2001

Dendritic cells in transplantation - Friend or foe?

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Dendritic Cells in Transplantation— Friend or Foe?

Review

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Introduction

Transplantation research has provided many insights into fundamental aspects of immunology, including the biology of dendritic cells (DCs). The critical tools of transplantation research—antibodies to major histocompatibility complex (MHC) class I and II products, the mixed leukocyte reaction (MLR), and rodent experimental models of skin and organ transplantation—were vital to the initial characterization of DCs as distinct leukocytes, specialized to initiate graft rejection (reviewed in Steinman, 1991). It was proposed that bone marrow–derived DCs in the allograft were the foes responsible for immunogenicity. An oversimplified concept arose that DC elimination, something that is very difficult to achieve, would lead to graft acceptance. In fact, the situation is far more complicated. DCs in both the graft donor and the recipient can act as the foe, stimulating rejection by what are termed the direct and indirect pathways, respectively. However, there is also emerging evidence that DCs can act as a friend, promoting graft acceptance. More broadly, the studies of DCs in transplantation are valuable for understanding their role in immunity to other antigens and, recently, in peripheral tolerance. This review addresses some potential roles of DCs in transplantation in the light of current knowledge concerning DC biology, immunity to alloantigens, and peripheral T cell tolerance.

Some Features of DCs

DCs were first distinguished from other white blood cells on the basis of their distinctive cell shape and an absence of critical lymphocyte and phagocyte properties. When these criteria were used to enrich DCs from different tissue sources, a high level of antigen-presenting MHC products, especially MHC class II ($>10^6$ molecules/cell when measured), was always noted. The capacity of T cells to respond to MHC products on DCs was tested in the MLR. The DCs proved to be remarkably potent, at least 100 times more active than MHC class II–bearing macrophages and B cells. Indeed, the MLR

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had to be renamed from mixed lymphocyte to mixed leukocyte reaction because MHC class II–bearing B cells from mouse spleen or from human blood were weak or inactive in stimulating the MLR. Purified CD4+ and CD8+ T cells each could be stimulated directly by the DCs, and this stimulation occurred in discrete cell aggregates in which the T cells began to grow while in contact with the DCs. Once activated T cells were produced, the T blasts responded vigorously to other allogeneic cells, such as B cells and macrophages. In other words, antigen presentation on either MHC class I or II in the MLR was occurring in two phases: in the "afferent" phase, DCs stimulated the growth and differentiation of CD4+ and CD8+ alloreactive T cells, while, in the "efferent" phase, the activated T cells responded to other types of antigen-presenting cells (APC) in an allogeneic MHC-specific manner. The same two stages in cell-mediated immunity were also observed in responses to nominal antigens, particularly in the induction of the primary antibody response (reviewed in Steinman, 1991).

Strong staining for MHC class II and an absence of B cell or macrophage markers were used to search for DCs in situ. In tissue sections from rats and humans, MHC class II–positive DCs were identified in the interstitial spaces of all organs examined except the brain parenchyma. In lymphoid tissues, DCs are abundant in the T cell areas of spleen and lymph nodes and the medulla of the thymus. The same criteria were used to identify DCs in human blood and in afferent lymph from many species, but DCs were absent in efferent lymph. When isolated DCs were reinfused into rodents, the DCs migrated to the T cell areas of the draining lymphoid organ. After antigen administration by intramuscular or intravenous routes, the DCs in the lymph and spleen were the main cell type able to present antigen to T cells. The concept arose that DCs could pick up antigens in the periphery and migrate to the T cell areas (in spleen via the blood, in lymph nodes via the lymph), where they could either initiate an immune response to the antigens that the DCs were presenting or simply die (Figure 1). As we will consider below, death is followed by re-presentation of proteins in the dying DCs by resident DC in the lymph node. In transplant models, DCs derived from transplanted allogeneic tissues and carrying donor MHC antigens could be identified in the peripheral lymphoid tissues of graft recipients. Further, many studies with nominal antigens showed that injections of antigen-charged DCs initiated T cell–mediated immunity, including protective antimicrobial and antitumor immunity. When tested, the T cell response was restricted to antigens presented by the MHC products of the injected DCs and not to the recipient's antigen-presenting cells, indicating that DCs could directly stimulate recipient T cells. In sum, the distribution and migration of DCs in vivo (Figure 1) correlated closely with their capacity to mediate the afferent phase of cell-mediated immunity (reviewed in Steinman, 1991).

DCs are continuously produced in the bone marrow, undergoing considerable turnover in most tissues except the epidermis. Immature DCs and their precursors
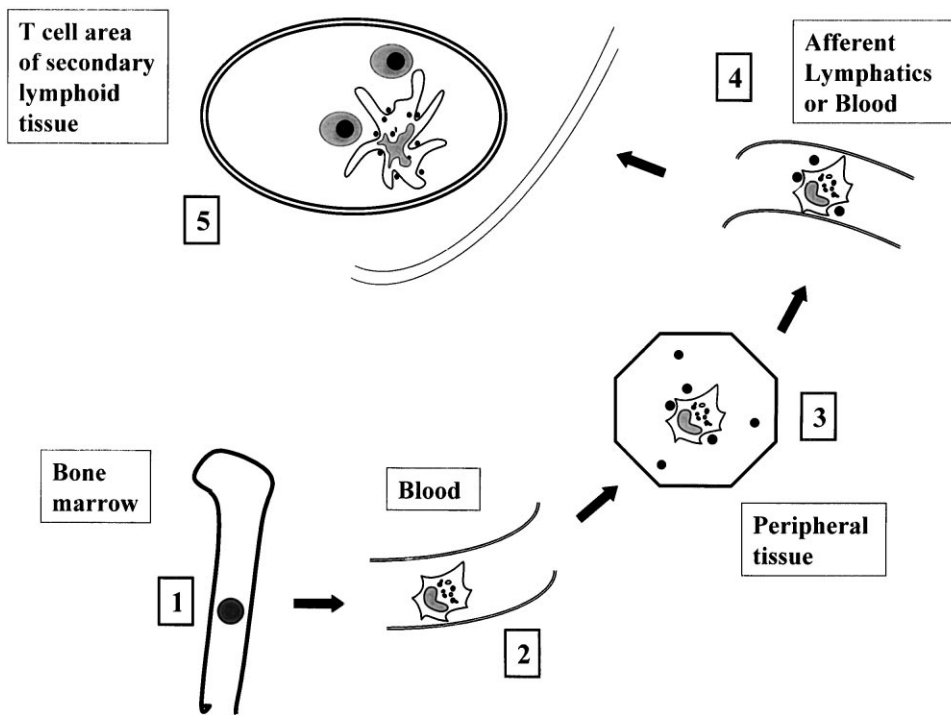
Figure 1. Distribution and Migration of Dendritic Cells

(1) Proliferating DC progenitors in the bone marrow release precursor and immature forms of DC into the blood.
(2) Precursors to DC in the blood migrate to peripheral tissues.
(3) Immature DC capture pathogens and self-antigens from the tissue.
(4) Antigen-loaded DC enter afferent lymphatics or the blood and thereby migrate to secondary lymphoid tissue; this process may be accompanied by maturation upon receipt of appropriate stimuli.
(5) Tissue DC present MHC-peptide (black circles) complexes to antigen-specific T cells in the secondary lymphoid organs.

are always trafficking through tissues in the steady state, in the absence of infection or inflammation, and then dying in the lymphoid organs. In the airways, for example, DCs have a half-life of <2 days (Holt et al., 1994). When afferent lymphatics have been cannulated in the limbs of sheep and humans, a flux of several thousand DCs per hour is noted, as is also the case with other lymphatics in rodents. Recent studies of the turnover of DCs in lymphoid tissues reveal half-lives of 1.5–3 days (Kamath et al., 2000)—even faster than the earliest measurements on the life span of these cells. Beyond the bone marrow, several possibly distinct sources of DCs are found in the blood (Table 1). However, the continued production and traffic of DCs in the steady state are not really analogous to granulocytes, which are constantly

being produced just in case infection should arise. Rather, DCs in the steady state may have a second role—transporting antigens from the periphery for the purpose of inducing and/or maintaining peripheral tolerance in the draining lymph node (Kurts et al., 1997a; Huang et al., 2000). This will be discussed in more detail below.

The mechanisms underlying the immunogenicity of DCs are beginning to be unravelled. The DCs in blood and tissues prove to be immature, not fully differentiated to act as potent stimulators of immunity. The first example was the Langerhans cell in the epidermis (Schuler and Steinman, 1985). Immature DCs can capture soluble and particulate antigens, especially by receptor-mediated pathways. Their MHC class II products lie primarily

Table 1. Bone Marrow–Derived Dendritic Cell Precursors in the Blood

Precursors	Stimuli for DC Development
Monocytes (CD14$^+$, CD11c$^+$)	GM-CSF and IL-4 followed by maturation stimuli (TNF, IL-1, CD40L, LPS, CpG oligos, necrosis) reverse migration across endothelia, possibly lymphatics particle uptake during inflammation
Plasmacytoid cells (CD14$^-$, CD11c$^-$)	enveloped viruses CD40L and IL-3 CpG oligos
Immature DCs and Langerhans cells (CD14$^-$, CD11c$^+$)	inflammatory cytokines infection CpG oligos

within MHC class II–positive endocytic vacuoles that also contain HLA-DM molecules, which facilitate the binding of high-affinity peptides. During maturation, MHC peptide complexes begin to form within the MHC class II– and HLA-DM-positive compartments, followed by transport in nonlysosomal vesicles to the cell surface in large amounts (Pierre et al., 1997). Several costimulatory molecules are also expressed, with especially high levels of CD86. The MHC-peptide complexes are found in clusters at the DC surface together with CD86 (Turley et al., 2000). It is postulated that these high levels of antigen-presenting and costimulatory molecules, in a clustered distribution, initiate the formation of the immunologic synapse, bringing together essential elements, like the T cell receptor (TCR) and CD28, that are required for T cell activation. Furthermore, mature DCs cease many of their endocytic activities, most likely accounting for the longevity of MHC-peptide complexes at the cell surface. Maturing DCs change in many other ways, including the production of cytokines like IL-12 and a reshaping of their chemokine receptors, the latter contributing to their migration to the T cell areas of lymphoid tissue (Cyster, 1999; Sallusto and Lanzavecchia, 1999). Multidrug transporters, by pumping cysteinyl leukotrienes, are newly recognized intermediaries for DC maturation and migration in vivo (Robbiani et al., 2000).

Maturation stimuli for DC development comprise inflammatory cytokines (TNFα, IL-1), microbial products (LPS, CpG deoxyoligonucleotides), and CD40L on activated T cells, platelets, and mast cells. Many of these maturation stimuli act on receptors that trigger NF-κB activation via the TNF receptor–associated factor TRAF 6. Receptors for maturation include IL-1R, TNF-R family members, and Toll receptors or TLRs. However, the act of transplantation itself appears to trigger the maturation and migration of DCs (Larsen et al., 1990), although the responsible stimuli are not yet known. CD40 is an intriguing player, since, in CD40L knockout mice, DCs are unable to migrate during contact allergy, another powerful T cell response in which DCs also mature and migrate to draining lymph nodes. In both transplantation (Barker and Billingham, 1968) and contact allergy (Frey and Wenk, 1957), the severing of afferent lymphatics reduces sensitization in response to skin grafts and allergens, suggesting that DC traffic is critical for immunization. Studies in corneal transplantation lend further support to these concepts, in that the likelihood of corneal allograft rejection was observed to correlate with the DC content of the transplanted tissue (Niederkorn, 1999). In addition, TNF mRNA was induced, even in syngeneic grafts, due to the trauma of surgery, providing a maturation signal for local DCs (King et al., 2000).

In summary, DCs are centrally involved in the initiation of T cell–dependent immune responses, such as transplant rejection. The roles of donor and recipient DCs and their contributions to sensitization and to the induction of tolerance in the two pathways of allorecognition are discussed separately below.

Contributions of Direct and Indirect Pathways of Anti-MHC Allorecognition to Transplant Rejection
The Direct Pathway
MHC alloantigens can be recognized by T cells via two distinct pathways, and DCs are likely to be central to both. The recognition events that lead to the vigor of responses observed when MHC-incompatible cells are cocultured are referred to as the "direct" pathway of allorecognition. This involves the ligation of T cell receptors on alloreactive T cells by foreign MHC molecules, intact, on the surface of allogeneic cells. It was noted several decades ago that underlying this strength of proliferative response was a uniquely high frequency of T cells with direct allospecificity. Evidence from a variety of sources, including a recent structural analysis of an alloreactive TCR (Daniel et al., 1998), indicates that this mode of allorecognition results from cross-reactivity by T cells specific for a self-MHC molecule, "A," with peptide "x" on an allogeneic MHC molecule, "B," with peptide "y." At face value, the recognition of allogeneic MHC breaks the rules of thymic positive selection for self-MHC restriction. However, the structural similarity between the TCR contact surfaces of many MHC alleles allows a substantial fraction of direct alloresponses to be accommodated within the framework of positive selection. In responder–stimulator combinations in which such structural similarities apply, allorecognition can be regarded as mimicking self-MHC restriction and as directed against novel peptides that are bound by the allogeneic but not the self-MHC molecules due to differences in the peptide binding groove (Lechler et al., 1990). In combinations in which there are multiple amino acid differences in the TCR contact surfaces, the alloresponse is likely to result from a chance higher affinity cross-reaction with the foreign MHC structure. Given the bias that appears to exist in TCR genes for MHC recognition (Merkenschlager et al., 1997), this is likely to occur in structurally dissimilar responder–stimulator combinations with sufficient frequency to account for the numbers of alloreactive T cells identified by limiting dilution analysis assays.

It has long been assumed that acute transplant rejection represents the in vivo correlate of the in vitro MLR; however, little evidence has been produced in support of this contention. Evidence that T cells with exclusively direct allospecificity can affect transplant rejection was provided in a very recent study. Reconstitution of severe combined immunodeficiency (SCID) or *Rag1*$^{-/-}$ mice with syngeneic CD4$^+$ T cells led to rejection of MHC class II–expressing cardiac allografts but not MHC class II–deficient grafts. Furthermore, *Rag1*$^{-/-}$ mice that were also MHC class II deficient rejected allogeneic cardiac transplants when reconstituted with CD4$^+$ T cells. Since these mice have no CD8$^+$ cells and lack the capacity for MHC class II–restricted indirect allorecognition (see below), these results indicate that direct pathway CD4$^+$ T cells were both necessary and sufficient to mediate allograft rejection (Pietra et al., 2000).

The Indirect Pathway of Anti-MHC Allorecognition and Transplant Rejection
The alternative pathway of allorecognition is referred to as the indirect pathway. This results from the recognition of allogeneic MHC molecules, predominantly MHC molecules, as peptides, presented in the context of self-MHC (Figure 2). Given that this corresponds to the manner by which T cells recognize all other nominal antigens, the term "indirect" may be misleading; however, it serves to contrast this mode of allorecognition with the direct pathway. In fact, MHC peptides are quite fre-
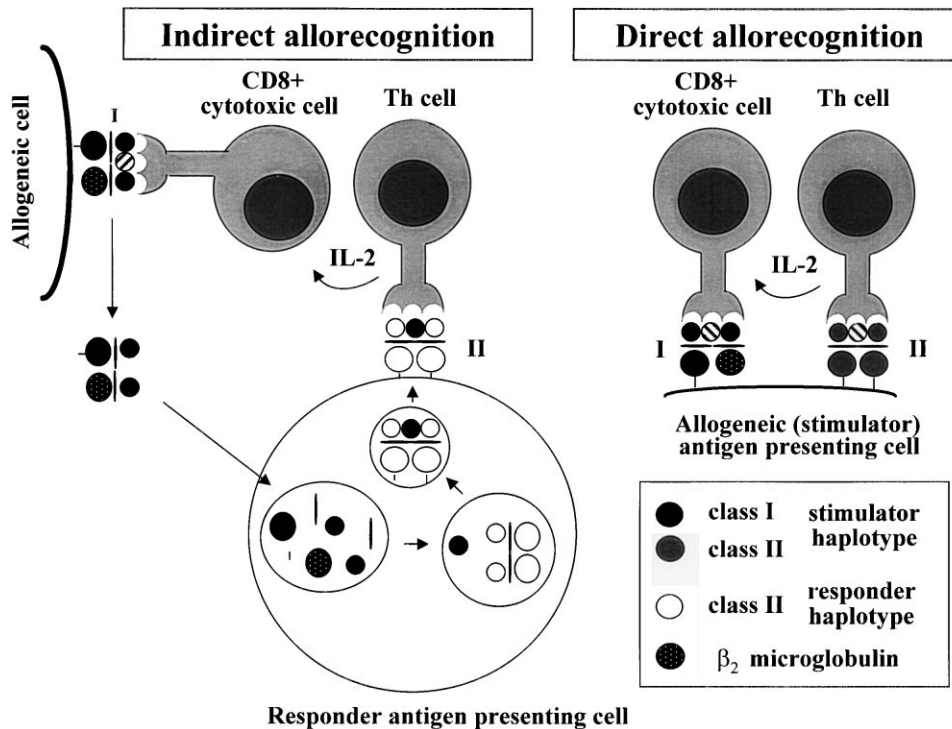
Figure 2. Direct and Indirect Allorecognition

(Indirect allorecognition) Alloantigens are shed from the donor cell surface or are taken up as dying allogeneic cells by host antigen-presenting cells (e.g., immature DCs). In the latter, peptides derived from allogeneic MHC molecules are re-presented on the self-MHC molecules (black circles), especially MHC class II, of the recipient antigen-presenting cells, much like conventional antigens. Helper cells recognizing donor MHC class I or II peptides on responder MHC then help the formation and function of CD8^{+} CTL that directly recognize donor allogeneic MHC molecules (e.g., by releasing IL-2 or other cytokines). (Direct allorecognition) Recipient T cells engage with complexes of intact allogeneic MHC molecules and bound peptides (striped circles) on the surface of donor cells.

quently eluted from the peptide binding grooves of MHC molecules (Rudensky et al., 1991; Chicz et al., 1993). The most striking demonstration of the propensity of MHC molecules to be presented in peptide form by other MHC molecules involved Y-Ae, the first monoclonal antibody that was shown to be specific for an MHC-peptide complex. This antibody sees a peptide fragment from the H2-Eα chain (highly conserved in homologous genes from rat and human) presented on H2-A^b products (Rudensky et al., 1991). The corresponding MHC-peptide complex is expressed at high levels on DCs and B cells from strains expressing H2-A^b and H2-E but not from strains expressing H2-A^b alone or H2-E with other H-2A alleles. The high level of staining obtained with this antibody illustrates the capacity of MHC molecules to present MHC-derived peptides. This has also been demonstrated in the DCs of the thymic medulla, an important site for the development of thymic or central tolerance by negative selection.

The original proposition that a second pathway of allorecognition exists and can contribute to transplant rejection arose from a DC depletion experiment involving rat kidney transplantation (Lechler and Batchelor, 1982a). These studies were based on the pioneering experiments of Lafferty and colleagues, who performed a series of transplants using thyroid or pancreatic islet tissues that had been depleted of bone marrow–derived cells by in vitro culture. They noted that the depleted

grafts enjoyed prolonged if not indefinite survival (Talmage et al., 1976; Bowen et al., 1980). This highlighted the distinction between antigenicity (the capacity to be recognized) and immunogenicity (the capacity to induce an effective immune response). Their interpretation of these findings was that bone marrow–derived cells were both antigenic and immunogenic, while the parenchymal cells of the graft were merely antigenic. The finding in the rat kidney model was that, in certain strain combinations in which primary kidney allografts were rapidly rejected, retransplanted grafts that had been "parked" in an intermediate recipient for 1 month or more under the cover of immunosuppression were spontaneously accepted without any exogenous immunosuppression (Lechler and Batchelor, 1982a). The relevance of DCs to these findings is discussed below. One of the strain combinations in which retransplanted kidney grafts were accepted without exogenous immunosuppression was (AS $\times$ AUG) F1 into AS. However, if the strain combination was changed and fully allogeneic AUG donors were used, the retransplanted grafts were invariably rejected, albeit at a slower tempo. Based on the assumption that the parenchymal tissues of the kidney were incapable of activating direct pathway antidonor T cells, it was proposed that a second, indirect, pathway of allorecognition was responsible for T cell sensitization.

Since the existence of the indirect pathway was first proposed, a large literature has accumulated illustrating

that this pathway can cause transplant rejection (reviewed in Auchincloss and Sultan, 1996). A comprehensive series of experiments has been performed by Auchincloss and colleagues using MHC class I and II knockout donor and recipient mice. Their most compelling evidence that the indirect pathway is sufficient to mediate transplant rejection was the observation that MHC class I knockout recipient mice could reject skin grafts from MHC class II knockout donor mice (Grusby et al., 1993). The recipient mice lacked CD8$^+$ cytotoxic T cells capable of recognizing donor MHC class I molecules directly, and the CD4$^+$ T cells in the recipient animals could only be stimulated by recognizing donor MHC class I molecules indirectly, presented in the context of recipient MHC class II molecules.

Returning to the original experiments with donor DC-depleted kidney grafts, in the strain combinations in which rejection occurred, the tempo was invariably slower, with a mean rejection time of 21 versus 10 days for DC-replete grafts. This was the basis for suggesting that the indirect pathway might be most prominent in later, more chronic forms of rejection. The hypothesis that T cells with indirect antidonor allospecificity are important drivers of chronic transplant rejection has received support from several clinical studies.

First, it appears that the strength of the direct antidonor alloresponse diminishes with time after transplantation. In renal and cardiac transplant patients, the frequencies of antidonor CD4$^+$ T cells proliferating and secreting cytokines in response to donor cells is substantially reduced compared with frequencies measured against third-party cells (Mason et al., 1996; Hornick et al., 1998). A similar fall in the frequencies of CD8$^+$ T cells has been documented. This is likely to reflect the consequences of alloantigen presentation by the nonimmunogenic parenchymal cells of the transplanted tissue. Indeed, culture of CD4$^+$CD4RO$^+$ T cells with HLA-mismatched, γ-interferon-treated primary epithelial cell cultures from human thyroid or kidney induced allospecific hyporesponsiveness (Marelli-Berg et al., 1997; Frasca et al., 1998). If this is the correct explanation for the decline of the direct antidonor alloresponse, the transplant-induced hyporesponsiveness should be most pronounced in the CD45RO$^+$ T cell population, in that alloreactive T cells of this phenotype are the ones that can cross the donor endothelium and enter the graft. We have recently tested this prediction by measuring antidonor and anti-third-party frequencies in CD45RA$^+$ and CD45RO$^+$ T cells at the time of transplantation and 4 months later. A significant fall in antidonor frequency was only seen in the RO$^+$ T cell fraction, in keeping with a role for transplant parenchymal cell antigen presentation in limiting the activity of the direct pathway response with time (Baker et al., 2001). Perhaps most significantly, the decline in the direct response was equally pronounced in patients with classical features of chronic rejection as in those with stable good function (Mason et al., 1996; Hornick et al., 1998). This finding suggests that the direct pathway of antidonor alloreactivity is not an important driver of chronic rejection.

The second series of clinical data comes from attempts to measure the indirect pathway in patients with established chronic rejection. Two approaches have been taken to reveal T cells with indirect alloreactivity.

One involves offering donor MHC molecules as synthetic peptides, thereby bypassing the need for antigen processing. Three groups have reported raised frequencies of T cells with indirect antidonor specificity in patients with chronic heart (Liu et al., 1996), kidney (Vella et al., 1997), and lung (SivaSai et al., 1999) transplant rejection. The other approach that has been used involves offering donor antigens in the form of lysed whole cells, thereby making no assumption as to which peptides will be recognized. Using this approach, we have detected raised frequencies of indirect pathway T cells in patients with but not without chronic heart transplant rejection (Hornick et al., 2000).

If the indirect pathway is critical in mediating transplant rejection, it follows that abolition of the direct pathway alone will not achieve allograft tolerance. More importantly, strategies that promote tolerance in the indirect pathway should enhance allograft survival. One strategy that has been employed to avoid direct pathway sensitization is to use, as donors, animals chimeric for recipient bone marrow. Krasinskas et al. created such bone marrow chimeras in mice and rats. This led to prolongation of graft survival in all cases, although the extent of the effect varied between different strain combinations. Despite this, allograft vasculopathy, an indication of chronic rejection, occurred in all recipients (Krasinskas et al., 2000). Although APC-depleted kidneys and skin grafts were rejected, APC-depleted islet grafts were accepted permanently without immunosuppression. It would appear at first sight that this was due to the lack of direct pathway stimulation; however, Coulombe and coworkers recently showed that the tolerance in such a model is CD4$^+$ T cell dependent (Coulombe et al., 1999). Since these islet grafts were essentially negative for MHC class II expression, this implied that the tolerance was maintained through the indirect pathway. Compelling evidence that induction of tolerance in the indirect pathway favors graft survival came from experiments in which recipient animals were pretreated with donor MHC peptides. The peptides were administered either intrathymically (Chowdhury et al., 1996) or orally (Zavazava et al., 2000) or as donor peptide-pulsed recipient APCs (Ali et al., 2000). All of these treatments induced graft prolongation. Although the mechanisms of these strategies have not been defined, the use of donor peptides means that the enhancement of graft survival can only be mediated through the indirect pathway. Equally interesting is the finding, in a murine skin allograft model, that tolerance cannot be induced in the absence of the indirect pathway. In this model, if normal MHC-mismatched skin was transplanted onto normal recipients, treatment with anti-CD40L, CTLA-4-Ig, and anti-CD8 led to long-term skin graft survival. Furthermore, the recipients became tolerant, as evidenced by the ability of their T cells to protect a fresh skin graft from the same donor strain following adoptive transfer. In contrast, if the response was confined to the direct pathway, due to the use of the so-called II$^-$4$^+$ mice as recipients (these mice express MHC class II molecules only in the thymus and not on peripheral APC) and if the same immunosuppressive protocol was used, all the grafts were rejected (Yamada and Auchincloss, personal communication). This suggests

that tolerance in the indirect pathway may be required to inhibit direct pathway T cells and to maintain tolerance.

The Role of Donor Dendritic Cells in Transplant Rejection or Tolerance

At face value, the contribution of DCs to transplant rejection may seem quite straightforward within this framework of direct and indirect allorecognition. Donor DCs initiate direct pathway responses, and recipient DCs initiate the activation of T cells with indirect allospecificity. Indeed, these were the concepts that grew out of the original "passenger cell" observations. As outlined above, the seminal finding was that depletion of donor leukocytes led to prolonged allograft survival. A variety of methods was used to deplete leukocytes, although, at the time, reagents such as monoclonal antibodies were not available to monitor and identify the types of depleted cells, distinguishing, for example, between tissue macrophages and tissue DCs. Lafferty used low-temperature culture or culture in hyperbaric oxygen to kill off passenger cells while preserving the viability of the tissue parenchymal cells. He applied these techniques to thyroid and pancreatic transplants (Talmage et al., 1976; Bowen et al., 1980). The rat kidney graft experiments involved parking the kidney in an intermediate recipient under the cover of immunosuppression. Support for the concept that the lack of rejection of these retransplanted grafts was due to the loss of immunogenic donor bone marrow–derived APC came from the finding that injection of as few as 10^4 donor strain DC at the time of retransplantation led to brisk rejection (Lechler and Batchelor, 1982a). Furthermore, if the intermediate recipient was made into a bone marrow chimera with donor strain bone marrow after acceptance of the primary graft, the retransplanted grafts were rejected as promptly as the primary graft would have been in the absence of immunosuppression (Lechler and Batchelor, 1982b).

The most intuitive interpretation of these experiments was that the donor DC made a vital contribution to sensitization of the direct pathway and that, in their absence, the fate of the tissue depended upon the strength of the indirect pathway, which would be predicted to be under classical *Ir* gene control. However, the assumptions underlying this interpretation were not tested. For example, it would be predicted that a DC-depleted graft would cause little amplification of the direct response, while a DC-replete graft would induce an increased frequency of T cells with direct antidonor allospecificity. Measurement of direct and indirect pathway sensitization was not undertaken in these studies.

In apparent contradiction to the classical passenger cell experiments, a series of groups have reported that depletion of donor bone marrow–derived cells prevents the induction of transplantation tolerance. For example, Cuturi noted that depletion of passenger leukocytes from rat donor heart allografts reversed the beneficial effects of donor-specific blood transfusion. Furthermore, tolerance was reestablished if donor-type DCs were cotransferred at the time of transplantation of APC-depleted cardiac grafts (Josien et al., 1998). Similarly, using rat strain combinations in which liver allografts are

spontaneously accepted, without immunosuppression, donor irradiation led to liver graft rejection (Sun et al., 1995). Although the possibility was not fully excluded that donor irradiation modified the graft in some deleterious way—altering vascular permeability, for example— these results probably indicate a need for donor leukocytes in the induction of tolerance.

Given the key role of the indirect pathway of allorecognition in transplant rejection and in transplantation tolerance, these results do not necessarily challenge the importance of the donor DC in priming direct pathway T cells. Rather, they may highlight the possibility that the donor DC, due to its propensity for migrating to draining lymph nodes, can provide a source of donor antigen, in the lymph node, for the induction of tolerance in T cells with indirect allospecificity. Support for this concept is provided by further experiments using the Y-Ae monoclonal antibody to monitor the processing and presentation of H2-Eα in vivo. When H2-E-bearing DCs were injected into H2-A^b recipients, within 2 days most of the recipient DCs in the draining lymph node became reactive with Y-Ae. The number of donor cells in the lymph node was very much smaller than the number of recipient DCs that had processed the donor H2-E (Inaba et al., 1998). This result implies that, when migratory donor DCs die upon reaching the lymph node, they are phagocytosed and processed by resident recipient DCs. However, these experiments have yet to elucidate if cross-presentation by DCs leads to immunity (cross-priming) or specific unresponsiveness (cross-tolerance) (Figure 3).

This interpretation of the role of the donor DC does not immediately resolve the conflicting data as to whether they are "friends" or "foes" of the transplanted tissue. However, in the models where donor leukocytes are required for the expression of tolerance induced by prior antigen exposure, donor DCs may be required to amplify indirect pathway regulatory cells primed by the earlier antigen treatment. In the case of spontaneously accepted liver grafts, the explanation may lie with the particular properties of Kuppfer cells, a liver-specific population of leukocytes, or, indeed, with the tolerance-promoting effects of the liver itself. It is also possible that distinct subsets of tolerogenic DCs exist and that they are differentially distributed in different organs.

Another set of data that relate to the role of donor DC in transplant immunity concerns the phenomenon of donor microchimerism, as observed by Starzl and co-workers. They have described the detection of donor cells in a variety of peripheral sites and in the thymus of recipient animals and in patients, following organ transplantation. This has been most pronounced in recipients of liver grafts, not surprisingly, due to the larger load of bone marrow–derived cells cotransplanted with a liver. It has been argued that the seeding of recipient tissues with such donor cells is instrumental in the induction of donor-specific tolerance for donor antigens (Starzl et al., 1996). Two major issues remain unresolved regarding this hypothesis. First, it is unclear whether the persistence of donor leukocytes in tolerant recipients represents cause or effect. If the recipient has become tolerant to donor alloantigens, this is likely to favor the
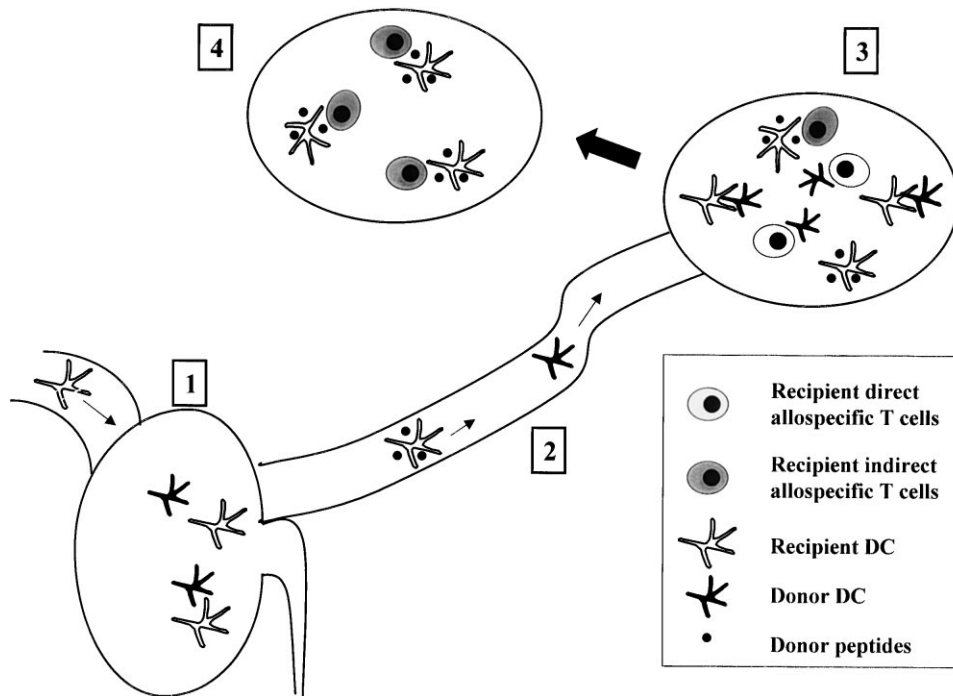
Figure 3. Roles of Donor and Recipient Dendritic Cells in Direct and Indirect Allorecognition

(1) The graft contains donor DCs (black) as well as recipient immature DCs (white).

(2) Recipient DCs that have captured antigens from the graft (e.g., dying cells) enter the lymph, much like donor graft-derived DCs.

(3) In the recipient lymph nodes, donor DCs and recipient DCs can stimulate the direct and indirect pathways of allorecognition. In addition, donor DCs can die in the lymph node, and their MHC products can be processed widely by the recipient DCs.

(4) As a result of (3), many more recipient DCs in the lymph nodes are able to capture and present donor peptides to indirect allospecific recipient T cells.

persistence of long-lived donor cells that would otherwise be destroyed. Second, it is not clear whether microchimerism is a mechanism for the induction of tolerance in the direct or the indirect pathway.

The Role of Recipient Dendritic Cells in Transplant Rejection or Tolerance

There can be little doubt that a major contribution of the recipient DC to transplant rejection is by sensitizing T cells with indirect antidonor specificity. A variety of experimental models have been designed to examine the pathways whereby DCs could stimulate the indirect pathway of rejection. The mechanism that leads to indirect allosensitization involves the internalization and processing of proteins from dead or dying donor cells and subsequent presentation by the MHC products of a recipient DC. Immature DCs are able to phagocytose apoptotic and necrotic cells and then present peptides on both MHC class I and II products (Albert et al., 1998; Inaba et al., 1998; Sauter et al., 2000). Successful presentation requires that the DCs mature after the initial uptake step (Albert et al., 1998; Inaba et al., 1998). Monocytes are, if anything, more phagocytic than mature DCs but seem to thoroughly degrade the ingested proteins rather than resurrect peptides from the dead cells (Albert et al., 1998). B cells, to date, are poor at cross-presentation (Munz et al., 2000). This may in part be due to limited phagocytic activity, but studies with different antigen-presenting cell lines (Regnault et al., 1999; Rodriguez et

al., 1999) suggest that DCs are peculiarly differentiated to cross-present antigens on MHC class I.

The processing of MHC class II donor alloantigens has primarily been studied using the Y-Ae antibody specific for the H2-A^b-Eα peptide complex as described above. Dead H2-E$^+$ donor B cells were fed to DCs that expressed H2-A^b, and indirect presentation was monitored by the development of the epitope recognized by the Y-Ae antibody. The H2-A^b DCs became reactive with Y-Ae, and the processing was sensitive to a blockade of proteolysis with ammonium chloride. The indirect pathway was unusually efficient in DCs. If one estimated the amount of H2-E protein in the dead cells that were offered to the DCs and compared this to the amount of H2-E peptide giving rise to the same amount of MHC peptide complex (Y-Ae epitope), the dead cells were several thousand times more effective as a source of antigen (Inaba et al., 1998). Interestingly, the sequence of the relevant H2-E peptide is conserved in HLA-DRα, and human B-LCL could also be processed by mouse DCs to form the Y-Ae epitope. In ongoing experiments, DCs are proving to be the main cell type in spleen that cross-presents H2-E protein when dead B cells are injected intravenously. Likewise, a recent report shows that DCs are the main cell type that cross-presents cell-associated donor proteins (in this case, ovalbumin) on MHC class I products (den Haan et al., 2000). The implication of these experiments is that, whenever dead cells are cleared from a graft via the blood or lymph, the MHC

products from the dead cells might undergo efficient processing and presentation by the recipient DCs.

As stated earlier, a tissue DC requires a maturation signal in order to become immunogenic. Such signals can be provided by invading pathogens or tissue inflammation or injury (Janeway, 1992; Matzinger, 1994). One difficulty with this "danger" theory is that it does not resolve a new view of the self–nonself dilemma that has emerged from studies of DC maturation. During infection—when DCs phagocytose dying infected cells, as well as proteins, in the infected environment, such as the lung or gut—how do the DCs selectively present microbial proteins and not proteins from dying self-tissue or the environment? For this reason, it has been proposed (Steinman et al., 2000) that, in the steady state, DCs have the capacity to tolerize the T cell repertoire peripherally to those self and environmental proteins that are readily processed. These proteins would be captured during the traffic of DCs through the tissues and lymph, especially from cells dying through the normal process of cell turnover. Uptake of dying intestinal epithelial cells has been observed in DCs trafficking in rat mesenteric lymph (Huang et al., 2000), and a subset of DCs in the lymph seems to be preferentially involved. Possibly, a subset of DCs, as proposed for CD8$^+$ mouse spleen DCs by Shortman (Kronin et al., 1996), have a tolerizing or regulatory role. Likewise, several laboratories (Forster and Lieberam, 1996; Kurts et al., 1997a; Adler et al., 1998; Morgan et al., 1999) have shown that bone marrow–derived cells in the lymph node (possibly DCs) can tolerize T cells, by deletion or anergy, to antigens cross-presented from nonhematopoietic cells. This tolerance is delicate. In the experiments of Kurts et al., an infusion of CD4$^+$ helper cells broke the tolerance of CD8$^+$ T cells (Kurts et al., 1997b). In a recent study by Bingaman et al., where a large number of T cells or thymocytes were introduced into nude animals bearing well-healed allografts, rejection was readily induced in the ostensible absence of danger (Bingaman et al., 2000). Perhaps these data can be reconciled by proposing that DCs in the steady state, in the absence of maturation stimuli, only have the capacity to silence and/or regulate small numbers of low-affinity T cells specific for self-peptides. If the number of T cells exceeds a certain limit (as in Kurts' experiments) or are of higher affinity (as in Bingaman's experiments), the tolerogenic effects of immature DCs may be overridden.

The implication of all of these observations is that the indirect pathway of allorecognition is driven by the efficient functioning of normal antigen presentation pathways. This may explain, in part, why chronic rejection is so refractory to conventional immunosuppression.

Dendritic Cells and Transplantation Tolerance

Returning to the question posed by the title of this review, it seems that the DC is almost inevitably a foe in the context of tissue transplantation. The donor DC is implicated as a foe by the passenger cell depletion experiments conducted with endocrine and kidney grafts. The recipient DC also appears to be responsible for priming and maintaining the indirect alloresponse.

Based on current knowledge of DC biology, this is predictable; surgeons are "dangerous," and the trauma of surgery and ischemia–reperfusion injury will invariably provide the maturation signals that tissue DC may require in order to migrate and induce T cell activation. The possible exception to this generalization is the liver, based on the irradiation experiments mentioned above. Nonetheless, it is not clear that elimination/inactivation of DCs is responsible for the beneficial effects of donor irradiation, and Kupffer cells and sinusoidal lining cells (Limmer et al., 2000) may well be important contributors to these events.

However, new research suggests that circumstances can be created in which DCs can be considered as friends capable of inducing peripheral tolerance. As summarized above, donor DCs may be required for the development of tolerance induced by donor antigen pretreatment (Josien et al., 1998), and exposure to immature DCs can prolong graft survival.

Thomson's group has explored this possibility in detail. They injected mice with allogeneic DCs, but they did so at the immature stage of development. A modest prolongation of graft survival was observed, and this was specific, since it was not seen following a challenge with third-party grafts (Fu et al., 1996). In experiments where DCs have been used to immunize mice with nominal antigens, mature DCs have been much more immunogenic than immature DCs (Stumbles et al., 1998; Inaba et al., 2000; Schuurhuis et al., 2000). However, the studies in transplantation raised the possibility that immature DCs were not simply ignored but could be actively tolerogenic. Likewise, studies on the presentation of self (Forster and Lieberam, 1996; Kurts et al., 1997a; Adler et al., 1998; Morgan et al., 1999) antigens in mice have shown that bone marrow–derived cells are capable of mediating peripheral tolerance by cross-presentation of self or tumor antigens. In these experiments, self- and tumor-reactive CD4$^+$ and CD8$^+$ T cells could be deleted (Kurts et al., 1997a) or anergized (Forster and Lieberam, 1996; Adler et al., 1998) upon encounter of antigens in the steady state, under "noninflammatory" conditions.

An alternative mechanism for transplantation tolerance has emerged from in vitro studies of the MLR, using DCs at an immature stage of differentiation. Again, it has been repeatedly observed that immature DCs are weak stimulators of the MLR, but recent data have significantly extended these findings (Jonuleit et al., 2000). Alloreactive CD4$^+$ cells of the Th1 type developed when mature DCs were used as stimulators. However, when immature DCs from the same donor were used to stimulate the same allogeneic T cells, the T cells proliferated minimally, lost the capacity to be stimulated by mature DCs, and, importantly, would significantly inhibit the responses of alloreactive Th1 cells to mature DCs. Therefore, T cells induced with immature allogeneic DCs had a regulatory function and also produced IL-10, as has been seen with cloned T regulatory cells. The mechanism of regulation in the Jonuleit et al. study also involved cell–cell contact.

A second in vivo study of regulation by immature DCs was just reported by Dhodapkar et al., using influenza viral peptide as a nominal antigen. They had shown that, when volunteers were given the peptide in saline, antigen-reactive CD8$^+$ T cells did not expand, but, when

stimulated with peptide-pulsed mature DCs given subcutaneously, the CD8$^+$ T cells responded rapidly. In contrast, when immature DCs were used in two individuals, the peptide-reactive CD8$^+$ T cells were silenced in terms of IFN-γ production and cytolytic function, but antigen-specific IL-10-producing CD8$^+$ T cells were now detected (Dhodapkar et al., 2001). This silencing of CD8$^+$ effectors by immature DCs was specific for the influenza peptide on the DCs, since other cytomegalovirus (CMV)-specific CD8$^+$ T cells were unperturbed.

These studies with human DCs have uncovered a potential role of immature DCs in the induction of regulatory T cells. The findings suggest that the injection of immature DCs, away from the site of the graft and therefore away from maturing inflammatory signals, may actively regulate alloreactivity. Two types of regulatory T cells, possibly related, are under study: the Tr1 regulatory cells that produce high levels of IL-10 (Groux et al., 1997) and the CD4$^+$CD25$^+$ suppressors for autoimmunity (reviewed in Shevach, 2000). These populations were both discovered in the investigation of in vivo tolerance. Tr1 cells were first appreciated in the setting of SCID bone marrow transplant recipients who were resistant to graft versus host (GVH), while CD4$^+$CD25$^+$ suppressors were identified as CD5 high, CD45RB, and RC low suppressors of several autoimmune diseases in rodents (Sakaguchi and Sakaguchi, 1990; Fowell and Mason, 1993).

Manipulating Dendritic Cells to Promote Tolerance Induction

The risk in using immature DC for tolerance induction is that they may inadvertently receive maturation signals in vivo and have the opposite effect to that intended. One solution to this problem is to manipulate the DC in vitro in order to inhibit its immunogenicity and potentiate its capacity to induce tolerance. There is both in vitro and in vivo evidence in support of this approach. For example, genetically engineered DC that constitutively express viral IL-10 (Takayama et al., 1998), TGF-β (Lee et al., 1998), FasL (Min et al., 2000), or CTLA-4-Ig (O'Rourke et al., 2000) can induce alloantigen-specific T cell hyporesponsiveness and enhance the survival of allografts in nonimmunosuppressed recipients. Other strategies that have been successful in generating "tolerogenic" DC include corticosteroids (Rea et al., 2000), vitamin D$_3$ (Penna and Adorini, 2000), and culture with a suboptimal dose of GM-CSF (Lutz et al., 2000). The common features of these tolerogenic DCs are the reduced expression of costimulatory molecules, in particular, CD86. The expression of both MHC class I and II molecules, maturation markers such as CD83, and adhesion molecules are also often downregulated. Thus, one possible explanation of their tolerogenic potential is the induction of donor-specific anergy of the direct pathway antidonor T cells as a result of antigen recognition in the absence of costimulatory signals. Furthermore, the tolerogenic effect of these manipulated DC may not be limited to the direct pathway. Finally, since many of these "designer" tolerogenic DC carry immunoregulatory substances (such as IL-10, TGF-β, and CTLA-4-Ig), this may have more generalized effects on other cell types.

Summary

The existence of two sets of dendritic cells in transplantation (of donor and recipient origin) poses unique problems in alloimmunity and tolerance. Donor DCs are potent stimulators of the direct pathway, in which recipient T cells respond to peptides presented on donor MHC products or to the donor MHC molecules themselves. Reduced DC function in the direct pathway is used to explain the acceptance of certain allografts that have been depleted of passenger leukocytes. Reciprocally, purified donor DCs powerfully stimulate the rejection of these grafts as well as the mixed leukocyte reaction by purified allogeneic T cells in culture. Donor DCs also can act as specialized antigen transport vehicles in cross-priming for the indirect pathway. Here, recipient DCs process MHC molecules from the donor. The indirect pathway of rejection is actually the rule for passenger leukocyte-depleted grafts. Furthermore, the indirect pathway seems to be the dominant alloresponse detected in long-term graft recipients, both in experimental models and clinical transplantation, particularly in those with chronic rejection. The stimulatory function of DCs in both direct and indirect pathways is regulated by their maturation in response to inflammatory stimuli, especially those delivered via IL-1, TNF, and Toll receptor families. Since the normal function of DCs is to generate immunity against invading pathogens, the indirect response to peptides continually derived from the allogeneic MHC molecules in a tissue allograft (a surgically introduced "pathogen" because of the associated inflammation and necrosis) may be more difficult to silence. However, in addition to their allostimulatory role, immature or in vitro–manipulated DCs also have the potential to downregulate direct and indirect antidonor responses through a variety of mechanisms. Intriguing new evidence shows that immature DCs can actively induce regulatory T cells. Given the probable role of the indirect pathway in driving chronic rejection, the induction of T cell tolerance (deletion, anergy, and regulation), especially in those T cells with indirect antidonor allospecificity, by the injection of immature DCs pretransplant, could serve as a critical therapeutic strategy.

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