20].

ORGAN TRANSPLANTATION: THE ROLE OF DENDRITIC CELLS IN DIRECT AND INDIRECT ALLORECOGNITION

Rejection of organ allografts has been associated traditionally with the migration of interstitial donor 'passenger' leucocytes to T cell areas of recipient lymphoid tissue. This trafficking of donor-derived DC allows direct presentation of highly immunogenic, donor-derived MHC Ags to recipient naïve T cells. Whilst donor DC are clearly important in direct allorecognition, host DC also play a significant role in graft rejection via the indirect pathway of allorecognition. This occurs when host APC present donor peptides to host T cells in the context of recipient MHC molecules. The relative contributions of direct and indirect allorecognition to murine skin graft rejection have been examined [44]. During acute rejection, <10% of T cells recognized allopeptides presented indirectly. By contrast, the remaining 90% of responding T cells responded to directly presented donor MHC peptides. This predominant role of the direct pathway provides a rational basis for manipulation of donor-derived DC (that are readily accessible in live-organ donation) to prevent acute graft rejection and to promote tolerance induction. Since indirect allorecognition is thought to be of greater significance in the pathogenesis of chronic rejection [45], manipulation of host-derived DC by pulsing with donor MHC class I peptide to promote their tolerogenicity [46,47] may be an equally important approach to therapy of this process that leads commonly to graft destruction. Approaches that have been adopted for treatment of allograft rejection based on utilization/targeting of DC are summarized in Table 1.

DENDRITIC CELLS THERAPY OF ALLOGRAFT REJECTION

Intrathymic injection of alloAg-pulsed DC

Injection of host BM-derived DC pulsed with a donor MHC class I peptide into the thymi of streptozotocin-induced diabetic Wistar-Furth rats, in combination with anti-lymphocyte serum therapy, results in permanent (>200 day) pancreatic islet allograft survival [46]. Recently, similar prolongation of heart allograft survival has been achieved using host BM-derived DC pulsed with immunodominant allopeptide delivered intravenously, thereby circumventing the limitations imposed by intrathymic DC administration [47].

Tolerogenic properties of immature dendritic cells in organ transplantation

Over the past several years, credence has been given to the concept that immature donor DC can promote organ or pancreatic

Table 1. Approaches to therapy of allograft rejection by using/targeting donor or host DC

Source of DC	Approach	Reference
Donor DC		
	Unmodified (*in vitro*-generated or *in vivo*-mobilized)	[48–50]
	Immature DC	[34,120]
	Specific DC subset (? plasmacytoid)	
	Manipulated	
	Immunological (e.g. IL-10; anti-IL-12 mAb; CTLA4Ig)	[121–123]
	Pharmacological (e.g. aspirin; dexamethasone; rapamycin; deoxyspergualin)	[51,54,124–126]
	Genetically engineered (e.g. viral vectors encoding IL-10; TGFβ1; FasL; CTLA4Ig)	[104–106,113,127]
	Antisense (e.g. NF-κB decoy oligodeoxyribonucleotides)	[53,114]
Recipient DC		
	Intrathymic injection	
	Promote central tolerance through administration of DC pulsed with donor MHC class I peptide	[46]
	Pharmacological manipulation	[54]
	Genetic engineering	
	Transgenic expression of donor MHC I	[115]
	Uptake of apoptotic cells	
	May promote induction of T reg cells	[128–130]

Table 2. Therapeutic effect of distinct DC subsets alone in the prolongation of transplant survival and prevention of autoimmune disease

DC subset	Condition	DC source	Therapeutic effect	Reference
Transplantation				
Immature donor myeloid ($CD8\alpha^-$) DC	Cardiac allograft	*In vitro*-generated	Prolonged graft survival	[49]
Immature donor myeloid DC ($GM^{low}DC$)	Cardiac allograft	*In vitro*-generated	Indefinite (>100 days) graft survival	[50]
$CD205^+B220^+CD19$ donor liver-derived DC	Cardiac allograft	*In vitro*-generated	Prolonged graft survival	[32]
Immature or mature 'lymphoid-related' $CD8\alpha^+$ donor DC†	Cardiac allograft	Freshly isolated	Prolonged graft survival	[120]
Autoimmune disease				
Myeloid ($CD8\alpha^-$) DC	Autoimune diabetes (NOD* mouse)	Freshly isolated from pancreatic lymph node	Prevention of diabetes	[77, 78]
Mature myeloid DC	Autoimmune diabetes (NOD mouse)	*In vitro* generated	Prevention of diabetes, induction of Th2 response	[79, 82]
'Semi-mature' DC	EAE† (C57BL/6 mice)	*In vitro* generated-matured with TNF-α	Prevention of EAE, induction of IL-10-producing T cells	[26]
'EAE'-DC	EAE (Lewis rats)	*In vitro* generated from rats with EAE	Prevention of EAE and resistance to subsequent challenge	[88]
Splenic DC	EAMG‡ (Lewis rats)	Freshly isolated, exposed to TGFβ	Prevention of EAMG, reduced levels of specific antibody	[92]

*Non-obese diabetic; †experimental allergic encephalomyelitis; ‡experimental autoimmune myasthenia gravis.

islet allograft survival [48,49], and induce donor-specific tolerance [50] (Table 2). Thus BM-derived DC generated from C57BL10 (B10) mice in low concentration GM-CSF for 8 days, retain an immature phenotype and are resistant to maturational stimuli such as bacterial lipopolysaccharide (LPS), TNF-α or CD40 ligation. When 5×10^5 of these immature donor DC are administered i.v. to fully allogeneic CBA recipients, 7 days before transplant, they demonstrate an impressive ability to prolong cardiac allograft survival indefinitely (>100 days) in the absence of immunosuppressive therapy [50]. Both anti-inflammatory (e.g. salicylates) [51] and immunosuppressive drugs (e.g. corticosteroids) [52] that inhibit DC maturation *in vitro* have been shown to suppress nuclear translocation of NF-κB that is critical for DC maturation. Another means to promote and maintain the immature state is to specifically target the NF-κB cell activation pathway using antisense oligonucleotides. Short oligodeoxynucleotides (ODN) with consensus binding sequences to NF-κB inhibit DC allostimulatory capacity by blocking NF-κB translocation [53]. This in turn, inhibits cell surface CM expression. BM-derived DC from B10 mice treated with NF-κB ODN for up to 36 h and administered to C3H recipients as a single dose (2×10^6) 7 days before organ transplantation, significantly prolong B10 vascularized heart graft survival [53].

A critical role of the maturational status of DC in allograft tolerance has been implicated in a nonhuman primate renal transplant model. Rhesus macaque monkeys were treated with a combination of anti-CD3 immunotoxin (IT) mAb and a 15-day course of deoxyspergualin (DSG), commencing 4 h before transplant [54]. This combination, but not IT alone, was associated with the development of long-term (>3 years) kidney allograft survival, without evidence of chronic graft nephropathy. Split skin grafts from third party donors were rejected promptly, indicating donor specificity of the IT-DSG therapy. Treatment of graft recipients with DSG inhibited nuclear translocation of NF-κB within secondary lymphoid tissue DC and their phenotypic maturation (up-regulation of CD86 and CD83 expression), maintaining these cells in an immature, potentially tolerogenic state. This effect on DC *in situ* was transient however, suggesting that the timing of DC immaturity may be critical in the induction of long-term tolerance. Interestingly, the DSG analogue LF15-0195 can induce donor DC to expand potent donor-specific regulatory $CD4^+$ $CD25^{+/-}$ T reg cells in rat MHC-mismatched heart allograft tolerance [55].

AUTOIMMUNITY: THE ROLE OF DENDRITIC CELLS

In view of the critical role that DC play in the maintenance of central and peripheral tolerance it is perhaps not surprising that abnormalities in DC function have been implicated in several autoimmune diseases [56]. In type-1 insulin-dependent diabetes, several groups have reported abnormalities in DC phenotype and function in both the mouse and human, which may result in the skewing of the response towards pathogenic Th1 cells [57–61]. In addition, non-obese diabetic (NOD) mice, the murine model for type-1 diabetes, exhibit defects in the number of regulatory T cells, including natural killer (NK)T cells, $CD4^+$ $CD25^+$ T cells and Th2 cells [62–64], – defects which may, in the future, be attributed to abnormalities of DC function. In systemic lupus erythematosus (SLE), recent reports have revealed abnormalities in the levels of interferon (IFN)-α production and in plasmacytoid DC that produce this important cytokine [65,66]. In murine SLE models, increased numbers of myeloid DC have been noted [67,68] and in one case, this was correlated with increases in the DC-mobilizing haematopoietic factor fms-like tyrosine 3 kinase ligand (Flt3L) in the BM [67]. Increased numbers of DC have also been observed in the affected joints of rheumatoid arthritis (RA) patients [69,70]. More recently, it was shown that RA synovial fluid contains DC precursors and myeloid DC growth factors, such as

GM-CSF, TNF, stem cell factor, and IL-13, which suggests that the RA joint could potentiate the differentiation of DC that could then further activate the autoimmune response [71].

Studies of DC in multiple sclerosis (MS) patients have revealed higher levels of IFNγ, TNFα and IL-6-secreting DC than in healthy subjects [72] whereas elevated expression of CCR5 (a chemokine receptor for CCL5 by circulating monocytoid DC may contribute to the recruitment of these cells to cerebrospinal fluid in MS [73]. Moreover, secretion of extracellular matrix-degrading metalloproteinases and their inhibitors by blood monocyte-derived DC is imbalanced in MS [74]. DC have also been implicated in Sjögrens syndrome [75] and thyroiditis [76].

DENDRITIC CELLS AS THERAPEUTIC AGENTS IN AUTOIMMUNE DISEASE

Autoimmune (type-1) diabetes

Ten years ago, Clare-Salzler *et al.* [77] demonstrated that local sc injection of DC isolated from draining lymph nodes of the pancreas (but not spleen DC) could protect NOD mice from disease development, and suggested that these DC could suppress autoimmunity by induction of regulatory cells. These observations have been confirmed and extended in more recent studies, in which an immunoregulatory role of CD1α-restricted invariant NKT cells has been attributed to the recruitment of tolerogenic myeloid DC to the pancreatic lymph nodes and inhibition of autoimmune inflammation [78]. Feili-Hariri *et al.* [79] have demonstrated that mature BM-derived DC are more effective in preventing diabetes in NOD mice than immature DC. These results have been confirmed in several other studies [80,81]. We have further demonstrated that the DC-mediated protection is a result of the induction of a regulatory Th2 response [82], and that this was correlated with the DC expressing high levels of CM and producing low levels of IL-12p70 [83]. This approach may have corrected the intrinsic regulatory defect in NOD mice that exhibit a strong Th1 bias in their generalized immune responsiveness [84] and may be applicable in humans with autoimmune diabetes.

Other therapeutic strategies have been shown to exert their effect through action on DC. The selective expression of IL-4 by β islet cells was found to act directly on local DC and alter the relative expression levels of CD80 and CD86, thereby inhibiting the differentiation of diabetogenic cytotoxic T lymphocytes (CTL) [85]. Recently, CD40L blockade in the rat insulin promoter-lymphochoriomeningitis virus model of autoimmune diabetes was found to completely prevent disease and the protection could be transferred by a cell population that had features of both DC and NK cells [86]. Therapeutic effects of DC that have been reported in models of type-1 diabetes and other autoimmune disorders are summarized in Table 2.

Experimental allergic encephalomyelitis (EAE) and multiple sclerosis (MS)

EAE is an animal model closely related to multiple sclerosis (MS). In a series of studies, Xiao *et al.* [87] have shown that tolerance can be induced against acute EAE in Lewis rats by BM-derived DC pulsed with encephalitogenic myelin basic protein (MBP) peptide 68–86 and injected s.c. into healthy rats before immunization with MBP 68–86 plus complete Freund's adjuvant (CFA). This therapeutic effect is associated with immature DC expressing high levels of IL-10 and low levels of IL-12, increased IFN-γ expression, nitric oxide (NO) production, gradually reduced proliferation and apoptosis of $CD4^+$ T cells and/or up-regulation of TGFβ-expressing cells in T cell areas of lymph nodes induced by DC-derived NO [88,89]. The same group has reported that DC-derived NO, that promotes apoptosis in autoreactive T cells, is involved in (intranasal) IL-4-induced suppression of EAE in the same Lewis rat model [90]. In mice, repetitive injection of wild-type DC matured with TNFα ('semimature' DC) and pulsed with autoantigenic peptide can induce peptide-specific, IL-10-producing $CD4^+$ T cells *in vivo* and prevent EAE [26]. Efficient suppression of EAE has also been demonstrated in mice using a minor splenic DC subset ($CD8\alpha^-$ $CD4^+$) capable of binding a disease-ameliorating Ig-chimeric molecule carrying the myelin-oligodendrocyte glycoprotein 35–55 peptide [91]. These findings have been taken as evidence that DC-based immunotherapy could be beneficial at least as a complement to conventional therapy of MS.

Experimental autoimmune myasthenia gravis (EAMG)

DC exposed *in vitro* to TGFβ1 (but not untreated DC) and administered s.c. (single injection only) to Lewis rats (2×10^6/rat) on day 5 after immunization with acetylcholine receptor (AChR) in CFA mediate protection against EAMG, and inhibit production of anti-AChR IgG Abs [92]. This finding indicates that DC-based therapy offers potential for inhibition of Ab-mediated autoimmune diseases.

DC as inducers of autoimmunity

While DC have been used effectively in several models of autoimmune disease as a means to prevent or treat disease, it is important to realize that under some circumstances, DC can induce autoimmunity. This has been shown in models of EAE [93,94], diabetes [95] and autoimmune thyroiditis [96]. The finding is perhaps not surprising in view of the efficiency of these cells as inducers of the immune response. These studies highlight the requirement for a thorough understanding of DC biology and function prior to exploiting these cells as therapeutic agents. In addition, it is important to comprehend the autoimmune response, such that specific DC populations may be used to achieve the desired therapeutic effect.

DENDRITIC CELLS IN ALLERGIC ASTHMA

There is recent evidence that mature pulmonary DC in bronchial lymph nodes of mice exposed to respiratory allergen induce the development of IL-10-producing T reg cells. This process is dependent on T cell costimulation via the inducible costimulator (ICOS)-ICOSL pathway [38]. These T reg cells, production of which is IL-10-dependent, block the development of airway hypersensitivity. Recent studies in patients with allergic asthma have revealed that DC are recruited rapidly to the bronchial mucosa following exposure to allergen [97]. Similar findings have been observed in animal models [98] and recently, a long-lived Ag-bearing DC population was observed in a model of allergic asthma in the mouse [99]. A newly described IL-7-like cytokine, thymic stromal lymphopoietin (TSLP), has been shown to act on DC to induce Th2-stimulating function. This factor may have an important role in allergic hypersensitivity since it is highly expressed in the skin of patients with atopic dermatitis [100]. To date, there have been no reports of the ability of DC subsets to prevent or treat asthma, rather they appear to contribute to the disease and could be future targets of therapeutic modalities

[101,102]. Thus, future therapies of allergic asthma may aim to recover the normal tolerogenic nature of airway DC, possibly using gene therapy approaches, as described below.

THERAPEUTIC POTENTIAL OF GENETICALLY ENGINEERED DENDRITIC CELLS

An attractive conceptual approach to enhancement/stabilization of the tolerogenic potential of DC is their genetic modification to express 'immunosuppressive' molecules that can either (i) inhibit or block cell surface CM expression (e.g. IL-10, TGFβ, or CTL Ag 4 [CTLA4]Ig) and skew the Ag-specific T cell response towards Th2 predominance) or (ii) promote the deletion (apoptosis) of Ag-specific T cell clones (e.g. FasL [CD95L] or TNF-related apoptosis-inducing ligand [TRAIL]) [103]. In principle, ectopic expression of these molecule(s) by DC trafficking to the precise microenvironment in which Ag presentation and Ag-specific T cell responses are initiated, minimizes systemic delivery of the immunosuppressive gene product, and diminishes potential undesired side-effects.

Progress in transplant models

These findings have prompted early evaluation of genetically engineered DC in experimental (murine) organ transplantation (Table 3). The best results following single gene transfer to donor DC have been obtained by Min *et al.* [104] who found that multiple intraperitoneal (i.p.) injections of DC transduced using lipofection to express human FasL 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 [105]. Coates *et al.* [106] showed that NOD-SCID mice engrafted with human skin and reconstituted with allogeneic human PBMC mixed with adenovirus (Ad)IL-10-transduced DC autologous to the skin donor, exhibited reduced evidence of graft rejection. To date, there have been no reports of donor-specific tolerance being achieved across MHC barriers using genetically modified donor-derived DC alone. In one report concerning IL-4-transduced donor DC, pretreatment of heart graft recipients with these cells exacerbated rejection [107]. Studies in large animals have been limited to preliminary work on AdIL-10-transduced DC in sheep [108]. A recent report indicates that rhesus monkey DC genetically modified to over-express TGFβ1 by targeting Ad to DC CD40 inhibit $CD4^+$ and $CD8^+$ T cell responses in an alloAg-specific manner [109].

The limited efficacy of genetically engineered DC observed in transplantation models to date can be ascribed to a number of non-exclusive factors that constitute important issues for further investigation. These include (i) unsustained immaturity of the potentially tolerogenic DC; (ii) inappropriate/inadequate numbers of injected DC; (iii) suboptimal route/frequency of cell injection; (iv) administration of a suboptimal/inadequate DC subset; (v) absence of persistent (long-term) transgene expression; (vi) transduction of only a minor population of administered DC (especially with retrovirus); (vii) adverse (immunostimulatory) effects of (Ad) vectors, and viii) failure of manipulation of donor DC to inhibit the indirect pathway of allorecognition. Interestingly, genetic modification of mouse embryonic stem-cell line-derived DC indicates therapeutic potential for this approach [110].

Innovative strategies

Retroviral transduction of mouse BM-derived myeloid DC to produce viral (v)IL-10 substantially impairs their allostimulatory activity [111], but the transduction strategy is comparatively inefficient. Generation of a 'sorting' retroviral vector, encoding both vIL-10 and enhanced green fluorescent protein, permits selection

Table 3. Evidence that genetically engineered myeloid DC can prolong allograft survival or inhibit autoimmune disease

Origin of DC	Gene transduced (method)	Regimen	Graft/disease	Reference
Transplantation				
Donor	FasL (lipofection)	Six postoperative i.p. injections of $5{\cdot}10^6$ DC	Heart	[104]
Donor	CTLA4Ig* (electroporation)	Twenty-five $\times 10^6$ DC i.v. on day 0 and 6	Pancreatic islets	[105]
Donor	TGFβ and IL-10† (adenovirus)	Portal venous injection of $5{\cdot}10^6$ DC 36 h before transplant	Kidney	[113]
Donor	CTLA4-Ig‡ (adenovirus)	Two $\times 10^6$ DC i.v. 7 days before transplant	Heart	[114]
Donor	IL-10 (adenovirus)	i.p. injection of 10^6 DC autologous to the skin donor + allogeneic human PBMC	Human skin in NOD-SCID mice	[106]
Donor	IL-4 (retrovirus)	Two $\times 10^6$ DC i.v. 7 days before transplant	Heart§	[107]
Donor	TGFβ (retrovirus)	Two $\times 10^6$ DC i.v. 7 days before transplant	Heart	[127]
Recipient	Donor MHC class I (adenovirus)	One month before transplant + anti-CD4 mAb	Heart	[115]
Autoimmune disease				
Autologous	IL-4 (adenovirus)	One $\times 10^6$ DC i.v. to mice with established disease	CIA¶	[116]
Autologous	IL-4 (retrovirus)	Three $\times 10^5$ DC s.c., i.v. or i.p. to mice 15 days after collagen immunization	CIA	[117]
Autologous	FasL (adenovirus)	One $\times 10^6$ DC i.v. to mice with established disease	CIA	[118]
Autologous	IL-4 (adenovirus)	Two $\times 5 \times 10^5$ DC i.v.to NOD mice at 10–11 weeks of age	Autoimmune diabetes	[119]

*DC cell line, †1:1 mixture of DC transduced with either Ad IL-10 or Ad TGFβ, ‡adenoviral transduction of DC treated with NFκB antisense oligodeoxynucleotides, §exacerbation of rejection, ¶CIA, collagen-induced arthritis.

(by flow cytometry) of positive transfectants with even further reduced T cell stimulatory activity (potentially tolerogenic, vIL-10-secreting DC) [112]. Combined use of DC populations transduced to overexpress different immunosuppressive genes offers an alternative approach. Thus, significantly prolonged renal allograft survival can be achieved in mice with a mixture (1:1): of donor-derived myeloid DC transduced with either AdTGFβ or AdIL-10 via the portal venous route, 36 h before transplantation. This effect correlates with inhibition of CTL induction and with enhancement of Th2 responses [113].

The capacity of Ad virus to induce DC maturation limits the therapeutic efficacy of recombinant Ad-transduced DC. An important recent finding is that NFκB-specific 'decoy' ODN stably inhibit DC maturation [53]. Moreover, they also markedly suppress DC maturation induced by various stimuli, including rAd vectors. Significantly, immunosuppressive transgene (CTLA4Ig) expression is not inhibited. Of even greater significance, is the finding that a single, pretransplant injection of NFκB ODN-treated, rAd CTLAIg-transduced donor myeloid DC can markedly prolong vascularized heart allograft survival. Forty percent of the animals exhibited long-term (>100 day) graft survival, and donor-specific skin graft tolerance [114].

As discussed above, cross-presentation of alloAg by recipient DC may play an important role in allotolerance. Billing *et al.* [115] have reported that administration of transgenic CBK ($H2^k + K^b$ as a transgene) DC to CBA ($H2^k$) recipient mice, 27 days before transplant in conjunction with two doses of anti-CD4 mAb on days –28 and –27, induces long-term survival in 75% of fully allogeneic B10 ($H2^b$) heart graft recipients. This group has further demonstrated that infusion of immature, recipient-derived DC transduced with an Ad vector encoding donor-type MHC class I gene ($H2K^b$) one month before transplant, together with anti-CD4 mAb, prolongs the survival of fully allogeneic cardiac grafts [115]. These findings suggest that genetically modified autologous DC may prove useful for delivery of donor alloAg in the induction of transplant tolerance.

Efficacy of genetically engineered dendritic cells in autoimmune disease models

The potential of genetically engineered DC for therapy of chronic systemic autoimmune disease (Table 3) has recently been demonstrated convincingly in a murine model of collagen-induced arthritis (CIA) [116,117]. Thus, a single systemic (i.v.) injection of 10^6 Ad IL-4-transduced BM-derived DC to mice with established CIA reduced the severity of the disease, and disease was completely ameliorated within 1 week in at least 50% of the animals. By contrast, mice injected with AdIL-10 DC showed continuous disease progression. In a separate study, a single injection of retrovirally transduced IL-4 DC also reduced the incidence and severity of CIA and suppressed established Th1 responses and associated humoral responses. These effects were achieved despite only transient persistence of the injected DC in the spleen. IL-4-transduced T cells or fibroblasts failed to alter the course of the disease, indicating that the therapeutic effect was restricted to DC. A recent study demonstrated that DC engineered to express FasL were also effective in the prevention of CIA [118].

We have also shown recently that DC engineered to express IL-4 can prevent autoimmune diabetes in NOD mice with already advanced insulitis. Between 8 and 10 weeks of age, insulitis progresses from a mild peri-insulitis to aggressive intraislet infiltration. At 10–11 weeks of age, unmodified DC are no longer effective, but those that express IL-4 prevent diabetes in a significant number of animals [119]. This protection is long-lasting and is associated with an increase in Th2 cytokines in the pancreas.

CONCLUSIONS

Over the last several years, it has become apparent that DC can function both as initiators of the immune response and as inducers of central and peripheral tolerance. The factors that determine how a given DC functions depends on its state of maturation and the local microenvironment. The use of DC in the treatment of allograft rejection is aimed at using the tolerogenic capacity of the cells to prevent the initiation of an immune response, either by deletion, the induction of anergy, or the stimulation of regulatory T cells. Thus, the goal would be to exploit the ability of DC to maintain tolerance in the steady state to inhibit the response to the 'novel' foreign allo-Ag. Since however, most autoimmune and allergic diseases are associated with an ongoing immune response, it is not sufficient to rely on the steady state tolerogenic function of DC. Rather it is important to utilize the ability of specific DC populations to modulate the immune response, thereby converting it from a pathogenic response to a protective one. This can be achieved via the induction of regulatory T cells, such as Th2, Tr1, suppressor cells and NKT cells. The most effective therapy will be determined by the nature of the pathogenic immune response. The challenge is to identify conditions that allow the generation and use of DC with defined characteristics, a task made more daunting by the highly plastic nature of DC themselves. The use of genetic modification of DC may be fruitful means to achieve this goal.

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REFERENCES

1 Lotze MT, Thomson AW (eds). *Dendritic Cells: Biology and Clinical Applications*, 2nd edn. London: Academic Press, 2001:1–794.
2 Banchereau J, Briere F, Caux C *et al.* Immunobiology of dendritic cells. Annu Rev Immunol 2000; **18**:767–811.
3 Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; **392**:245–52.
4 Jenkinson EJ, Jhittay P, Kingston R *et al.* Studies of the role of the thymic environment in the induction of tolerance to MHC antigens. Transplantation 1985; **39**:331–3.
5 Matzinger P, Guerder S. Does T-cell tolerance require a dedicated antigen-presenting cell? Nature 1989; **338**:74–6.
6 Steinman RM, Turley S, Mellman I *et al.* The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000; **191**:411–6.
7 Thomson AW, Lu L, Murase N *et al.* Microchimerism, dendritic cell progenitors and transplantation tolerance. Stem Cells 1995; **13**:622–39.
8 Pulendran B, Banchereau J, Maraskovsky E *et al.* Modulating the immune response with dendritic cells and their growth factors. Trends Immunol 2001; **22**:41–7.
9 Ardavin C, Martinez del Hoyo G, Martin P *et al.* Origin and differentiation of dendritic cells. Trends Immunol 2001; **22**:691–700.

10 Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol 2002; **2**:151–61.
11 Kamath AT, Henri S, Battye F *et al.* Developmental kinetics and lifespan of dendritic cells in mouse lymphoid organs. Blood 2002; **100**:1734–41.
12 Ruedl C, Koebel P, Bachmann M *et al.* Anatomical origin of dendritic cells determines their life span in peripheral lymph nodes. J Immunol 2000; **165**:4910–6.
13 Holt PG, Haining S, Nelson DJ *et al.* Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. J Immunol 1994; **153**:256–61.
14 Huang FP, Platt N, Wykes M *et al.* A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J Exp Med 2000; **191**:435–44.
15 Scheinecker C, McHugh R, Shevach EM *et al.* Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. J Exp Med 2002; **196**:1079–90.
16 Belz GT, Behrens GM, Smith CM *et al.* The CD8alpha (+) dendritic cell is responsible for inducing peripheral self-tolerance to tissue-associated antigens. J Exp Med 2002; **196**:1099–104.
17 Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol 2001; **19**:47–64.
18 Hawiger D, Inaba K, Dorsett Y *et al.* Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med 2001; **194**:769–79.
19 Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. J Exp Med 2002; **195**:1653–9.
20 Chang CC, Ciubotariu R, Manavalan JS *et al.* Tolerization of dendritic cells by T (S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. Nat Immunol 2002; **3**:237–43.
21 Munn DH, Sharma MD, Lee JR *et al.* Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 2002; **297**:1867–70.
22 Fallarino F, Vacca C, Orabona C *et al.* Functional expression of indoleamine 2,3-dioxygenase by murine CD8 alpha (+) dendritic cells. Int Immunol 2002; **14**:65–8.
23 Grohmann U, Orabona C, Fallarino F *et al.* CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol 2002; **3**:1097–101.
24 Kawahata K, Misaki Y, Yamauchi M *et al.* Peripheral tolerance to a nuclear autoantigen. dendritic cells expressing a nuclear autoantigen lead to persistent anergic state of CD4+ autoreactive T cells after proliferation. J Immunol 2002; **168**:1103–12.
25 Shevach EM. Regulatory T cells in autoimmmunity*. Annu Rev Immunol 2000; **18**:423–49.
26 Menges M, Rossner S, Voigtlander C *et al.* Repetitive injections of dendritic cells matured with tumor necrosis factor alpha induce antigen-specific protection of mice from autoimmunity. J Exp Med 2002; **195**:15–21.
27 Gilliet M, Boonstra A, Paturel C *et al.* The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. J Exp Med 2002; **195**:953–8.
28 Kuwana M, Kaburaki J, Wright TM *et al.* Induction of antigen-specific human CD4 (+) T cell anergy by peripheral blood DC2 precursors. Eur J Immunol 2001; **31**:2547–57.
29 Roncarolo MG, Levings MK, Traversari C. Differentiation of T regulatory cells by immature dendritic cells. J Exp Med 2001; **193**:F5–9.
30 Dhodapkar MV, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8 (+) regulatory T cells in vivo in humans. Blood 2002; **100**:174–7.
31 Jonuleit H, Schmitt E, Schuler G *et al.* 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**:1213–22.
32 Lu L, Bonham CA, Liang X *et al.* Liver-derived DEC205+B220+ CD19- dendritic cells regulate T cell responses. J Immunol 2001; **166**:7042–52.
33 Dhodapkar MV, Steinman RM, Krasovsky J *et al.* Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J Exp Med 2001; **193**:233–8.
34 Arpinati M, Green CL, Heimfeld S *et al.* Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. Blood 2000; **95**:2484–90.
35 Alpan O, Rudomen G, Matzinger P. The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. J Immunol 2001; **166**:4843–52.
36 Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. J Exp Med 1999; **190**:229–39.
37 Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2001; **2**:725–31.
38 Akbari O, Freeman GJ, Meyer EH *et al.* Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 2002; **8**:1024–32.
39 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**:587–92.
40 Rissoan MC, Soumelis V, Kadowaki N *et al.* Reciprocal control of T helper cell and dendritic cell differentiation. Science 1999; **283**:1183–6.
41 Morikawa Y, Tohya K, Ishida H *et al.* Different migration patterns of antigen-presenting cells correlate with Th1/Th2-type responses in mice. Immunology 1995; **85**:575–81.
42 Kalinski P, Hilkens CM, Snijders A *et al.* 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**:28–35.
43 De Smedt T, Van Mechelen M, De Becker G *et al.* Effect of interleukin-10 on dendritic cell maturation and function. Eur J Immunol 1997; **27**:1229–35.
44 Benichou G, Valujskikh A, Heeger PS. Contributions of direct and indirect T cell alloreactivity during allograft rejection in mice. J Immunol 1999; **162**:352–8.
45 Auchincloss H Jr, Lee R, Shea S *et al.* The role of 'indirect' recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. Proc Natl Acad Sci USA 1993; **90**:3373–7.
46 Ali A, Garrovillo M, Jin MX *et al.* Major histocompatibility complex class I peptide-pulsed host dendritic cells induce antigen-specific acquired thymic tolerance to islet cells. Transplantation 2000; **69**:221–6.
47 Garrovillo M, Ali A, Depaz HA *et al.* Induction of transplant tolerance with immunodominant allopeptide-pulsed host lymphoid and myeloid dendritic cells. Am J Transplant 2001; **1**:129–37.
48 Rastellini C, Lu L, Ricordi C *et al.* Granulocyte/macrophage colony-stimulating factor-stimulated hepatic dendritic cell progenitors prolong pancreatic islet allograft survival. Transplantation 1995; **60**:1366–70.
49 Fu F, Li Y, Qian S *et al.* Costimulatory molecule-deficient dendritic cell progenitors (MHC class II^+, $CD80^{dim}$, $CD86^-$) prolong cardiac allograft survival in nonimmunosuppressed recipients. Transplantation 1996; **62**:659–65.
50 Lutz MB, Suri RM, Niimi M *et al.* 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**:1813–22.
51 Hackstein H, Morelli AE, Larregina AT *et al.* Aspirin inhibits in vitro maturation and in vivo immunostimulatory function of murine myeloid dendritic cells. J Immunol 2001; **166**:7053–62.
52 Woltman AM, de Fijter JW, Kamerling SW *et al.* The effect of calcineurin inhibitors and corticosteroids on the differentiation of human dendritic cells. Eur J Immunol 2000; **30**:1807–12.

53 Giannoukakis N, Bonham CA, Qian S *et al.* Prolongation of cardiac allograft survival using dendritic cells treated with NF-*k*B decoy oligodeoxyribonucleotides. Mol Ther 2000; **1**:430–7.
54 Thomas JM, Contreras JL, Jiang XL *et al.* Peritransplant tolerance induction in macaques. early events reflecting the unique synergy between immunotoxin and deoxyspergualin. Transplantation 1999; **68**:1660–73.
55 Chiffoleau E, Beriou G, Dutartre P *et al.* Role for thymic and splenic regulatory CD4+ T cells induced by donor dendritic cells in allograft tolerance by LF15-0195 treatment. J Immunol 2002; **168**:5058–69.
56 Turley SJ. Dendritic cells. inciting and inhibiting autoimmunity. Curr Opin Immunol 2002; **14**:765–70.
57 Poligone B, Weaver DJ Jr, Sen P *et al.* Elevated NF-kappaB activation in nonobese diabetic mouse dendritic cells results in enhanced APC function. J Immunol 2002; **168**:188–96.
58 Steptoe RJ, Ritchie JM, Harrison LC. Increased generation of dendritic cells from myeloid progenitors in autoimmune-prone nonobese diabetic mice. J Immunol 2002; **168**:5032–41.
59 Marleau AM, Singh B. Myeloid dendritic cells in non-obese diabetic mice have elevated costimulatory and T helper-1-inducing abilities. J Autoimmun 2002; **19**:23–35.
60 Prasad SJ, Goodnow CC. Intrinsic in vitro abnormalities in dendritic cell generation caused by non-MHC non-obese diabetic genes. Immunol Cell Biol 2002; **80**:198–206.
61 Liu J, Beller D. Aberrant production of IL-12 by macrophages from several autoimmune-prone mouse strains is characterized by intrinsic and unique patterns of NF-kappa B expression and binding to the IL-12 p40 promoter. J Immunol 2002; **169**:581–6.
62 Baxter AG, Kinder SJ, Hammond KJ *et al.* Association between alphabetaTCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. Diabetes 1997; **46**:572–82.
63 Salomon B, Lenschow DJ, Rhee L *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity 2000; **12**:431–40.
64 Poulton LD, Smyth MJ, Hawke CG *et al.* Cytometric and functional analyses of NK and NKT cell deficiencies in NOD mice. Int Immunol 2001; **13**:887–96.
65 Blanco P, Palucka AK, Gill M *et al.* Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science 2001; **294**:1540–3.
66 Scheinecker C, Zwolfer B, Koller M *et al.* Alterations of dendritic cells in systemic lupus erythematosus: phenotypic and functional deficiencies. Arthritis Rheum 2001; **44**:856–65.
67 Adachi Y, Taketani S, Toki J *et al.* Marked increase in number of dendritic cells in autoimmune-prone (NZW × BXSB) F1 mice with age. Stem Cells 2002; **20**:61–72.
68 Kalled SL, Cutler AH, Burkly LC. Apoptosis and altered dendritic cell homeostasis in lupus nephritis are limited by anti-CD154 treatment. J Immunol 2001; **167**:1740–7.
69 Summers KL, Daniel PB, O'Donnell JL *et al.* Dendritic cells in synovial fluid of chronic inflammatory arthritis lack CD80 surface expression. Clin Exp Immunol 1995; **100**:81–9.
70 Pettit AR, Thomas R. Dendritic cells: the driving force behind autoimmunity in rheumatoid arthritis? Immunol Cell Biol 1999; **77**:420–7.
71 Santiago-Schwarz F, Anand P, Liu S *et al.* Dendritic cells (DCs) in rheumatoid arthritis (RA). progenitor cells and soluble factors contained in RA synovial fluid yield a subset of myeloid DCs that preferentially activate Th1 inflammatory-type responses. J Immunol 2001; **167**:1758–68.
72 Huang YM, Xiao BG, Ozenci V *et al.* Multiple sclerosis is associated with high levels of circulating dendritic cells secreting pro-inflammatory cytokines. J Neuroimmunol 1999; **99**:82–90.
73 Pashenkov M, Teleshova N, Kouwenhoven M *et al.* Elevated expression of CCR5 by myeloid (CD11c+) blood dendritic cells in multiple sclerosis and acute optic neuritis. Clin Exp Immunol 2002; **127**:519–26.
74 Kouwenhoven M, Ozenci V, Tjernlund A *et al.* Monocyte-derived dendritic cells express and secrete matrix-degrading metalloproteinases and their inhibitors and are imbalanced in multiple sclerosis. J Neuroimmunol 2002; **126**:161–71.
75 van Blokland SC, van Helden-Meeuwsen CG, Wierenga-Wolf AF *et al.* Two different types of sialoadenitis in the NOD- and MRL/lpr mouse models for Sjogren's syndrome: a differential role for dendritic cells in the initiation of sialoadenitis? Laboratory Invest 2000; **80**:575–85.
76 Simons PJ, Delemarre FG, Drexhage HA. A functional and phenotypic study on immune accessory cells isolated from the thyroids of Wistar and autoimmune-prone BB-DP rats. J Autoimmun 2000; **15**:417–24.
77 Clare-Salzler MJ, Brooks J, Chai A *et al.* Prevention of diabetes in nonobese diabetic mice by dendritic cell transfer. J Clin Invest 1992; **90**:741–8.
78 Naumov YN, Bahjat KS, Gausling R *et al.* Activation of CD1d-restricted T cells protects NOD mice from developing diabetes by regulating dendritic cell subsets. Proc Natl Acad Sci U S A 2001; **98**:13838–43.
79 Feili-Hariri M, Dong X, Alber SM *et al.* Immunotherapy of NOD mice with bone marrow-derived dendritic cells. Diabetes 1999; **48**:2300–8.
80 Papaccio G, Nicoletti F, Pisanti FA *et al.* Prevention of spontaneous autoimmune diabetes in NOD mice by transferring in vitro antigen-pulsed syngeneic dendritic cells. Endocrinology 2000; **141**:1500–5.
81 Shinomiya M, Fazle Akbar SM, Shinomiya H *et al.* Transfer of dendritic cells (DC) ex vivo stimulated with interferon-gamma (IFN-gamma) down-modulates autoimmune diabetes in non-obese diabetic (NOD) mice. Clin Exp Immunol 1999; **117**:38–43.
82 Feili-Hariri M, Falkner DH, Morel PA. Regulatory Th2 response induced following adoptive transfer of dendritic cells in prediabetic NOD mice. Eur J Immunol 2002; **32**:2021–30.
83 Feili-Hariri M, Morel PA. Phenotypic and functional characteristics of BM-derived DC from NOD and non-diabetes-prone strains. Clin Immunol 2001; **98**:133–42.
84 Koarada S, Wu Y, Ridgway WM. Increased entry into the IFN-gamma effector pathway by CD4+ T cells selected by I-Ag7 on a nonobese diabetic versus C57BL/6 genetic background. J Immunol 2001; **167**:1693–702.
85 King C, Mueller Hoenger R, Malo Cleary M *et al.* Interleukin-4 acts at the locus of the antigen-presenting dendritic cell to counter-regulate cytotoxic CD8+ T-cell responses. Nat Med 2001; **7**:206–14.
86 Homann D, Jahreis A, Wolfe T *et al.* CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. Immunity 2002; **16**:403–15.
87 Xiao BG, Huang YM, Xu LY *et al.* Mechanisms of recovery from experimental allergic encephalomyelitis induced with myelin basic protein peptide 68–86 in Lewis rats: a role for dendritic cells in inducing apoptosis of CD4+ T cells. J Neuroimmunol 1999; **97**:25–36.
88 Xiao BG, Huang YM, Yang JS *et al.* Bone marrow-derived dendritic cells from experimental allergic encephalomyelitis induce immune tolerance to EAE in Lewis rats. Clin Exp Immunol 2001; **125**:300–9.
89 Yang JS, Xu LY, Huang YM *et al.* Adherent dendritic cells expressing high levels of interleukin-10 and low levels of interleukin-12 induce antigen-specific tolerance to experimental autoimmune encephalomyelitis. Immunology 2000; **101**:397–403.
90 Xu L, Huang Y, Yang J *et al.* Dendritic cell-derived nitric oxide is involved in IL-4-induced suppression of experimental allergic encephalomyelitis (EAE) in Lewis rats. Clin Exp Immunol 1999; **118**:115–21.
91 Legge KL, Gregg RK, Maldonado-Lopez R *et al.* On the role of dendritic cells in peripheral T cell tolerance and modulation of autoimmunity. J Exp Med 2002; **196**:217–27.
92 Yarilin D, Duan R, Huang YM *et al.* Dendritic cells exposed in vitro to TGF-beta1 ameliorate experimental autoimmune myasthenia gravis. Clin Exp Immunol 2002; **127**:214–9.

93 Weir CR, Nicolson K, Backstrom BT. Experimental autoimmune encephalomyelitis induction in naive mice by dendritic cells presenting a self-peptide. Immunol Cell Biol 2002; **80**:14–20.

94 Dittel BN, Visintin I, Merchant RM *et al.* Presentation of the self antigen myelin basic protein by dendritic cells leads to experimental autoimmune encephalomyelitis. J Immunol 1999; **163**:32–9.

95 Ludewig B, Odermatt B, Landmann S *et al.* Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. J Exp Med 1998; **188**:1493–501.

96 Knight SC, Farrant J, Chan J *et al.* Induction of autoimmunity with dendritic cells: studies on thyroiditis in mice. Clin Immunol Immunopathol 1988; **48**:277–89.

97 Jahnsen FL, Moloney ED, Hogan T *et al.* Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. Thorax 2001; **56**:823–6.

98 Lambrecht BN, Carro-Muino I, Vermaelen K *et al.* Allergen-induced changes in bone-marrow progenitor and airway dendritic cells in sensitized rats. Am J Respir Cell Mol Biol 1999; **20**:1165–74.

99 Julia V, Hessel EM, Malherbe L *et al.* A restricted subset of dendritic cells captures airborne antigens and remains able to activate specific T cells long after antigen exposure. Immunity 2002; **16**:271–83.

100 Soumelis V, Reche PA, Kanzler H *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002; **3**:673–80.

101 Graffi SJ, Dekan G, Stingl G *et al.* Systemic administration of antigen-pulsed dendritic cells induces experimental allergic asthma in mice upon aerosol antigen rechallenge. Clin Immunol 2002; **103**:176–84.

102 Lambrecht BN. The dendritic cell in allergic airway diseases: a new player to the game. Clin Exp Allergy 2001; **31**:206–18.

103 Lu L, Thomson AW. Genetic engineering of dendritic cells to enhance their tolerogenic potential. Graft 2002; **5**:308–15.

104 Min WP, Gorczynski R, Huang XY *et al.* Dendritic cells genetically engineered to express Fas ligand induce donor-specific hyporesponsiveness and prolong allograft survival. J Immunol 2000; **164**:161–7.

105 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**:1440–6.

106 Coates PT, Krishnan R, Kireta S *et al.* 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**:1224–33.

107 Kaneko K, Wang Z, Kim SH *et al.* Dendritic cells genetically engineered to express IL-4 exhibit enhanced IL-12p70 production in response to CD40 ligation and enhance organ allograft rejection. Gene Ther 2003; **10**:143–52.

108 Coates T, Krishnan R, Chew G *et al.* In: Transplantation Proceedings. Elsevier, Rome, Italy, 2001.

109 Asiedu C, Dong SS, Pereboev A *et al.* Rhesus monocyte-derived dendritic cells modified to over-express TGF-beta1 exhibit potent veto activity. Transplantation 2002; **74**:629–37.

110 Fairchild PJ, Brook FA, Gardner RL *et al.* Directed differentiation of dendritic cells from mouse embryonic stem cells. Curr Biol 2000; **10**:1515–8.

111 Takayama T, Nishioka Y, Lu L *et al.* 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**:1567–74.

112 Takayama T, Tahara H, Thomson AW. Transduction of dendritic cell progenitors with a retroviral vector encoding viral interleukin-10 and enhanced green fluorescent protein allows purification of potentially tolerogenic antigen-presenting cells. Transplantation 1999; **68**:1903–9.

113 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**:182–9.

114 Bonham CA, Peng L, Liang X *et al.* 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**:3382–91.

115 Billing J, Fry JW, Wheeler PR *et al.* Donor-specific dendritic cells modulate cardiac allograft rejection. *Immunology* 2001 104: Abstract L28.

116 Kim SH, Kim S, Evans CH *et al.* Effective treatment of established murine collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express IL-4. J Immunol 2001; **166**:3499–505.

117 Morita Y, Yang J, Gupta R *et al.* Dendritic cells genetically engineered to express IL-4 inhibit murine collagen-induced arthritis. J Clin Invest 2001; **107**:1275–84.

118 Kim SH, Kim S, Oligino TJ *et al.* Effective treatment of established mouse collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express FasL. Mol Ther 2002; **6**:584–90.

119 Feili-Hariri M, Falkner D, Gambotto A *et al.* Dendritic cells transduced to express IL-4 prevent diabetes in nonobese diabetes with advanced insulitis. *Human Gene Therapy* 2003; **14**:13–23.

120 O'Connell PJ, Li W, Wang Z *et al.* Immature and mature CD8alpha+ dendritic cells prolong the survival of vascularized heart allografts. J Immunol 2002; **168**:143–54.

121 Steinbrink K, Wolfl M, Jonuleit H *et al.* Induction of tolerance by IL-10-treated dendritic cells. J Immunol 1997; **159**:4772–80.

122 Gorczynski RM, Cohen Z, Fu XM *et al.* 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**:1592–600.

123 Lu L, Qian S, Hershberger PA *et al.* Fas ligand (CD95L) and B7 expression on dendritic cells provide counter-regulatory signals for T cell survival and proliferation. J Immunol 1997; **158**:5676–84.

124 Hackstein H, Taner T, Zahorchak AF *et al.* Rapamycin inhibits IL-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. *Blood* 2003.

125 Matsue H, Yang C, Matsue K *et al.* Contrasting impacts of immunosuppressive agents (rapamycin, FK506, cyclosporin A, and dexamethasone) on bidirectional dendritic cell–T cell interaction during antigen presentation. J Immunol 2002; **169**:3555–64.

126 Min WP, Zhou D, Ichim TE *et al.* Inhibitory feedback loop between tolerogenic dendritic cells and regulatory T cells in transplant tolerance. J Immunol 2003; **170**:1304–12.

127 Takayama T, Kaneko K, Morelli AE *et al.* Retroviral delivery of transforming growth factor-β1 to myeloid dendritic cells: inhibition of T cell priming ability and influence on allograft survival. Transplantation 2002; **74**:112–9.

128 Bittencourt MC, Perruche S, Contassot E *et al.* Intravenous injection of apoptotic leukocytes enhances bone marrow engraftment across major histocompatibility barriers. Blood 2001; **98**:224–30.

129 Ferguson TA, Herndon J, Elzey B *et al.* Uptake of apoptotic antigen-coupled cells by lymphoid dendritic cells and cross-priming of CD8 (+) T cells produce active immune unresponsiveness. J Immunol 2002; **168**:5589–95.

130 Morelli AE, Larregina AT, Shufesky WJ *et al.* Internalization of circulating apoptotic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. Blood 2003; **101**:611–20.