Dendritic cells and the balance between transplant tolerance and immunity

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Introduction

Dendritic cells (DC) are a distinct lineage of unusually potent accessory cells for immune responses (reviewed in [1–4]) that are derived from CD34+ bone marrow precursors. They possess, or can acquire, the capacity to initiate primary adaptive immune responses in vitro and in vivo. This property is ascribed to expression of high cell surface levels of major histocompatibility complex (MHC) class II and costimulatory molecules. DC are required for the activation of naive T cells [5] and they also activate memory T cells. Evidence also exists however, for DC tolerogenicity. DC appear to play a critical role in central tolerance [6] and evidence has accumulated that, under defined experimental conditions, they can also induce systemic tolerance. DC lacking sufficient cell-surface expression of costimulatory molecules for naive T-cell activation, such as B7-1 (CD80) or B7-2 (CD86), induce alloantigen-specific anergy [7]. Moreover, we have reported that, in mice, costimulatory molecule-deficient DC progenitors can promote the survival both of pancreatic islet [8] and organ allografts [9] in otherwise non-immunosuppressed hosts. There is also evidence that the cytokine microenvironment to which DC are exposed dictates the nature and magnitude of the resultant immune response. Thus, the 'immunosuppressive' cytokines – interleukin-10 (IL-10) and transforming growth factor-β (TGFβ) – inhibit DC maturation. In addition, the chimeric fusion protein cytotoxic T-lymphocyte antigen-4 (CTLA-4)–Ig can markedly inhibit the capacity of DC to activate T cells. In addition, we have shown for the first time that DC induced to produce nitric oxide (NO) or that express Fas/Apo-1 (CD95) ligand (FasL) can inhibit growth and/or induce programmed cell death (apoptosis) in alloactivated T cells. This chapter reviews the immunobiology of DC in relation to organ transplantation with special emphasis on the conditions that may determine their tolerogenicity.

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DC in commonly transplanted organs

Members of the DC lineage are thought to be distributed ubiquitously. Although DC isolated from lymphoid tissue have been studied extensively, we are only beginning to understand the properties of DC resident in commonly transplanted non-lymphoid organs, *ie*, kidney, liver and heart. The migration of DC from cardiac [10] or liver allografts [11] to T-dependent areas of recipient spleens has been described in rodents, but there is little information on the functional properties of these 'chimeric' cells or of the precursors from which they are derived. The best characterized DC isolated from non-lymphoid tissues of normal individuals are MHC class II$^+$ epidermal Langerhans cells (LC) [12]. When freshly-isolated, these cells have a phenotype distinct from that of 'mature' lymphoid tissue DC, and can process protein-containing antigens. They lack critical costimulatory molecules (B7-1 and B7-2) and thus cannot initiate responses in naive T cells. Such cells may have tolerizing potential.

When cultured in granulocyte-macrophage–colony-stimulating factor (GM–CSF), LC can mature into cells resembling lymphoid tissue DC [13–16]: their phagocytic and antigen-processing activities are lost. They up-regulate cell-surface MHC class II antigen expression and become mature, potent antigen-presenting cells (APC) with costimulatory activity for T cells [16]. Recent evidence indicates that the expression of at least some costimulatory molecules (B7-1 and B7-2) on LC is upregulated in conjunction with increased immunostimulatory function of mouse DC from spleen, skin, kidney and heart [17–19]. In various other non-lymphoid tissues, including the respiratory tract, presumptive DC have been identified on the basis of their dendritic profiles and MHC class II expression [20, 21]. These potential migratory cells are located, as in skin, below the epithelial lining, where they are ideally suited to acquire antigens (pathogens) [22]. In most cases, these non-lymphoid DC cannot stimulate primary T-cell responses (particularly, allogeneic mixed lymphocyte reaction (MLR)). Recently, it has been shown that, like fresh LC, DC isolated from mouse kidneys and hearts cannot stimulate allogeneic T cells, unless cultured overnight [19]. These DC from non-lymphoid organs thus resemble immature rather than mature DC. It is possible that the immunostimulatory function reported for all DC isolated from non-lymphoid organs may be a consequence of in vitro maturation in culture.

DC transport antigens rapidly from peripheral tissues, predominantly via lymph, but also apparently via blood, to regional (secondary) lymphoid tissues [23–25]. Upon arrival in lymphoid tissues, DC accumulate in the T cell-dependent regions, where they effectively prime antigen-specific immune responses. This ability to 'home' to sites of T-cell activation is central to the identification of DC as potential immunomodulators of resistance to cancer or microbial infection and also of allograft rejection or autoimmune disorders. Homing affords DC with the opportunity to interact with the largest number of potential alloreactive T cells. In principle, the homing capacity of DC also provides an opportunity to deliver to the site of primary T-cell activation signals that are the result of DC manipulation.

DC and tolerance induction

While extensive studies have resulted in a greater understanding of the critical role of DC in the stimulation of primary immune responses, evidence has accumulated that DC can also play a role in tolerance induction (summarized in table I).

Table I. Observations of the tolerogenic capacity of dendritic cells (DC)

Dendritic cell type	Tolerogenic effect reported	Reference
Thymus DC	Tolerance acquired to host MHC in chimeric thymi	[26]
	Encephalitogen pulsing and adoptive transfer prevents EAE*	[27]
Splenic DC	Reconstitution of DC-depleted thymuses restores ability to delete thymocytes in vivo	[6]
	Large numbers of DC reduce local HVG* reaction in vivo	[28]
	Large numbers of DC/high antigen load inhibit antitumor immunity in vivo	[29]
	CD8$^+$/FasL$^+$ DC induce apoptosis in activated T cells in vitro	[30]
Langerhans cells	Depletion enhances effector phase of CH* in vivo	[31]
Pancreatic lymph-node DC	Transfer reduces incidence of diabetes in NOD mice in vivo	[32]
Costimulator-deficient DC progenitors	Induction of alloantigen-specific unresponsiveness in vitro	[7]
	Adoptive transfer prolongs allograft survival in vivo	[8, 9, 33]

*EAE: experimental allergic encephalomyelitis,*HVG: host-versus-graft; CH: contact hypersensitivity.

DC in central tolerance

The thymus plays a major role in immune homeostasis by the deletion of self-reactive T cells, thus contributing to the maintenance of self-tolerance [34]. A number of cell types present within the thymus including DC [26, 34] may potentially provide signals responsible for the negative selection of developing T cells (reviewed in [35, 36]). However, two lines of evidence suggest that a substantial proportion of this activity is provided by thymic DC. When T cells differentiate in MHC-disparate thymus grafts depleted of bone marrow-derived APC, these T cells show only limited tolerance to the MHC antigens of the grafted thymuses [37, 38]. Matzinger and Guerder [6] outlined the critical role of DC in central tolerance by demonstrating that the tolerogenic properties of the APC-depleted thymi could be restored by reconstitution with purified splenic DC. Similar results have been reported in parents to F_1 bone marrow chimeras and in transgenic mice [39, 40]. These findings indicate that the role of DC in deleting potentially autoreactive developing T cells in the thymus is not dependent upon unique characteristics of the thymic DC, but may be mediated by signals provided by DC from other tissue sites [6]. More recent studies directed at investigating the possible amelioration of autoimmunity or allograft rejection have indicated that the tolerance exhibited following intrathymic inoculation of exogenous antigen is dependent upon thymic DC [41, 42]. Studies of lipopolysaccharide (LPS)-treated mice have suggested that NO production by presumptive thymic DC could represent one mechanism for thymocyte apoptosis and possibly self-tolerance [43]. These findings are consistent with our own recent observations of NO production by DC following treatment with interferon-γ (IFN-γ) or LPS or interaction with allogeneic T cells (see below and [44]).

DC and peripheral (systemic) tolerance

While central tolerance may reflect the unique nature of the intrathymic environment or of T-cell development, more recent studies suggest that tolerance induction mediated by DC is not confined to the intrathymic compartment. Protection from autoimmunity induced by the encephalitogenic autoantigen myelin basic protein (MBP) can be achieved by intravenous injection of thymic DC either pulsed with the immunodominant peptide of MBP or isolated

from thymi inoculated in vivo with MBP [27]. This protection was similar to that invoked by direct intrathymic inoculation of MBP [27]. The ability to induce tolerance is not restricted to thymic DC, as intravenous administration of antigen-pulsed LC or splenic DC has been reported to result in antigen-specific suppression of delayed-type hypersensitivity responses [45, 46], possibly via a mechanism involving selective activation of T_h2-like T-cell subsets [47]. A further illustration of the ability of DC to shape a protective effect against autoimmunity has been provided by the demonstration that DC isolated from pancreatic lymph nodes of non-obese diabetic (NOD) mice and transferred into pre-diabetic NOD mice can limit the expression of diabetes [32], possibly via a mechanism involving the enhanced induction of regulatory T cells.

While the in vivo administration of DC via particular routes, or of certain DC populations, has resulted in the generation of tolerance or certain tolerance-like states, it is likely that the nature of on-going immune responses is dependent upon signals provided by DC. This suggestion is consistent with observations that DC exhibit a dichotomous potential to regulate immune responsiveness. Thus, in vivo experimental studies have indicated that administration of DC loaded with low doses of tumor antigen can enhance anti-tumor immunity, while administration of DC loaded with high doses of tumor antigen or administration of high numbers of tumor antigen-pulsed DC inhibit the development of protective anti-tumor immunity [29]. Likewise, inoculation of high numbers of semi-allogeneic DC reduces the expression of host-vs-graft reactivity in a local in vivo assay [28]. Depletion of LC from epidermal surfaces of mice results in markedly stronger expression of the effector phase of contact hypersensitivity, suggesting these cells may provide both stimulatory and down-modulatory signals [31].

It is possible that DC from specific peripheral tissue sites may possess the capacity to induce forms of tolerance whereby certain aspects of immune responsiveness are selectively inhibited, while others remain intact. Thus, considerable evidence has accumulated that an APC bearing an antigen-specific signal migrates from the eye following administration of soluble antigen into the anterior chamber (AC) and results in the activation of regulatory T cells in the spleen. It is proposed that this phenomenon accounts for aspects of the 'immune-privilege' of the AC (reviewed in [48]) apparently via the selective activation of a T_h2-type response [49]. Recent examination of the APC populations present in the tissues surrounding the AC has provided a strong indication that the APC migrating from the AC is an MHC class II-positive DC [50, 51]. It is likely that specific components of the local tissue microenvironment, for example the cytokine $TGF\beta$ [48], are responsible for imparting DC with such properties, but the roles of various other microenvironmental influences on DC function remain to be clearly defined. Examination of other tissue sites may help to clarify this. There is also evidence that expression of immune unresponsiveness observed following application of antigen to mucosal surfaces (reviewed in [52, 53]) is the result of selective activation of T-cell subsets during primary immune stimulation. Whether DC from these tissue sites provide signals that result in the selective activation of T-cell subsets has not yet been determined, but on-going studies may help to elucidate the immunoregulatory role of these DC.

Experimental manipulations rendering tolerogenicity to DC

Experimental modulation of DC function, for example by exposure to ultraviolet (UV) irradiation, the immunosuppressive chimeric fusion protein CTLA-4–Ig, or to the immunomodulatory effects of IL-10, has also been reported to induce antigen-specific hyporesponsiveness in vitro [54, 55]. The relevance of such in vitro experimental treatments to the in vivo function

of DC in conditions such as the tolerance that may be observed to UV-induced tumors is unclear. Interestingly, evidence is emerging that alteration of the immunostimulatory capacity of DC by the cytokine milieu within tumors [56] may contribute to the tolerance often observed with many tumor types [57]. Therefore, the ability to manipulate the tolerogenic potential of DC is likely both to further our understanding of immune regulation and to lead to potential therapeutic strategies to enhance allograft acceptance and suppress autoimmune disease.

Molecular regulation of the immunologic activity of DC

Studies examining the phenotypic characteristics of DC isolated from mouse thymus and spleen have demonstrated the presence of a major DC subpopulation in these tissues expressing significant levels of CD8 [58], comprised predominantly of the CD8α homodimer [58]. It has been proposed that expression of the CD8 molecule by APC may provide these cells with a 'veto' function, enabling them to inactivate cytotoxic T-cell precursors with which they interact [59, 60]. Expression of this molecule by major subpopulations of thymus and spleen DC may allow these cells to perform a regulatory role in immune responsiveness by both presenting antigen and inactivating T cells [58]. While experimental evidence is yet to be provided confirming the role of CD8$^+$ DC as veto cells, further work has demonstrated that the CD8α^+ population of murine splenic DC express CD95L (FasL) and that these cells are capable of killing activated CD4$^+$ T cells via Fas–FasL interactions [30]. The elucidation of the role of costimulation in T-cell activation (reviewed in [61]) has provided insight into one potential mechanism by which 'tolerogenic APC' may function. Our current understanding of T-cell activation indicates that presentation of antigen to T cells by APC in the absence of essential co-stimulatory signals induces a state of antigen-specific unresponsiveness (anergy) in these T cells [62], and thus also tolerance. Such a proposal leads to speculation that DC in peripheral tissue sites, which exhibit low levels of costimulatory molecule expression in the steady-state [18, 63], may play a potentially important role in peripheral tolerance. Such a scheme would allow peripheral DC populations to perform a dual role, by contributing to the maintenance of peripheral tolerance in the steady-state, and also by acting as effective sentinels following activation signals, provided for example, by microbial products (reviewed in [64]). While experimental studies have yet to provide evidence, MHC class II-negative DC precursors described in the airway epithelium of adult rats [65], which are poor allostimulators [66], and LC precursors described in the epidermis [67] may represent examples of endogenous populations of peripheral DC that possess the potential to function in such a manner.

Recent studies from our laboratory support the notion that DC deficient in costimulatory signals may effect tolerance induction [7–9, 33]. Thus DC progenitors propagated in vitro from mouse bone marrow in the presence of low concentrations of GM–CSF express low levels of costimulatory molecules (B7-1^{low}, B7-2$^-$) and induce alloantigen-specific unresponsiveness (anergy) in T cells in vitro [7]. This T-cell unresponsiveness induced by in vitro-propagated costimulator molecule-deficient DC is not restricted to in vitro assays. Thus, administration of these cells or costimulatory molecule-deficient DC propagated from the liver under similar conditions prolongs cardiac [9] or pancreatic islet [8] allograft survival. Likewise, DC on which the activity of surface B7-1 and B7-2 costimulatory molecules has been blocked by CTLA-4–Ig have been shown to protect animals from the induction of experimental allergic encephalomyelitis (EAE) [68]. It has also been suggested that rhesus monkey bone marrow-derived DC progenitors (MHC class II$^{-/dim}$, CD8$^+$) may exert tolerance promoting activity both in vitro and in vivo [69].

DC and impaired allogeneic T-cell responses (a–i)

Over the past several years, we have established model systems that have allowed us evaluate, both in vitro and in vivo, factors that regulate DC growth, phenotype and function. These data are now summarized in the context of both the interaction of these cells with allogeneic T cells and the potential tolerogenicity of DC.

a) Growth of donor-derived DC from the lymphoid tissue of spontaneously tolerant mouse liver allograft recipients [70]

Livers are accepted 'spontaneously' (without immunosuppression) between many MHC mismatched mouse strains [11]. We hypothesized that DC progenitors might be seeded from the liver graft to recipient lymphoid tissues after orthotopic liver transplantation. To test this hypothesis, bone marrow or spleen cells were isolated from unmodified mouse orthotopic liver transplant (OLTx) recipients (male B10 [H-2^b]→female C3H [H-2^k]) 14 days after transplantation. The cells were cultured for 10 days in GM–CSF, employing the techniques developed in our laboratory to enrich for DC lineage cells. Using flow cytometric and immunocytochemical analyses for donor MHC class I$^+$ and II$^+$ cells, respectively, a minor population of cells of donor phenotype (in addition to recipient cells) was found to propagate in culture. Radioactive polymerase chain reaction (PCR) analysis, using a probe specific to the sex-determining region of male donor Y chromosome, revealed growth of cells of donor origin in bone marrow (especially) and in spleen cell cultures propagated from female recipients of male livers. No signal for donor-derived cells could be detected in 10-day GM–CSF-stimulated cultures of thymocytes from liver-allograft recipients. This suggested that, although the thymus is a site in which chimeric cells are detected after OLTx, it may not be a destination for adequate numbers of donor-derived DC progenitors. We next sought direct evidence that the donor-derived cells propagated from the bone marrow of liver-allograft recipients were of DC lineage. Sorting of donor-positive cells was considered, but the anticipated yield of cells was calculated to be too low for subsequent functional analysis. Instead, 10-day GM–CSF-stimulated bone marrow-derived cells were harvested, DEC 205$^+$ cells were sorted (at least 90% purity by morphological and FACScan® analyses) and then investigated for the presence of donor Y chromosome. PCR analyses demonstrated convincingly that the highly purified DC population comprised approximately 1–10% Y-chromosome positive (donor-derived) DC. Further evidence for the presence of donor-derived DC was obtained by testing the allostimulatory activity of sorted, DEC 205$^+$ GM-CSF+IL-4-stimulated bone marrow-derived cells in primary MLR. Here, IL-4 was used to promote ex vivo DC maturation, since this was necessary for the detection (exhibition) of allostimulatory activity. The purified DEC 205$^+$ population propagated from C3H recipients of B10 allografts strongly stimulated B10 (donor strain) responders, but also stimulated a response in recipient strain (C3H) T cells. The extent of stimulation ($P < 0.01$ compared with negatively sorted cells or syngeneic DC) was similar to that achieved with 'artificial mixtures' containing 1% GM–CSF+IL-4-stimulated donor strain (B10) DC [70].

A key additional observation was our failure to propagate donor-derived cells from the bone marrow of mice rejecting heart allografts. In contrast to liver-allograft recipients, non-immunosuppressed C3H mice reject heart allografts from the same donor strain (B10) within 8 days [11]. PCR analysis for donor Y chromosome was performed on 10-day GM–CSF-stimulated cultures of bone marrow cells harvested from heterotopic cardiac allograft recipients 8 days

after transplant. In contrast to the results obtained from liver-graft recipients, no evidence was obtained for the propagation of donor-derived cells from the bone marrow of animals rejecting their cardiac grafts, despite evidence of small numbers of chimeric cells in freshly-isolated bone marrow. Thus heart-derived cells, in contrast to those detected in fresh bone marrow of liver-allograft recipients, appear not to contain sufficient numbers of GM–CSF-responsive progenitors for growth of donor-derived cells (as opposed to amplification in the case of liver grafts) after 10 days of culture. These findings suggest that donor-derived alloantigen-presenting DC progenitors may play a key role in the induction of organ transplant tolerance.

b) DC deficient in cell surface expression of T-cell costimulatory molecules (B7-1, B7-2) induce T-cell hyporesponsiveness (anergy) in vitro [7]

DC were propagated from B10 mouse bone marrow in GM–CSF or GM–CSF + IL-4, as described [7]. Cells propagated in GM–CSF + IL-4 exhibited potent allostimulatory activity in primary mixed leukocytes cultures. In contrast, the cells stimulated with GM-CSF alone were very weak stimulators. They induced alloantigen-specific hyporesponsiveness in allogeneic T cells (C3H) detected upon restimulation in secondary MLR. This was associated with blockade of IL-2 production. Reactivity to third party stimulators was intact [7]. The hyporesponsiveness induced by the GM–CSF-stimulated, costimulatory molecule-deficient DC progenitors was prevented by incorporation of anti-CD28 monoclonal antibody (mAb) into the primary MLR. It was reversed by the addition of IL-2 to restimulated T cells. These findings show that MHC class II$^+$ B7-2$^-$ DC progenitors can induce alloantigen-specific hyporesponsiveness in T cells in vitro. Under the appropriate conditions, donor-derived, costimulatory molecule-deficient DC progenitors could therefore contribute to the induction of donor-specific unresponsiveness in vivo.

c) Costimulatory molecule-deficient DC prolong the survival of pancreatic islet allografts in non-immunosuppressed recipients in a donor-specific manner [8]

We have tested the in vivo relevance and therapeutic potential of cultured DC progenitors (MHC class II$^+$, B7-2$^{-/dim}$) in a pancreatic islet-allograft model. Two days after rendering groups of B10 (H-2^b) mice diabetic with streptozotocin and 7 days before transplantation with 800 islets (99% pure) under the left renal capsule, the animals received iv either culture medium, 2.5 × 10^6 allogeneic (B10.BR; H-2^k) or syngeneic, 10-day cultured, GM–CSF-stimulated DC progenitors (immature DC) or 10-day cultured, GM–CSF-stimulated mature spleen DC. Blood glucose and body weights were recorded daily. The GM-CSF-stimulated DC progenitors prolonged allograft survival from 11.9 ± 0.9 days (control) to 30.3 ± 17.1 days (P < 0.001).

d) MHC class II$^+$ B7-2$^-$ DC progenitors prolong cardiac allograft survival in non-immunosuppressed hosts [9]

We have also found that GM–CSF-stimulated DC progenitors (B7-2$^-$) capable of inducing T-cell hyporesponsiveness in vitro, can prolong heart-allograft survival. In the B10→C3H model, 2 × 10^6 B7-2$^-$ B10 DC progenitors were injected iv 7 days before transplantation. These cells significantly prolonged heart graft survival from 10.1 ± 2.2 (control) to 23.3 ± 5.5 days compared with syngeneic (C3H; 12.5 ± 0.6 days) or third party cells (BALB/c; 16.8 ± 2.8 days (P < 0.01).

In contrast and as expected, mature (B7-2⁺) DC reduced mean graft survival time. The non-specific effect of third party DC progenitors (although significantly less than that of allogeneic cells) is of interest, but at present not understood; one simple explanation is that the C3H anti-B10 and C3H anti-BALB/c responses are partially cross-reactive so that BALB/c DC progenitors partially tolerize the anti-B10 response. We are pursuing this finding and plan to include third party controls in all such future experiments testing in vivo DC function.

e) DC treated with an immunosuppressive cytokine (TGFβ) exhibit markedly depressed ability to stimulate naive allogeneic T cells

Two prototypic genes that we are presently transferring into DC are those which encode IL-10 and TGFβ. Preliminary studies were performed to evaluate the likely effects of TGFβ production by gene-engineered DC on their allostimulatory capacity in vitro. Mature (GM–CSF + IL-4-propagated) mouse (C57BL/10) bone marrow-derived DC exposed to (h)TGFβ1 (0.1 ng/ml) for a period of 18 h exhibited a markedly reduced ability to stimulate a primary allogeneic MLR when compared to untreated controls (fig 1).

f) Blockade of costimulatory B7 molecule expression on DC using the chimeric fusion protein CTLA-4–Ig suppresses T-cell allostimulatory activity in a dose-dependent manner

The interaction of CD28 on T cells with CD28 counter-receptors on APC provides critical costimulatory signals that are required for T-cell activation. We have demonstrated that mouse DC deficient in CD28 counter-receptors (DEC 205⁺, B7-1ˡᵒʷ[CD80ˡᵒʷ], B7-2⁻[CD86⁻]) are able to induce T-cell hyporesponsiveness in vitro and, when adoptively transferred into normal mice, can extend the survival of pancreatic islet allografts and cardiac allografts. Blockade of
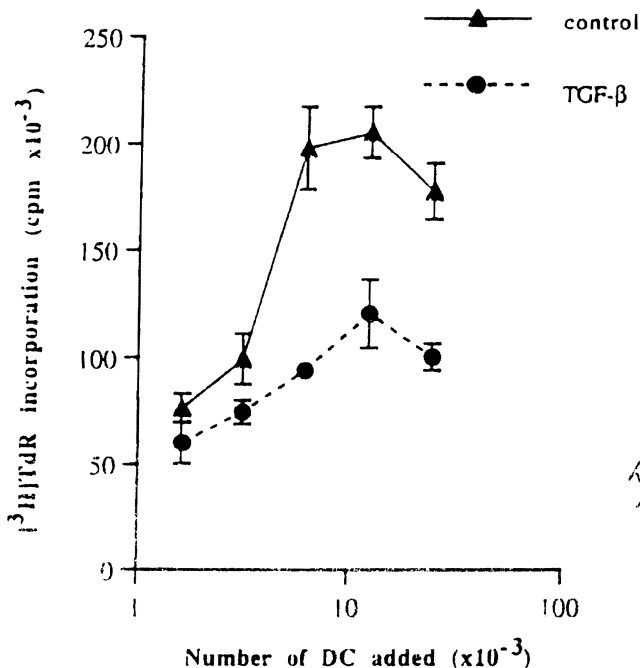

Fig 1. Allostimulatory activity of mouse DC exposed to TGFβ. DC were propagated from bone marrow (C57BL/10) in the presence of GM–CSF + IL-4, harvested (d 5) and washed (2x). Following overnight culture in RPMI/10% FCS + (rm)GM-CSF (4 ng/mL) in the presence or absence of (rh)TGFβ1 (0.1 ng/ml) DC were washed (2x). Three-day MLR cultures were established using 2×10^5 allogeneic (B10.BR) nylon wool-passaged spleen cells and graded numbers of γ-irradiated DC. Data presented are mean [³H]TdR incorporations of triplicate cultures.

CD28 counter-receptors with the chimeric fusion protein mCTLA-4–Ig inhibits the in vitro allostimulatory of bone marrow-derived GM–CSF + IL-4-propagated murine DC. Addition of CTLA-4–Ig to primary one-way MLR cultures (fig 2A) resulted in marked dose-dependent reduction of the proliferation of allogeneic T cells observed in this system. Pre-incubation of DC with CTLA-4–Ig (at the submaximal dose of 100 ng/ml) also markedly reduced the ability of DC to stimulate naive allogeneic T cells (data not shown).

g) Highly purified DC can be induced by IFNγ or LPS to synthesize nitric oxide (NO) synthase (NOS); NO production suppresses allogeneic T-cell proliferation in MLR and induces apoptosis both in activated T cells and DC themselves [44]

NO is an important effector molecule involved in immune regulation and host defense. We have recently found that highly purified DEC 205$^+$ MHC class II^{bright} B7-2$^+$ DC propagated from normal mouse bone marrow in response to GM–CSF + IL-4 can be induced to produce NO by IFNγ or LPS. NO production was inhibited by the NOS inhibitor, N^G-monomethyl-L-arginine (NMMA). Similar amounts of NO_2^- were detected in supernatants of IFNγ (±LPS)-stimulated B7-2$^-$ DC. Nitrite also accumulated in mixed leukocyte-culture supernatants as the result of coculture of DC with purified naive allogeneic T cells. Suboptimal T-cell proliferation observed at high relative concentrations of DC correlated with increased NO production and was mitigated by NMMA. Induction of mRNA for an inducible (i) NOS in DC was confirmed by Northern blotting, whereas intracellular iNOS was visualized by 2-color flow cytometry and by both immunofluorescent and immunogold labeling in a subpopulation of IFNγ- and LPS-stimulated cells. Both endogenous NO production and exposure of unstimulated DC to the NO donor S-nitroso-N-acetylpenicillamine (SNAP) resulted in DC apoptosis. Thus, although DC function initially as the most potent APC for T-cell activation, DC induced to
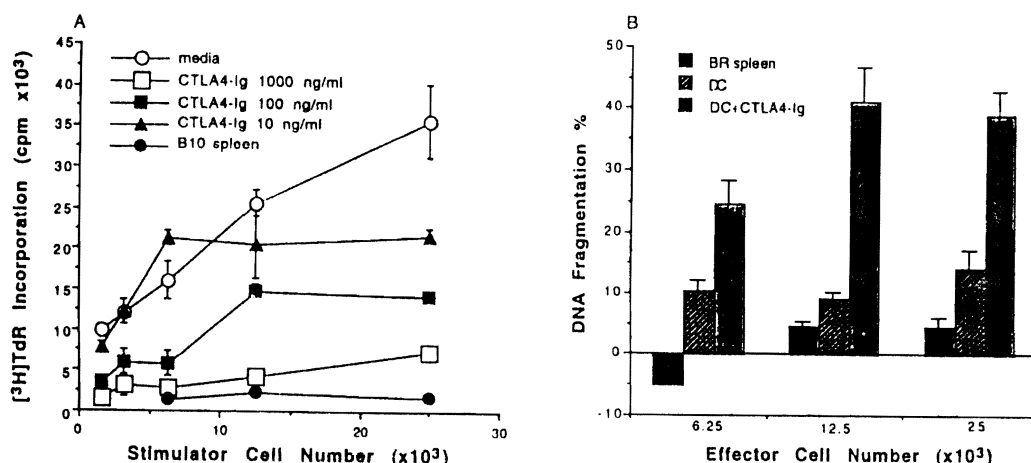

Fig 2. Blockade of the B7–CD28 pathway augments apoptosis induced in allogeneic T cells by DC. MLR cultures were established with graded concentrations of bone marrow-derived DC (B10.BR) and allogeneic (C57BL/10) splenic T cells (2 × 10^5). A. Blockade of B7–CD28 interactions with 1000 ng/ml CTLA-4–Ig was effective in providing almost complete inhibition of T-cell proliferation. B. Blockade of B7–CD28 interactions by treatment of DC with 1000 ng/mL CTLA-4–Ig also resulted in a marked increased in DNA fragmentation of allogeneic T cells in MLR cultures, compared with untreated DC.

synthesize NOS by IFNγ may inhibit (allogeneic) T-cell proliferation: NO may suppress lymphocyte proliferation and also induce apoptosis of the most potent source of alloantigenic stimulation.

h) Sorted DC propagated from mouse bone marrow express Fas (CD95) ligand and can induce apoptosis in alloactivated T cells

During primary activation, mature T cells change from an activation-induced cell death (AICD)-resistant to an AICD-sensitive phenotype. The complete molecular basis for this transition remains to be determined, but CD95 (Fas/Apo-1) and CD95L (Fas ligand; FasL) appear to play an important role in the homeostatic regulation of T-cell responses. We have recently determined that certain DC populations can induce T-cell apoptosis through CD95 ligation and that costimulatory molecule expression on DC affects apoptosis of T cells induced by these APC. Highly purified DEC 205$^+$ MHC class II^{bright} B7-2$^+$ DC propagated from normal mouse bone marrow in culture medium containing GM–CSF+IL-4 express significant cell-surface levels of CD95L, as shown by immunocytochemical staining and FACS analysis using Fas–Fc fusion protein (provided by David H Lynch PhD, Immunex, Seattle, WA, USA) and reverse transcriptase-PCR.

i) CTLA-4–Ig–treated 'immature' DC induce higher levels of apoptosis in allogeneic T cells than fully differentiated DC

Bone marrow-derived DC were tested for their capacity to induce apoptosis in Con A- or alloantigen-activated T cells by DNA fragmentation and in situ TdT-catalyzed DNA nick-end labeling (TUNEL) assay. Inclusion of murine CTLA-4–Ig (a molecule that blocks the B7–CD28 costimulatory pathway) into cultures of DC and activated T cells resulted in an increased level of apoptosis in the allostimulated T cells both in short- (4 h) and long-term (18 h) cultures (fig 2B). A role for CD95L is suggested by the observation that DC propagated from CD95L-defective B6-*gld* mice do not induce significant levels of apoptosis in cultures of alloactivated T cells. These findings suggest that B7/CD28 and CD95/CD95L interactions may play a key role in regulating the outcome of allogeneic DC–T-cell interaction.

Conclusion

These observations provide evidence that DC whose allostimulatory function is impeded either by incomplete phenotypic maturation, selective blockade of costimulatory molecules (eg, using CTLA-4–Ig) or the influence of specific cytokine-gene products (*ie*, IL-10, TGFβ) exhibit activity consistent with the induction of tolerance rather than an immunogenic DC function. In addition, the expression by DC of key molecules associated with inhibition of T-cell growth or induction T-cell apoptosis (*ie*, NO, FasL) suggests that these important APC have potential to impose restraint on immune reactivity.

Acknowledgments

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