AN OFFPRINT FROM

2027

DENDRITIC CELLS
Biology and Clinical Applications

Edited by

Michael T. Lotze
Departments of Surgery and Molecular Genetics and Biochemistry
University of Pittsburgh Cancer Institute
USA

Angus W. Thomson
Departments of Surgery and Molecular Genetics and Biochemistry
Thomas E. Starzl Transplantation Institute
University of Pittsburgh
USA

Academic Press
San Diego London Boston New York Sydney Tokyo Toronto

CHAPTER 20
Dendritic Cells in Rejection and Acceptance of Solid Organ Allografts

A.J. Demetris[1], N. Murase[2], J.J. Fung[2] and T.E. Starzl[2]
Thomas E. Starzl Transplantation Institute, Departments of [1]Pathology and [2]Surgery, Divisions of Transplantation, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

INTRODUCTION

The field of solid organ transplantation is based largely on the concept that replacement of irreversibly damaged organ in an otherwise 'healthy' recipient can significantly prolong survival or even cure some diseases. Although the hope for clinical success is based on this premise, many allograft recipients suffer from recurrence of the original disease. For example, chronic viral hepatitis types B and C almost invariably attack the new liver after hepatic replacement [1, 2]. This unfortunate reality illustrates the importance of a systemic, or nonlocal, perspective in transplantation biology: a specific disease may primarily manifest in a single organ, but for many disorders this simply represents a local manifestation of a more pervasive problem. An exception to this generalization is organ-specific toxic injury or organ-based metabolic diseases where replacement of the defective organ corrects a systemic problem and brings about a true cure.

A systemic perspective is also of importance in the study of transplantation physiology, including allograft immunobiology. For example, insulin secreted by islet allografts can regulate the recipient blood sugar; and a new liver will receive blood and nutrients from the recipient intestines, from which it will synthesize cholesterol used to construct new 'recipient' cells. Fortunately, clinical physicians rarely have to be concerned about physiological compatibility between the donor and recipient. Most of the complex systems involved have 'nonlocal' properties that enable a donor organ to spontaneously adjust to its new environment. The integration occurs so naturally that one rarely even thinks about the interface between donor and recipient, unless something goes wrong.

The most important exception to the above generalization is the immune system: its 'local' properties result in an inability to spontaneously integrate the donor with the recipient, and vice versa, which in turn leads to significant problems for the transplant surgeon. Unless the patient is heavily immunosuppressed, the allograft is eventually rejected. There are, however, situations in experimental animals where allografts are accepted without immunosuppression, and in humans where immunosuppression can

Dendritic Cells: Biology and Clinical Applications
ISBN 0-12-455283

be completely withdrawn and the allografts function for the lifetime of the recipient. Thus, the local properties of the immune system were not designed to frustrate transplant clinicians, instead, they can be predictably redesigned under certain circumstances.

Consequently, the entire field of clinical transplantation could be thought of as an experiment conducted to investigate the local properties of the immune system, among others, including the role of MHC antigens and the consequences of MHC antigen expression on different cell types. Since these antigens, as well as sex chromosomes, can be used to mark the genetic fidelity of an individual cell, double labeling techniques can be used to trace specific donor and recipient cell types after solid organ transplantation to assess problems with integration. Discoveries made using this technology have brought together two previously unlinked research fields in transplantation immunology: the deleterious and the beneficial functions of donor hematolymphoid cells. The sections below overview both lines of investigation and highlight the role of dendritic cells (DC) in allograft rejection and acceptance.

ORGAN-BASED IMMUNE NETWORKS

Before proceeding directly to a discussion of the role of DC in transplantation, it is important first to briefly overview organ-based immune physiology. This refers to a dynamic network of hematolymphoid cells that travel into and out of, and transiently occupy, the interstitium of all solid organs. These cells monitor the microenvironment and communicate with central lymphoid organs, and regional lymph nodes via the circulatory system and lymphatics [3]. An understanding of this system is of particular importance in organ transplantation, since problems with assimilation of the donor immune system affect many aspects of transplantation biology, as well as patient management. Examples include the susceptibility to rejection, the clinical and histopathological manifestations of both acute and chronic rejection, infections, and the interface between infection and rejection.

Organ-based immune cells are primarily derived from progenitors that migrate from the bone marrow, although maturation from local precursors, including intraorgan hematopoietic stem cells, can also contribute to this pool. Even in an adult, the liver has enough hematopoietic stem cells to fully reconstitute, for the long term, a lethally irradiated recipient [4, 5]. The important point is that intraorgan immune cells are a dynamic population: they continuously mature and migrate and therefore must ultimately be maintained by a stem cell population.

Normal physiology accounts for the considerable variation among different organs in the quantity and quality of organ-based immune cells [6, 7]. Organs in direct contact with the external environment, such as the lungs and intestines, have an exaggerated complement of organ-associated lymphoid tissue, generally termed 'mucosal-associated lymphoid tissue'. After contact with commensal bacteria in neonatal life, the immune cells spontaneously arrange into an organized complex structure, which is identical to that seen in lymph nodes, replete with B cell follicles and T cell-rich interfollicular zones rich in DC [8–10]. Thereafter, a delicate balance is maintained between reaction to antigens from the environment and local tissue damage. The liver, on the other hand, is indirectly exposed to the external environment, since it drains blood from the intestines. It is richly endowed with a large component of cells from the monocyte/macrophage lineage, consistent with its role as a filter of various opsonized materials,

including microorganisms, activated platelet aggregates, and coagulated proteins [11]. Heart [6, 7, 12, 13] and kidneys [6, 14, 15] also have considerable, but less well developed immune networks in comparison to the above organs.

Common to vascularized organs is a population of DC precursors at various stages of maturation located in the interstitial connective tissue [14–19]. The 'immature' state of many intraorgan DC [18, 20–22], except for those in the lymphoid tissue of the lung and intestines [18, 19], is characterized by phagocytic capacity, which is absent in mature DC [18, 21, 22]; relatively inefficient stimulatory capacity in a mixed leukocyte reaction (MLR) [22]; and low density of MHC class II [21] or costimulatory molecule expression [19]. Organ-based DC precursors can also be identified with a series of monoclonal antibodies directed at certain differentiation markers, covered elsewhere in this book.

Dendritic cells and their precursors reside near terminal lymphatics throughout the interstitium of organs, and are concentrated near draining efferent lymphatic vessels in the adventitia of arteries; epithelial-lined conduits that are in contact with the external environment, such as the mucosal-associated lymphoid tissue of the lungs [23] and intestines [18, 19]; and portal tracts of the liver [17, 21, 24]. At these sites, they monitor the microenvironment for foreign, or dangerous antigens [25], exposure to which stimulates DC maturation and migration via efferent lymphatics to the paracortex of regional lymph nodes where they stimulate a T cell response [18, 22, 26–30]. The factors involved in DC activation, maturation, and migration are covered in more detail in Chapter 11. Thus, the vasculature and lymphatics represent important lines of communication between the intraorgan immune network, the regional lymph nodes, and eventually the thymus and spleen.

HEMATOLYMPHOID AND DENDRITIC CELLS AS POTENTIATORS AND FACILITATORS OF REJECTION

Acute 'Cellular' Rejection

When an organ becomes an allograft, the nascent hematolymphoid cells become known as 'passenger leukocytes'. The idea that these particular donor cells are especially immunogenic was first proposed by Snell [31]. Steinmuller [32] provided experimental evidence for this concept by showing that chimeric donor skin allografts in which the passenger leukocytes were allogeneic, but the epidermal cells were syngeneic to the recipient, resulted in permanent graft acceptance. However, the recipients became sensitized to the alloantigens on the hematopoietic cells.

The relative importance of passenger leukocytes in precipitating rejection was further addressed by Guttmann *et al.* [33], who constructed chimeric donor organs by lethally irradiating rats and reconstituting them with allogeneic bone marrow. He showed that the 'immunogenicity' of the organs could be greatly reduced when the passenger leukocytes were syngeneic to the recipient. Several other experimental manipulations also showed conclusively that the passenger leukocytes were more immunogenic than the parenchyma. These included pretransplant *in vitro* culturing to eliminate or reduce the complement of passenger leukocytes [34–36], and so-called 'parking' experiments [37–40]. In the latter, allografts are transplanted into intermediate hosts that are kept

immunosuppressed or are naturally immuno-incompetent for one reason or another. Once the original passenger leukocytes have been replaced, the composite grafts are then retransplanted into a second recipient, that is syngeneic with either the parenchymal and stroma or the passenger leukocyte population. Regardless of the technique used, the important concept was that depletion of allogeneic passenger leukocytes led to prolonged allograft survival.

Lechler and Batchelor [40] provided a key piece of evidence bringing DC to the forefront as the passenger leukocyte prototype. They showed that the immunogenicity of long-surviving enhanced (AS X AUG)F1 renal allografts retransplanted into secondary AS recipients could be restored by the injection of as few as 1×10^4 to 5×10^4 DC of donor strain derived from afferent lymph [40]. In contrast, neither the passenger volume of donor strain blood, nor 5×10^6 T- or B-lymphocytes were able to do so, thereby demonstrating more than a 100-fold difference in immunogenic potency. These findings were consistent with observations about the functional activities of DC originally made by Steinman and colleagues [41–43] and later confirmed by many others.

Disrupted Local Lymphatics

It should be remembered that donor organ harvesting and reimplantation disrupts the efferent lymphatic channels. This blocks an important migratory circuit for immune cells that are either already present in or enter the allograft interstitium, until connections with regional lymph nodes are reestablished within 2–3 weeks [44–48]. In fact, efferent lymphatic disruption, along with ischemic injury contributes to a 'reimplantation' response, which by itself can precipitate activation of the intraorgan immune network [46–51]. Thus, this purely mechanical problem can, in some respects, simulate an antigen-driven immune response [51] and is one of the first difficulties, but by no means the only one, encountered when the immune system of the donor attempts assimilation with that of the recipient.

The presence of MHC, adhesion and costimulating molecules on DC causes them to spontaneously form clusters with and directly activate allogeneic lymphocytes [24, 52]. In an allograft, activated recipient T cells in such clusters proliferate [24] and produce cytokines that recruit other immune cells that have the potential to damage the organ. In fact, the distribution of mononuclear cells at the initiation of acute rejection reflects the intraorgan distribution of DC [24, 52]: in kidney allografts it preferentially localizes to the cortex and outer medulla [14, 15], whereas, in the liver, portal tracts and perivenular regions are preferentially targeted [17, 24, 53]. Conversely, the absence of renal cortex or portal tracts in an allograft kidney or liver biopsy, respectively, renders such samples inadequate for the evaluation of rejection. Altogether, this process is referred to as 'peripheral sensitization', or recognition of the allograft in the periphery via direct alloantigenic stimulation. Subsequently, upregulation of adhesion molecules on the surrounding vasculature and recruitment of lymphoid and nonlymphoid effector cells and local tissue damage signal the development of acute 'cellular' rejection.

In organs with mucosal-associated lymphoid tissue, such as the lung and intestines, recipient lymphocytes trafficking into the allograft following the same migratory routes as they normally would: they enter the T cell-dependent areas of the bronchial (BALT) and mucosal (MALT) associated lymphoid tissue, respectively [12, 54–58]. Here they encounter donor DC and participate in a bidirectional '*in vivo* mixed leukocyte

response' [12, 54–59], which is the same type of response as described above for the interstitium of organs except that the surrounding microenvironment contains immunologically active donor T- and B-lymphocytes.

Passenger Leukocytes

Passenger leukocytes from the donor immune network, including DC, leave the allograft, either hematogenously or via intact regional efferent lymphatics that drain to *donor* regional lymph nodes transplanted *en bloc* with the allograft [56, 57, 59]. Larsen and colleagues [60, 61] were the first to show that donor DC from a heart allograft migrate hematogenously to the recipient spleen. The same thing occurs after transplantation of a liver allo- or xenograft [24, 62]. Donor DC leaving these allografts migrate to the periarterial lymphatic sheath and marginal zone of the spleen, where their appearance is associated with a proliferative response in the recipient lymphoid cells (Plate 20.1) [24, 63]. Altogether, this process is referred to as 'central sensitization' [24, 60, 61, 63], or central recognition of the allograft, again via direct alloantigenic stimulation. The passenger leukocyte population also contains hematopoietic stem cells, as well as T and B cells, macrophages, and mature and progenitor DC (Plate 20.2). This is evidenced by their ability to reconstitute lethally irradiated experimental animal recipients [4, 5] and to convert the blood type of human recipients [64]. Thus, at the progenitor level, donor cells will have access to the recipient bone marrow, to regional and distant lymph nodes, and even to the thymic medulla [3, 63, 65–68].

Once recipient T cells are activated within the allograft and rejection effector mechanisms begin to damage the organ, there is an increased production of lymph and disruption of the lymphatic and capillary microvascular endothelial junctions. This retards immune cell traffic and lymph flow [45–48, 69–71] and contributes to the reappearance of graft edema and swelling typically seen during acute rejection [45, 69–72]. The result is an endless cycle of immune activation and damage, graft edema, retarded immune cell trafficking and diminished blood flow. Unless interrupted by increased immunosuppression, the process usually leads to allograft failure.

Considering the completeness of the data outlined above, it was reasonable to conclude that the passenger leukocyte population should be removed from the allograft before transplantation. Thus, various types of irradiation [73] and monoclonal antibodies directed at common leukocyte [74–76] or MHC class II [77–79] antigens were used in an effort to deplete these cells. In general, all of these pretreatment regimens prolonged allograft survival, but the long-term beneficial effects were less than expected.

Targeting Donor DC

Blocking costimulatory molecules such as the B7 family [80–85] and CD40 [81, 86] interrupts cell signaling mediated by interactions of these molecules with their ligands and significantly diminishes acute rejection, resulting in prolongation of graft survival [81–88]. In some models, there is even an absence [81] or amelioration of chronic rejection (CR) [82–86]. However, in some of the models used, CR develops directly from acute rejection, which is nonlethal because of genetic compatibility between the donor and recipient [83–85], and may not reflect the clinical scenario in most cases of CR [89]. In addition, the presence of infiltrates within the treated allografts [82, 84] or

actual allograft vasculopathy [82–85] suggests that tolerance without a susceptibility to CR has not been achieved. Nevertheless, this line of research represents an important advance in our understanding of how acute rejection damages an allograft.

It is interesting to note that the beneficial effect of costimulatory molecule blockade was *diminished* by immunosuppression and was *augmented* by the addition of donor antigen in the form of donor splenocytes [85]. In addition, the B7-CD28 blockade does not completely stop cytokine mRNA production within the allograft. Instead it is shifted toward a T_H2-type 'tolerogenic' pathway [87]. Paradoxically, the beneficial effect is still observed in IL-4 deficient mice [88]. These observations suggest, but do not prove, that tolerance requires T cell activation, and that stimulation is best provided by the passenger leukocyte population [36, 90, 91].

Nonetheless, the logical conclusion of this line of reasoning is to construct allografts completely devoid of passenger leukocytes. The idea is that the stimulatory DC are removed and forms of donor antigen other than hematopoietic cells, such as the allograft parenchyma and stroma [25, 92, 93] and soluble MHC antigens [94, 95] or peptides [94–97], may actually be tolerogenic [90]. Although this line of reasoning has several important conceptual flaws, it is based on the concept that more than one signal is needed to trigger T cell activation and proliferation [25].

In vitro, presentation of antigen without proper costimulation can result in anergy [25]. When this concept is tested *in vivo* using transgenic mice, the results are not clear cut. For example, the immune system of mice carrying allogeneic MHC transgenes on nonimmune cells, such as islets of Langerhans, simply 'ignores' alloantigen expression until it is presented to the immune system in the proper context by DC and other antigen-presenting cells. This most often occurs during tissue damage from viral infection or other insults that cause local immune stimulation [98–100].

In a vascularized allograft, immune activation, and thus immunological cognition of the allograft, is extremely difficult, if not impossible to avoid. Organ harvesting and reimplantation, ischemia, preexisting donor diseases and efferent lymphatic disruption can all potentially contribute to intragraft immune activation, which in turn creates an immune environment conducive to allorecognition, even if donor DC are not present. Migration of potently allostimulatory cells from the allograft assures central allorecognition. Thus, from a practical perspective in clinical transplantation, it is our opinion that, initially, very few allografts are 'ignored', which is evidenced by the fact that all allograft recipients require immune suppression to prevent acute rejection.

Nevertheless, certain tissue allografts, such as pure epithelial [35, 101, 102], fibroblast [103], and corneal [104] allografts, can be completely depleted of donor hematopoietic cells. Unfortunately, these allografts are still often rejected [35, 101, 102], particularly if they are placed into an immunologically active environment [105], similar to the observations described above for the transgenic mice. Thus, despite an absence of passenger leukocytes or DC, rejection still occurs, albeit more slowly, and probably via the indirect pathway of alloantigen presentation [35, 104, 106–109].

Transitional Phase

Since passenger leukocytes are bone marrow-derived or stem cell-dependent, they exist for a relatively short period in the periphery. Thus, replacement of the donor intraorgan immune network (including DC) after transplantation with similar recipient cells is an

expected finding [57, 110–118]. Indeed, this occurs to some extent in all allografts, since trafficking of immune cells is part of the normal 'nonlocal' immune physiology. However, as discussed above, when recipient T cells encounter donor DC (and vice versa), the transitional process has to be chaperoned by immunosuppressive drugs because it usually precipitates acute rejection. For DC, the replacement phenomenon likely occurs at a precursor cell stage, since mature recipient DC do not appear to home to allograft tissues [119].

It is likely that the same factors that control activation, maturation, and migration of DC precursors in nonallograft organs contribute to donor and recipient DC trafficking in allografts. For example, it is known that the intraorgan cytokine milieu contributes to activation, recruitment, and migration of both macrophages and DC. In allografts, the cytokine-rich milieu of rejection [120] quickens the rate and extent of donor macrophage replacement [111, 121]. Infiltrates associated with T_H1-type cytokines, such IFN-γ and macrophage-activating chemokines like IL-12, mobilize donor macrophages and foster the influx of activated recipient cells (unpublished observation).

An orderly transition from donor to recipient cells in the intraorgan immune network, however, is dependent on preventing architectural damage during the transition [57]. If this can be accomplished, recipient cells can function alongside donor ones, as a chimeric intraorgan immune network. If not, irreversible structural damage may prevent reestablishment of a normally functioning intraorgan immune network, with lines of communication to regional lymph nodes. The architectural damage can occur at the level of the BALT [116, 122, 123] or GALT [57, 118, 124], lymphatic drainage from the organ, and/or the regional donor lymph nodes [57]. Subsequently, any cause of allograft inflammation, such as environmental irritants or infection, may result in an ineffectual local immune response and persistence of the insult(s). This, in turn, can cause cytokine release that facilitates alloimmune injury, and the allograft then becomes trapped in a relentless downward spiral of declining organ function that eventually ends in allograft failure (see below).

Chronic Rejection

Chronic rejection (CR), in any organ, can be broadly defined as a largely indolent but progressive form of allograft injury, characterized primarily by persistent but patchy inflammation of the allograft, interstitial fibrosis, fibrointimal hyperplasia of arteries, and destruction and atrophy of parenchymal elements and organ-associated lymphoid tissue [89, 120, 125]. The term chronic implies a temporally prolonged course and, in general, CR more indolently compromises organ function than acute rejection. However, it clearly develops in many cases from inadequately controlled acute rejection and in patients not compliant with immunosuppressive therapy. In others, CR more indolently compromises allograft function over a period of months to years, without an apparent precipitating event [89]. Our emphasis here will be on DC. The reader interested in a broader perspective of CR is referred to several recent reviews [89, 126–128].

The intragraft inflammatory infiltrates associated with CR are often arranged into nodular aggregates, some of which contain germinal centers [89] reminiscent of the development of mucosal-associated lymphoid tissue, discussed above. Immunophenotypic analysis reveals a predominance of $CD4^+$ and $CD8^+$ T cells and macrophages with fewer B cells, although those present can form small primary and secondary

follicles. This is in contrast to the infiltrates associated with acute rejection, which have fewer B cells and no follicles and tend to be more diffusely distributed throughout the interstitium, lacking an organized structure.

Few studies have specifically investigated DC in chronically rejecting organs. Oguma *et al.* (129) suggested that DC of *recipient* origin participated in the CR process by coordinating antigenic presentation in arteries affected by obliterative arteriopathy and in the interstitium. Subsequent studies have verified these findings and found that the number of recipient DC in chronically rejecting organs correlates directly with the overall severity of inflammation [116, 120, 130]. Moreover, the *recipient* DC are concentrated amidst the lymphoid aggregates [116, 120, 122, 129], suggesting that they are coordinating antigen presentation, an assumption based on the spatial relationship between the DC and the lymphoid infiltrates. These morphological and immunohistochemical observations are consistent with the concept that indirect (rather than direct), MHC-restricted alloantigen presentation importantly contributes to CR [106–108], and that chronic antigenic stimulation occurring outside the lymph nodes can result in the development of intraorgan lymphoid tissue [131, 132], similarly to autoimmune disorders such as Hashimoto's thyroiditis, primary biliary cirrhosis, and Crohn's disease [131].

As alluded to above, chronically rejecting allografts also develop another significant problem: the persistent or severe injury during the transitional phase selectively damages mucosal-associated lymphoid tissue normally present in the lung [72, 133] and intestines [56, 58] and focally disrupts intraorgan lymphatics in other organs [46, 89, 120]. Eventually these structures can be completely destroyed and replaced by fibrosis [56, 58, 133, 134]. Consequently, the organizational structure of the immune network and migratory routes of DC are disrupted. This undoubtedly contributes to the inability of the intraorgan immune network to adequately process infectious and other antigens. Thus, it is tempting to speculate that this accounts for the frequent association between infection and CR [123, 133, 135–139]: chronically rejecting organs may simply be unable to adequately handle infections or other antigenic insults.

HEMATOLYMPHOID AND DENDRITIC CELLS AS FACILITATORS OF TOLERANCE INDUCTION

There are two lines of transplantation research in which studies show how donor hematopoietic cells in general, and DC specifically, might induce tolerance to solid organ allografts. The first of these is activation-induced clonal deletion and the second is induction of hematopoietic chimerism.

Donor Dendritic Cells as Mediators of Peripheral Clonal Deletion

Implantation of any solid organ allograft results in a characteristic cycle of heightened immune activation, followed by evolution toward a more stable relationship between the allograft and the recipient when immunosuppression can be considerably lowered or even withdrawn [140, 141]. With the understanding brought about by the appreciation of donor hematopoietic cell migration after transplantation [24, 63, 113, 142], this prototypic series of events can likely be attributed, in large part, to the initial engagement of donor and recipient immune cells in the allograft and recipient lymphoid tissues.

During this time, it is possible that the intense immune activation results in a form of clonal deletion called clonal stripping [143], deletion or purging through apoptosis [144–146]. It might be crudely thought of as the peripheral equivalent of negative selection in the thymus, which is a very efficient means of controlling reactivity in a lymphocyte population. Current research suggests that DC may be involved in this process: blocking of costimulation on mature DC allows exhibition of their apoptosis-inducing potential and they thus may be particularly adept at mediating such a process [27, 147, 148]. In fact, it is tempting to speculate that the combination of clonal deletion via apoptosis after a strong rejection reaction, followed by replacement of the intra-graft immune network (graft adaptation), ultimately causes the immune system to ignore the allograft.

Unfortunately, on a practical level, the harsh reality is that, even in combination, the above mechanisms are unable to prevent rejection in the majority of long-term survivors without the aid of exogenous immunosuppression [89]. At best, there exists an uneasy truce between an adapted allograft and a recipient that can be triggered into a rejection reaction at the slightest provocation. In the worst case, most long-term recipients are slowly rejecting their organs. This also holds true for liver allograft recipients, who are resistant to CR compared to recipients of other vascularized allografts [89, 149]: even this 'favorable' recipient population requires chronic immunosuppression in over 80% of long-term survivors [141]. Thus, while clonal deletion or stripping, veto or regulatory cells, or other mechanisms may contribute to graft acceptance in immunosuppressed recipients, they are insufficient in the majority of patients to allow cessation of immunosuppressive therapy.

Dendritic Cells as Mediators of the Effects of Hematopoietic Chimerism

Owen [150] was the first to show that twin cattle sharing a placental circulation develop chimeric immune systems, each composed of immune cells from both individuals [150]. He found that this condition enabled them to exchange other tissues without the fear of rejection, or a need for immunosuppression. When attempts were made to create chimeric immune systems in adult animals, it was quickly realized that lethal or sublethal irradiation and other harsh conditioning regimens were required to ablate the recipient immune system and to make 'physiological space' for the engraftment of infused donor bone marrow [151–159]. Unfortunately, this limits clinical implementation of the concept. Two major problems exist: graft-versus-host disease [160, 161] and the morbidity associated with the conditioning regimens [158, 162]. Current approaches to decreasing morbidity without compromising donor stem cell engraftment include lower doses of irradiation, facilitator cells [163, 164], and/or higher doses or repeated infusions of donor bone marrow [159, 162, 165].

Nevertheless, when donor stem cell engraftment results in long-term mixed hematopoietic chimerism, there is complete assimilation of the donor immune system in some cases, and an undeniable association with tolerance. In the mixed chimeric animals, intraorgan immune networks are composed of cells from both individuals [154, 166], and tolerance is strictly dependent on the persistence of hematopoietic chimerism: loss of chimerism and loss of tolerance go together [167, 168]. This has been observed in humans given fetal liver cell allografts [169–171], and in a number of small experimental animal models [151–155, 157–159].

The experimental models have been useful in determining the role of DC in tolerance induction. For example, mixed chimeric animals specifically lack donor responsiveness in a MLR, accept allografts without immunosuppression, and are resistant to CR [172, 173]. Central deletional tolerance is primarily responsible for these observations [154, 155, 158], although peripheral mechanisms also likely contribute to the process [174, 175]. Thus, the situation is similar to the nontransplantation setting, where both central and peripheral mechanisms contribute to 'self' tolerance, which is a 'local' property of the immune system.

In the nontransplantation setting, central or thymic tolerance involves a complex set of thymic epithelial and stromal interactions with immature T cells that first mediate positive selection of developing thymocytes, based on 'self' reactivity [176–179]. Subsequently, thymic medullary DC play a predominant role in negative selection [176, 178, 180–186], where cells that react too strongly with the DC are deleted. Similar observations have been made in the mixed allogeneic chimeras: thymic stromal and epithelial elements appear to mediate positive selection, even if there is MHC mismatching between the lymphoid and nonlymphoid populations [169, 171, 187], whereas donor thymic hematopoietic cells appear to mediate negative selection [158, 166, 168, 169, 171, 174, 187]. Although more studies are needed to identify the population of donor cells that mediate negative selection in chimeras, the characteristics identified to date make donor DC a likely candidate [3, 63, 65–68].

FINDINGS THAT BRIDGE THE GAP BETWEEN IMMUNOGENIC AND TOLEROGENIC DC

In the late 1980s, a series of experiments were carried out to examine the sequence of histopathological changes associated with acute intestinal allograft rejection [56, 57]. During these studies, it was necessary to distinguish between donor and recipient lymphoid cells, so that one could unravel the underlying immune pathophysiology. This was achieved with the development of a monoclonal antibody that reacted with the class II major histocompatibility antigens of most rat strains, except the Brown Norway (BN) [188]. Subsequently, it was possible to show that Lewis cells emigrated into the BN GALT and mesenteric lymph node of intestinal allografts, where they replaced cells of donor origin in the organ-based immune network. This occurred within the first several weeks after transplantation [57]. Similar findings were observed in human small-intestinal allograft recipients [112].

The above studies prompted an investigation of the fate of donor cells that had emigrated from the allograft. Up to that time, very few such studies had appeared in the literature [24, 60, 65, 189, 190], and all came to the same conclusion discussed above—donor passenger leukocytes, especially DC, were essentially deleterious to allograft survival because they served only to precipitate and/or amplify a rejection reaction. However, all of these studies were conducted in nonimmunosuppressed recipients, which resulted in rapid rejection of the allografts, as expected. Had similar studies been carried out on the fate of passenger leukocytes in either transiently or continuously immunosuppressed recipients, the conclusion about the role of DC in organ transplantation might have been different. In essence, that is what was done in the early 1990s.

A quick survey of available long-term experimental animal and several humans organ allograft recipients revealed a surprising finding—the donor hematolymphoid

cells persisted in long-term survivors (Plate 20.3), some of whom were chronically free of immunosuppressive therapy [65, 113, 166]. Moreover, the donor cells were ubiquitously distributed throughout the lymphoid and nonlymphoid tissues, and some had the characteristics of DC [65, 166]. This included a strong surface expression of MHC class II antigens, a dendritic shape, and a location in the interstitium of organs, the paracortex of lymph nodes, the thymic medulla [63, 65–68], and the periarterial lymphatic sheath of the spleen. These are all sites where DC normally reside. Further studies by Lu and Thomson [21, 66, 191, 192] showed conclusively that DC and their precursors were included among the passenger leukocytes persisting in organ allograft recipients.

These observations led to the suggestion that 'microchimerism' or the persistence of donor hematopoietic cells was necessary, but alone not sufficient, for the induction of tolerance [113–115, 193]. Since the donor cells persisted for decades in some patients, it was assumed that they are sustained by 'engrafted' donor stem cells transplanted with the organ (Plate 20.2). The important conceptual point is that donor hematolymphoid cells appear to have integrated successfully into the recipient immune system, similarly to the chimeras made by irradiation. However, the number of donor cells present is much smaller in microchimeras than in macrochimeras made by irradiation, and this likely affects the relative contribution of various mechanisms to the tolerant phenotype. For example, it is unlikely that only a few donor DC that make their way to the recipient thymus would be able to completely delete donor reactive T cells (Plate 20.3), but there may be enough to activate autoregulatory circuits. In the meanwhile, other studies confirmed that passenger leukocytes persisted in experimental animals and in humans, but the authors did not necessarily agree with our interpretation [92, 93, 194–201].

Initially this discovery did not fit easily into the current understanding of donor DC in transplantation immunobiology. It was not immediately clear how the most potentially potent allogeneic simulator could survive long term in a recipient who no longer required immunosuppression. This was especially true for DC in the periphery, even though freshly isolated DC from nonlymphoid tissues are relatively inefficient antigen-presenting cells. It was clear, however, that long-term persistence of donor DC in the allograft was associated with freedom from chronic rejection [120] and, therefore, they might mediate tolerogenic reactions. In addition, trafficking of donor hematopoietic stem and progenitor cells from the allograft to the recipient bone marrow and thymus was not widely appreciated. However, analogies were drawn between this situation and that of mixed allogeneic chimeras achieved with irradiation. In the periphery, only a few studies had suggested that DC might mediate tolerogenic reactions [202, 203]. More recently, however, there is an increasing awareness that DC can and do mediate eventual 'nonresponsiveness' [27, 204–207]. How this is achieved is covered in greater detail in Chapter 26.

In the nontransplantation setting, there is now convincing evidence that DC can mediate both stimulatory and tolerogenic immune reactions. The context of presentation is certainly of great importance in determining which pathway is chosen. This is also true for transplantation. Without transient immunosuppression, mature DC invariably precipitate rejection that causes graft failure. With immunosuppression, acute rejection is avoided and the persistence of donor DC is associated with allograft protection from CR [120]. These probably arise from engrafted stem cells and/or other

precursors. Emerging details about the mechanism(s) in stimulatory and tolerogenic reactions involved are covered in Chapter 26.

What, then, has been learned about the immune system, MHC antigens and the role of DC in the experiment of transplantation? First, it is clear that an immune system has 'local' properties, which greatly complicate the ability to take an organ from one person and implant it into another one. We have also learned that MHC antigens and DC both play important roles in defining these local properties, and successful long-term freedom from rejection probably requires transfer of the donor immune system. DC appear to exert this effect via their ability to stimulate immune reactions that either prevent or facilitate assimilation of the donor immune system and its accompanying organ into the recipient, which in turn depends on their phenotype and maturational stage. Thus, in a generic sense, at least part of the 'local' properties of the immune system are self-defined by DC, and the other immune cells with which they interact, both in the thymus and in the periphery. However, this occurs in the context of the environment and is dependent on, but not strictly limited by, the recipient MHC genes/antigens. Consequently, it appears that the local properties of the immune system and MHC antigens were not designed for the purpose of preventing allogeneic transplantation but to enable species adaptability [208–210]. The key to understanding, and thus controlling, the immune system for the purpose of transplantation will come from a knowledge of its local and nonlocal properties so that the latter can be exploited [90, 211].

ACKNOWLEDGMENT

Supported by NIH 1 RO1 DK49615-01 and NIH RO1 AI40329-02.

REFERENCES

1. Demetris, A.J., Jaffe, R., Sheahan, D.D. *et al.* (1986). Recurrent hepatitis B in liver allograft recipients. Differentiation between viral hepatitis B and rejection. *Am. J. Pathol.* **125** (1), 161–172.
2. Randhawa, P.S. and Demetris, A.J. (1995). Hepatitis C virus infection in liver allografts. [Review]. *Pathol. Annu.* **2**, 203–226.
3. Beschorner, W.E., Yao, X. and Divic, J. (1995). Recruitment of semiallogeneic dendritic cells to the thymus during post-cyclosporine thymic regeneration. *Transplantation* **60** (11), 1326–1330.
4. Murase, N., Starzl, T.E., Ye, O. *et al.* (1996). Multilineage hematopoietic reconstitution of supralethally irradiated rats by syngeneic whole organ transplanation: with particular reference to the liver. *Transplantation* **61**, 1–4.
5. Taniguchi, H., Toyoshima, T., Fukao, K. and Nakauchi, H. (1996). Presence of hematopoietic stem cells in the adult liver. *Nature Medicine* **2**, 198–203.
6. Murase, N., Starzl, T.E., Tanabe, M. *et al.* (1995). Variable chimerism, graft-versus-host disease, and tolerance after different kinds of cell and whole organ transplantation from Lewis to brown Norway rats. *Transplantation* **60** (2), 158–171.
7. Holzinger, C., Zuckermann, A., Reinwald, C. *et al.* (1996). Are T cells from healthy heart really only passengers? Characterization of cardiac tissue T cells. *Immunol. Lett.* **53** (2–3), 63–67.
8. Anderson, J.C. (1977). The response of gut-associated lymphoid tissue in gnotobiotic piglets to the presence of bacterial antigen in the alimentary tract. *J. Anat.* **124** (3), 555–562.
9. Gordon, J.I., Hooper, L.V., McNevin, M.S., Wong, M. and Bry, L. (1997). Epithelial cell growth and differentiation. III. Promoting diversity in the intestine: conversations between the microflora, epithelium, and diffuse GALT. *Am. J. Physiol.* **273** (3 Pt 1), G565–570.

10. Woolverton, C.J., Holt, L.C., Mitchell, D. and Sartor, R.B. (1992). Identification and characterization of rat intestinal lamina propria cells: consequences of microbial colonization. *Vet. Immunol. Immunopathol.* **34** (1–2), 127–138.
11. Wardle, E.N. (1987). Kupffer cells and their function. *Liver* **7**, 63–75.
12. Prop, J., Kuijpers, K., Petersen, A.H., Bartels, H.L., Nieuwenhuis, P. and Wildevuur, C.R. (1985). Why are lung allografts more vigorously rejected than hearts? *J. Heart Transplant.* **4** (4), 433–436.
13. Eiref, S.D., Zhang, W., Popma, S.H., Shah, L.J., Moore, J.S. and Rosengard, B.R. (1997). Creation of chimeric hearts: a tool for testing the 'passenger leukocyte' hypothesis. *Ann. Thorac. Surg.* **4** (3), 628–633.
14. Kaissling, B. and Le Hir, M. (1994). Characterization and distribution of interstitial cell types in the renal cortex of rats. *Kidney Int.* **45** (3), 709–720.
15. Kaissling, B., Hegyi, I., Loffing, J. and Le Hir, M. (1996). Morphology of interstitial cells in the healthy kidney. *Anat Embryol. (Berl)* **193** (4), 303–318.
16. Hart, D.N.J. and McKenzie, J.L. (1990). Interstitial dendritic cells. *Int. Rev. Immunol.* **6**, 128–149.
17. Prickett, T.C.R., McKenzie, J.L. and Hart, D.N.J. (1988). Characterization of interstitial dendritic cells in human liver. *Transplantation* **46**, 754–761.
18. Rao, A.S., Roake, J.A., Larsen, C.P., Hankins, D.F., Morris, P.J. and Austyn, J.M. (1993). Isolation of dendritic leukocytes from non-lymphoid organs. *Adv. Exp. Med. Biol.* **329**, 507–512.
19. Inaba, K., Witmer-Pack, M., Inaba, M. *et al.* (1994). The tissue distribution of the B7-2 costimulator in mice: abundant expression on dendritic cells in situ and during maturation in vitro. *J. Exp. Med.* **180** (5), 1849–1860.
20. Roake, J.A. and Austyn, J.M. (1993). The role of dendritic cells and T cell activation in allograft rejection. *Exp. Nephrol.* **1** (2), 90–101.
21. Lu, L, Woo, J., Rao, A.S. *et al.* (1994). Propagation of dendritic cell progenitors from normal mouse liver using granulocyte/macrophage colony-stimulating factor and their maturational development in the presence of type-1 collagen. *J. Exp. Med.* **179** (6), 1823–1834.
22. Austyn, J.M., Hankins, D.F., Larsen, C.P., Morris, P.J., Rao, A.S. and Roake, J.A. (1994). Isolation and characterization of dendritic cells from mouse heart and kidney. *J. Immunol.* **152** (5), 2401–2410.
23. Gong, J.L., McCarthy, K.M., Telford, J., Tamatani, T., Miyasaka, M. and Schneeberger, E.E. (1992). Intraepithelial airway dendritic cells: a distinct subset of pulmonary dendritic cells obtained by microdissection. *J. Exp. Med.* **175** (3), 797–807.
24. Demetris, A., Qian, S., Sun, H. *et al.* (1991). Early events in liver allograft rejection: delineation of sites of simultaneous intragraft and recipient lymphoid tissue sensitization. *Am. J. Pathol.* **138**, 609.
25. Matzinger, P. (1994). Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045.
26. Steinman, R.M. (1991). The dendritic cell system and its role in immunogenicity. [Review]. *Annu. Rev. Immunol.* **9**, 271–296.
27. Steinman, R.M., Pack, M. and Inaba, K. (1997). Dendritic cells in the T-cell areas of lymphoid organs. *Immunol. Rev.* **156**, 25–37.
28. Wright-Browne, V., McClain, K.L., Talpaz, M., Ordonez, N. and Estrov, Z. (1997). Physiology and pathophysiology of dendritic cells. *Hum. Pathol.* **28** (5), 563–579.
29. Ni, K. and O'Neill, H.C. (1997). The role of dendritic cells in T cell activation. *Immunol. Cell. Biol.* **75** (3), 223–230.
30. Cella, M., Sallusto, F. and Lanzavecchia, A. (1997). Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* **9** (1), 10–16.
31. Snell, G.D. (1957). The homograft reaction. *Annu. Rev. Microbiol.* **11**, 439–458.
32. Steinmuller, D. (1967). Immunization with skin isografts taken from tolerant mice. *Science* **158**, 127–129.
33. Guttmann, R.D., Lindquist, R.R. and Ockner, S.A. (1969). Renal transplantation in the inbred rat. IX. Hematopoietic origin of an immunogenic stimulus of rejection. *Transplantation* **8** (4), 472–484.
34. Lafferty, K.J., Cooley, M.A., Woolnough, J. and Walker, K.Z. (1975). Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science* **188** (4185), 259–261.
35. Rouabhia, M., Germain, L., Belanger, F. and Auger, F.A. (1993). Cultured epithelium allografts: Langerhans cell and Thy-1$^+$ dendritic epidermal cell depletion effects on allograft rejection. *Transplantation* **56** (2), 259–264.

36. Coulombe, M., Yang, H., Guerder, S., Flavell, R.A., Lafferty, K.J. and Gill, R.G. (1996). Tissue immunogenicity: the role of MHC antigen and the lymphocyte costimulator B7-1. *J. Immunol.* **157** (11), 4790–4795.
37. Stuart, F.P., Bastien, E., Holter, A., Fitch, F.W. and Elkins, W.L. (1971). Role of passenger leukocytes in the rejection of renal allografts. *Transplant. Proc.* **3** (1), 461–464.
38. Hart, D.N., Winearls, C.G. and Fabre, J.W. (1980). Graft adaptation: studies on possible mechanisms in long-term surviving rat renal allografts. *Transplantation* **30** (1), 73–80.
39. Lechler, R.I. and Batchelor, J.R. (1982). Immunogenicity of retransplanted rat kidney allografts. Effect of inducing chimerism in the frst recipient and quantitative studies on immunosuppression of the second recipient. *J. Exp. Med.* **156** (6), 1835–1841.
40. Lechler, R.I. and Batchelor, J.R. (1982). Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. *J. Exp. Med.* **155** (1), 31–41.
41. Steinman, R.M. and Cohn, Z.A. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology. *J. Exp. Med.* **137**, 1142–1162.
42. Steinman, R.M. and Cohn, Z.A. (1974). Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. *J. Exp. Med.* **139**, 380–397.
43. Steinman, R.M., Lustig, D.S. and Cohn, Z.A. (1974). Identification of a novel cell in peripheral lymphoid organs in mice. III. Functional properties in vivo. *J. Exp. Med.* **139**, 1431–1445.
44. Kocandrle, V., Houttuin, E. and Prohaska, J.V. (1966). Regeneration of the lymphatics after autotransplantation and homotransplantation of the entire small intestine. *Surg. Gynecol. Obstet.* **122** (3), 587–592.
45. Malek, P., Vrubel, J. and Kolc, J. (1969). Lymphatic aspects of experimental and clinical renal transplantation. *Bull. Soc. Int. Chirurg.* **28** (1), 110–114.
46. Kline, I.K. and Thomas, P.A. (1976). Canine lung allograft lymphatic alterations. *Ann. Thorac. Surg.* **21** (6), 532–535.
47. Cuttino, J.T., Jr, Clark, R.L., Mandel, S.R., Webster, W.P. and Jaques, P.F. (1978). Lymphatic visualization during renal transplant rejection. *Invest. Radiol.* **13** (4), 328–333.
48. Rabin, A.M., Abramson, A.F., Manzarbeitia, C. *et al.* (1991). Dilated periportal lymphatics mimicking an anastomotic bile leak after liver transplantation. *Gastrointest. Radiol.* **16** (4), 337–338.
49. Shibuya, H., Ohkohchi, N., Tsukamoto, S. and Satomi, S. (1997). Tumor necrosis factor-induced, superoxide-mediated neutrophil accumulation in cold ischemic/reperfused rat liver. *Hepatology* **26** (1), 113–120.
50. Rao, P.N., Liu, T., Synder, J.T., Platt, J.L. and Starzl, T.E. (1991). Reperfusion injury following cold ischemia activates rat liver Kupffer cells. *Transplant. Proc* **23** (1 Pt 1), 666–669.
51. Galkowska, H. and Olszewski, W.L. (1992). Spontaneous cluster formation of dendritic (veiled) cells and lymphocytes from skin lymph obtained from dogs with chronic lymphedema. *Lymphology* **25** (3), 106–113.
52. Forbes, R.D., Parfrey, N.A., Gomersail, M., Darden, A.G. and Guttmann, R.D. (1986). Dendritic cell-lymphoid aggregation and major histocompatibility antigen expression during rat cardiac allograft rejection. *J. Exp. Med.* **164**, 1239–1258.
53. van den Oord, J.J., Volpes, R. and Desmet, V.J. Dendritic cells and the liver. [Review]. *Apmis* (Supplement) **23**, 68–76.
54. Prop, J., Nieuwenhuis, P. and Wildevuur, C.R. (1985). Lung allograft rejection in the rat. I. Accelerated rejection caused by graft lymphocytes. *Transplantation* **40** (1), 25–30.
55. Prop, J., Wildevuur, C.R. and Nieuwenhuis, P. (1985). Lung allograft rejection in the rat. II. Specific immunological properties of lung grafts. *Transplantation* **40** (2), 126–131.
56. Murase, N., Demetris, A.J., Kim, D.G., Todo, S., Fung, J.J. and Starzl, T.E. (1990). Rejection of multivisceral allografts in rats: a sequential analysis with comparison to isolated orthotopic small-bowel and liver grafts. *Surgery* **108** (5), 880–889.
57. Murase, N., Demetris, A.J., Matsuzaki, T. *et al.* (1991). Long survival in rats after multivisceral versus isolated small-bowel allotransplantation under FK 506. *Surgery* **110** (1), 87–98.
58. Lee, R.G., Nakamura, K., Tsamandas, A.C. *et al.* (1996). Pathology of human intestinal transplantation [see comments]. *Gastroenterology* **110** (6), 1820–1834.
59. Fung, J., Zeevi, A., Demetris, A.J. *et al.* (1989). Origin of lymph node derived lymphocytes in human hepatic allografts. *Clin. Transplant.* **3**, 316–324.

60. Larsen, C.P., Morris, P.J. and Austyn, J.M. (1990). Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel route for initiation of rejection. *J. Exp. Med.* **171**, 307–314.
61. Larsen, C.P., Austyn, J.M. and Morris, P.J. (1990). The role of graft-derived dendritic leukocytes in the rejection of vascularized organ allografts. Recent findings on the migration and function of dendritic leukocytes after transplantation. [Review]. *Ann. Surg.* **212** (3), 308–315.
62. Langer, A., Valdivia, L.A., Murase, N. *et al.* (1993). Humoral and cellular immunopathology of hepatic and cardiac hamster-into-rat xenograft rejection. Marked stimulation of IgM^{++}/bright/IgD^{+}dull splenic B cells. *Am. J. Pathol.* **143** (1), 85–98.
63. Demetris, A.J., Murase, N., Fujisaki, S., Fung, J.J., Rao, A.S. and Starzl, T.E. (1993). Hematolymphoid cell trafficking, microchimerism, and GVH reactions after liver, bone marrow, and heart transplantation. *Transplant. Proc.* **25** (6), 3337–3344.
64. Collins, R.H., Anastasi, J., Terstappen, L.W.W.M. *et al.* (1993). Brief report: Donor-derived long-term multilineage hematopoiesis in a liver-transplant recipient. *N. Engl. J. Med.* **328**, 762–765.
65. Demetris, A.J., Murase, N. and Starzl, T.E. (1992). Donor dendritic cells after liver and heart allotransplantation under short-term immunosuppression [letter]. *Lancet* **339** (8809), 1610.
66. Lu, L., Rudert, W.A., Qian, S. *et al.* (1995). Growth of donor-derived dendritic cells from the bone marrow of murine liver allograft recipients in response to granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* **182** (2), 379–387.
67. Kobayashi, E., Kamada, N., Delriviere, L. *et al.* (1995). Migration of donor cells into the thymus is not essential for induction and maintenance of systemic tolerance after liver transplantation in the rat. *Immunology* **84** (2), 333–336.
68. Lord, R., Goto, S., Vari, F. *et al.* (1997). Differences in the rate of donor leucocyte migration between natural and drug-assisted tolerance following rat liver transplantation. *Clin. Exp. Immunol.* **108** (2), 358-365.
69. Malek, P. and Vrubel, J. (1968). Lymphatic system and organ transplantation. [Review]. *Lymphology* **1** (1), 4–22.
70. Cockett, A.T., Sakai, A. and Netto, I.C. (1973). Kidney lymphatics: an important network in transplantation. *Trans. Am. Ass. Genito Urinary, Surg.* **65**, 73–76.
71. Eliska, O., Eliskova, M. and Mirejovsky, P. (1986). Lymph vessels of the transplanted kidney. *Nephron* **44** (2), 136–141.
72. Ruggiero, R., Fietsam, R., Jr, Thomas, G.A. *et al.* (1994). Detection of canine allograft lung rejection by pulmonary lymphoscintigraphy. *J. Thorac. Cardiovasc. Surg.* **108** (2), 253–258.
73. von Gaudecker, B., Petersen, R., Epstein, M., Kaden, J. and Oesterwitz, H. (1993). Down-regulation of MHC-expression on dendritic cells in rat kidney grafts by PUVA pretreatment. *Adv. Exp. Med. Biol.* **329**, 495–499.
74. Brewer, Y., Palmer, A., Taube, D. *et al.* (1989). Effect of graft perfusion with two CD45 monoclonal antibodies on incidence of kidney allograft rejection. *Lancet* **2** (8669), 935–937.
75. Goldberg, L.C., Bradley, J.A., Connolly, J. *et al.* (1995). Anti-CD45 monoclonal antibody perfusion of human renal allografts prior to transplantation. A safety and immunohistological study. CD45 Study Group. *Transplantation* **59** (9), 1285–1293.
76. Goldberg, L.C., Cook, T. and Taube, D. (1994). Pretreatment of renal transplants with anti-CD45 antibodies: optimization of perfusion technique. *Transplant. Immunol.* **2** (1), 27–34.
77. Lloyd, D.M., Cotler, S.J., Letai, A.G., Stuart, F.P. and Thistlethwaite, J.R. Jr. (1989). Pancreas-graft immunogenicity and pretreatment with anti-class II monoclonal antibodies. *Diabetes* **38** (Suppl. 1), 104–108.
78. Krzymanski, M., Waaga, A.M., Ulrichs, K. *et al.* (1991). The influence of MHC class II antigen blockade by perfusion with a monoclonal antibody on rat renal graft survival. *Transplant. Int.* **4** (3), 180–185.
79. Krzymanski, M. and Muller-Ruchholtz, W. (1992). Tissue distribution of MHC class II-positive cells, their down-manipulation by monoclonal antibodies and potential role in organ allograft immunogenicity. *Arch. Immunol. Ther. Exp. (Warsz)* **40** (3–4), 177–181.
80. Larsen, C.P., Ritchie, S.C., Pearson, T.C., Linsley, P.S. and Lowry, R.P. (1992). Functional expression of the costimulatory molecule, B7/BB1, on murine dendritic cell populations. *J. Exp. Med.* **176** (4), 1215–1220.
81. Larsen, C.P., Elwood, E.T., Alexander, D.Z. *et al.* (1996). Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* **381** (6581), 434–438.

82. Steurer, W., Nickerson, P.W., Steele, A.W., Steiger, J., Zheng, X.X. and Strom, T.B. (1995). Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. *J. Immunol.* **155** (3), 1165–1174.
83. Russell, M.E., Hancock, W.W., Akalin, E. *et al.* (1996). Chronic cardiac rejection in the LEW to F344 rat model. Blockade of CD2B-B7 costimulation by CTLA4Ig modulates T cell and macrophage activation and attenuates arteriosclerosis. *J. Clin. Invest.* **97** (3), 833–838.
84. Azuma, H., Chandraker, A., Nadeau, K. *et al.* (1996). Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection [see comments]. *Proc. Natl Acad. Sci. USA* **93** (22), 12439–12444.
85. Chandraker, A., Russell, M.E., Glysing-Jensen, T., Willett, T.A. and Sayegh, M.H. (1997). T-cell costimulatory blockade in experimental chronic cardiac allograft rejection: effects of cyclosporine and donor antigen. *Transplantation* **63** (8), 1053–1058.
86. Larsen, C.P., Alexander, D.Z., Hollenbaugh, D. *et al.* (1996). CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation* **61** (1), 4–9.
87. Lin, H., Wei, R.Q., Goodman, R.E. and Bolling, S.F. (1997). CD28 blockade alters cytokine mRNA profiles in cardiac transplantation. *Surgery* **122** (2), 129–137.
88. Lakkis, F.G., Konieczny, B.T., Saleem, S. *et al.* (1997). Blocking the CD28-B7 T cell costimulation pathway induces long term cardiac allograft acceptance in the absence of IL-4. *J. Immunol.* **158** (5), 2443–2448.
89. Demetris, A.J., Murase, N., Lee, R.G. *et al.* (1997). Chronic rejection. A general overview of histopathology and pathophysiology with emphasis on liver heart and intestinal allografts. *Transplant. Ann.* **2**, 27–44.
90. Demetris, A.J., Murase, N., Rao, A.S. and Starzl, T.E. (1994). The role of passenger leukocytes in rejection and 'tolerance' after solld organ transplantation: a potential explanation of a paradox. In: *Rejection and tolerance* (eds. J.L. Touraine *et al.*) Kluwer Academic, Dordrecht, pp. 325–392.
91. Gill, R.G., Coulombe, M. and Lafferty, K.J. (1996). Pancreatic islet allograft immunity and tolerance: the two-signal hypothesis revisited. *Immunol. Rev.* **149**, 75–96.
92. Bushell, A., Pearson, T.C., Morris, P.J. and Wood, K.J. (1995). Donor–recipient microchimerism is not required for tolerance induction following recipient pretreatment with donor-specific transfusion and anti-CD4 antibody. Evidence of a clear role for short-term antigen persistence [see comments]. *Transplantation* **59** (10), 1367–1371.
93. Shirwan, H., Wang, H.K., Barwari, L., Makowka, L. and Cramer, D.V. (1996). Pretransplant injection of allograft recipients with donor blood or lymphocytes permits allograft tolerance without the presence of persistent donor microchimerism. *Transplantation* **61** (9), 1382–1386.
94. Oluwole, S.F., Jin, M.X., Chowdhury, N.C., Engelstad, K., Ohajekwe, O.A. and James, T. (1995). Induction of peripheral tolerance by intrathymic inoculation of soluble alloantigens: evidence for the role of host antigen-presenting cells and suppressor cell mechanism. *Cell. Immunol.* **162** (1), 33–41.
95. Oluwole, S.F., Chowdhury, N.C., Jin, M.X. and Hardy, M.A. (1993). Induction of transplantation tolerance to rat cardiac allografts by intrathymic inoculation of allogeneic soluble peptides. *Transplantation* **56** (6), 1523–1527.
96. Sayegh, M.H., Perico, N., Imberti, O., Hancock, W.W., Carpenter, C.B. and Remuzzi, G. (1993). Thymic recognition of class II major histocompatibility complex allopeptides induces donor-specific unresponsiveness to renal allografts. *Transplantation* **56** (2), 461–465.
97. Sayegh, M.H., Khoury, S.J., Hancock, W.W., Weiner, H.L. and Carpenter, C.B. (1996). Mechanisms of oral tolerance by MHC peptides. *Ann. NY Acad. Sci.* **778**, 338–345.
98. Heath, W.R., Karamalis, F., Donoghue, J. and Miller, J.F. (1995). Autoimmunity caused by ignorant $CD8^+$ T cells is transient and depends on avidity. *J. Immunol.* **155** (5), 2339–2349.
99. Miller, J.F. and Heath, W.R. (1993). Self-ignorance in the peripheral T-cell pool. [Review]. *Immunol. Rev.* **133**, 131–150.
100. Nossal, G.J., Herold, K.C. and Goodnow, C.C. (1992). Autoimmune tolerance and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* **35** (Suppl. 2), S49–59.
101. Hoffman, D.K., Sibley, R.K., Korman, J.M. and Press, B.H. (1994). Light microscopic and immunohistochemical features in serial biopsies of epidermal versus dermal allografts. *Ann. Plast. Surg.* **33** (3), 295–299.

102. Kawai, K., Ikarashi, Y., Tomiyama, K., Matsumoto, Y. and Fujiwara, M. (1993). Rejection of cultured keratinocyte allografts in presensitized mice. *Transplantation* **56** (2), 265–269.
103. Hultman, C.S., Brinson, G.M., Siltharm, S. *et al.* (1996). Allogeneic fibroblasts used to grow cultured epidermal autografts persist in vivo and sensitize the graft recipient for accelerated second-set rejection. *J. Trauma* **41** (1), 51–58; discussion, 58–60.
104. Sano, Y., Ksander, B.R. and Streilein, J.W. (1996). Minor H, rather than MHC, alloantigens offer the greater barrier to successful orthotopic corneal transplantation in mice. *Transplant. Immunol.* **4** (1), 53–56.
105. Sano, Y., Ksander, B.R. and Streilein, J.W. (1997). Murine orthotopic corneal transplantation in high-risk eyes. Rejection is dictated primarily by weak rather than strong alloantigens. *Invest. Ophthalmol. Vis. Sci.* **38** (6), 1130–1138.
106. Braun, M.Y., McCormack, A., Webb, G. and Batchelor, J.R. (1993). Mediation of acute but not chronic rejection of MHC-incompatible rat kidney grafts by alloreactive CD4 T cells activated by the direct pathway of sensitization. *Transplantation* **55** (1), 177–182.
107. Batchelor, J.R. and Braun, M.Y. (1994). Distinct T cells mediating acute and chronic rejection. In: *Rejection and Tolerance* (ed. Touraine, J.J., *et al.*), vol. 25. Kluwer Academic, Dordrecht, pp. 103–110.
108. Bradley, J.A. (1996). Indirect T cell recognition in allograft rejection. *Int. Rev. Immunol.* **13** (3), 245–255.
109. Sayegh, M.H. and Carpenter, C.B. (1996). Role of indirect allorecognition in allograft rejection. *Int. Rev. Immunol.* **13** (3), 221–229.
110. Porter, K.A. (1969). Pathology of the orthotopic homograft and heterograft. *Experience in Hepatic Transplantation* (ed. Starzl, T.E.) W.B. Saunders, pp. 422–471.
111. Gouw, A.S., Houthoff, H.J., Huitema, S., Beelen, J.M., Gips, C.H. and Poppema, S. (1987). Expression of major histocompatibility complex antigens and replacement of donor cells by recipient ones in human liver grafts. *Transplantation* **43** (2), 291–296.
112. Iwaki, Y., Starzl, T.E., Yagihashi, A. *et al.* (1991). Replacement of donor lymphoid tissue in small-bowel transplants. *Lancet* **337** (8745), 818–819.
113. Starzl, T.E., Demetris, A.J., Murase, N., Ildstad, S., Ricordi, C. and Trucco, M. (1992). Cell migration, chimerism, and graft acceptance [see comments]. [Review]. *Lancet* **339** (8809), 1579–1582.
114. Starzl, T.E., Demetris, A.J., Trucco, M. *et al.* (1993). Chimerism after liver transplantation for type IV glycogen storage disease and type 1 Gaucher's disease [see comments]. *N. Engl. J. Med.* **328** (11), 745–749.
115. Starzl, T.E., Demetris, A.J., Trucco, M. *et al.* (1993). Cell migration and chimerism after whole-organ transplantation: the basis of graft acceptance [see comments]. [Review]. *Hepatology* **17** (6), 1127–1152.
116. Uyama, T., Winter, J.B., Sakiyama, S., Monden, Y., Groen, G. and Prop, J. (1993). Replacement of dendritic cells in the airways of rat lung allografts. *Am. Rev. Respir. Dis.* **148** (3), 760–767.
117. Valdivia, L.A., Demetris, A.J., Langer, A.M., Celli, S., Fung, J.J. and Starzl, T.E. (1993). Dendritic cell replacement in long-surviving liver and cardiac xenografts. *Transplantation* **56** (2), 482–484.
118. Langrehr, J.M., Demetris, A.J., Banner, B. *et al.* (1994). Mucosal recipient-type mononuclear repopulation and low-grade chronic rejection occur simultaneously in indefinitely surviving recipients of small bowel allografts. *Transplant. Int.* **7** (2), 71–78.
119. Larsen, C.P., Barker, H., Morris, P.J. and Austyn, J.M. (1990). Failure of mature dendritic cells of the host to migrate from the blood into cardiac or skin allografts. *Transplantation* **50** (2), 294–301.
120. Demetris, A.J., Murase, N., Ye, Q. *et al.* (1997). Analysis of chronic rejection and obliterative arteriopathy. Possible contributions of donor antigen-presenting cells and lymphatic disruption. *Am. J. Pathol.* **150** (2), 563–578.
121. Steinhoff, G., Wonigeit, K., Sorg, C. *et al.* (1989). Patterns of macrophage immigration and differentiation in human liver grafts. *Transplant. Proc.* **21**, 398–400.
122. Uyama, T., Winter, J.B., Groen, G., Wildevuur, C.R., Monden, Y. and Prop, J. (1992). Late airway changes caused by chronic rejection in rat lung allografts. *Transplantation* **54** (5), 809–812.
123. Siddiqui, M.T., Garrity, E.R. and Husain, A.N. (1996). Bronchiolitis obliterans organizing pneumonia-like reactions: a nonspecific response or an atypical form of rejection or infection in lung allograft recipients? *Hum. Pathol.* **27** (7), 714–719.

124. Li, X.C., Tucker, J., Zhong, R., Jevnikar, A. and Grant, D. (1993). The role of gut-associated lymphoid tissue in intestinal rejection. Allogeneic response to rat intestinal lymphocytes. *Transplantation* **56** (1), 244–247.
125. Hayry, P., Isoniemi, H., Yilmaz, S. *et al.* (1993). Chronic allograft rejection. *Immunol. Rev.* **134**, 33–81.
126. Azuma, H. and Tilney, N.L. (1994). Chronic graft rejection. [Review]. *Curr. Opin. Immunol.* **6** (5), 770–776.
127. Hayry, P., Mennander, A., Yilmaz, S. *et al.* (1992). Towards understanding the pathophysiology of chronic rejection. [Review]. *Clin. Invest.* **70** (9), 780–790.
128. Paul, L.C. (1995). Immunobiology of chronic renal transplant rejection. [Review]. *Blood Purif.* **13** (3–4), 206–218.
129. Oguma, S., Banner, B., Zerbe, T., Starzl, T. and Demetris, A.J. (1988). Participation of dendritic cells in vascular lesions of chronic rejection of human allografts. *Lancet* **2** (8617), 933–936.
130. Wakabayashi, T. and Onoda, H. (1991). Interdigitating reticulum cells in human renal grafts. *Virchows Arch. A: Pathol. Anat. Histopathol.* **418** (2), 105–110.
131. Zinkernagel, R.M., Ehl, S., Aichele, P., Oehen, S., Kundig, T. and Hengartner, H. (1997). Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. *Immunol. Rev.* **156**, 199–209.
132. Zinkernagel, R.M. (1997). Immunology and immunity studied with viruses. *Ciba Found. Symp.* **204**, 105–125; discussion, 125–129.
133. Hruban, R.H., Beschorner, W.E., Baumgartner, W.A. *et al.* (1988). Depletion of bronchus-associated lymphoid tissue associated with lung allograft rejection. *Am. J. Pathol.* **132** (1), 6–11.
134. MacPherson, G.G., Murphy, M.J. Jr, and Morris, B. (1977). The traffic of mononuclear phagocytes through renal allografts in sheep. *Transplantation* **24** (1), 16–28.
135. Durham, J.R., Nakhleh, R.E., Levine, A. and Levine, T.B. (1995). Persistence of interstitial inflammation after episodes of cardiac rejection associated with systemic infection. *J. Heart Lung Transplant.* **14** (4), 774–780.
136. Heemann, U.W., Tullius, S.G., Schmid, C., Philipp, T. and Tilney, N.L. (1996). Infection-associated cellular activation accelerates chronic renal allograft rejection in rats. *Transplant. Int.* **9** (2), 137–140.
137. Manez, R., White, L.T., Linden, P. *et al.* The influence of HLA matching on cytomegalovirus hepatitis and chronic rejection after liver transplantation. *Transplantation* **55** (5), 1067–1071.
138. Wallwork, J. (1994). Risk factors for chronic rejection in heart and lungs—why do hearts and lungs rot? *Clin. Transplant.* **8** (3 Pt 2), 341–344.
139. Whitehead, B., Rees, P., Sorensen, K. *et al.* Incidence of obliterative bronchiolitis after heart-lung transplantation in children. *J. Heart Lung Transplant.* **12** (6 Pt 1), 903–908.
140. Starzl, T.E., Marchioro, T.L. and Waddell, W.R. (1963). The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. *Surg. Gynecol. Obstet.* **117**, 385–395.
141. Mazariegos, G.V., Reyes, J., Marino, I.R. *et al.* (1997). Weaning of immunosuppression in liver transplant recipients. *Transplantation* **63** (2), 243–249.
142. Larsen, C.P., Morris, P.J. and Austyn, J.M. (1990). Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel pathway for initiation of rejection. *J. Exp. Med.* **171** (1), 307–314.
143. Starzl, TE. (1964). *Host-Graft Adaptation. Experience in Renal Transplantation.* W.B. Saunders, Philadelphia PA, pp. 164–170.
144. Webb, S., Morris, C. and Sprent, J. (1990). Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* **63** (6), 1249–1256.
145. Sprent, J., Gao, E.K. and Webb, S.R. (1990). T cell reactivity to MHC molecules: immunity versus tolerance. *Science* 1990, **248** (4961), 1357–1363.
146. Webb, S.R., Hutchinson, J., Hayden, K. and Sprent, J. (1994). Expansion/deletion of mature T cells exposed to endogenous superantigens in vivo. *J. Immunol.* **152** (2), 586–597.
147. Lu, L., Qian, S., Starzl, T.E., Lynch, D.H. and Thomson, A.W. (1997). Blocking of the B7-CD28 pathway increases the capacity of FasL$^+$ (CD95L$^+$) dendritic cells to kill alloactivated T cells. *Adv. Exp. Med. Biol.* **417**, 275–282.
148. Lu, L., Qian, S., Hershberger, P.A., Rudert, W.A., Lynch, D.H. and Thomson, A.W. (1997). Fas ligand (CD95L) and B7 expression on dendritic cells provide counter-regulatory signals for T cell survival and proliferation. *J. Immunol.* **158** (12), 5676–5684.

149. Demetris, A.J., Murase, N., Delaney, C.P., Woan, M., Fung, J.J. and Starzl, T.E. (1995). The liver allograft, chronic (ductopenic) rejection, and microchimerism: what can they teach us? *Transplant. Proc.* **27** (1), 67–70.
150. Owen, R.D. (1945). Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* **102**, 400–401.
151. Main, J.M. and Prehn, R.T. (1955). Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *J. Natl Cancer Inst.* **15**, 1023–1029.
152. Monaco, A.E., Wood, M.L. and Russell, P.S. (1966). Studies of heterologous anti-lymphocyte serum in mice. III. Immunologic tolerance and chimerism produced across the H-2 locus with adult thymectomy and anti-lymphocyte serum. *Ann. NY Acad. Sci.* **129**, 190–209.
153. Slavin, S., Fuks, Z., Weiss, L. and Morecki, S. (1989). Mechanisms of tolerance in chimeric mice prepared with total lymphoid irradiation. *ICN-UCLA Symp. Mol. Cell. Biol.* **17**, 383.
154. Ildstad, S.T. and Sachs, D.H. (1984). Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature*, **307** (5947), 168–170.
155. Ildstad, S.T., Wren, S.M., Bluestone, J.A., Barbieri, S.A. and Sachs, D.H. (1985). Characterization of mixed allogeneic chimeras. Immunocompetence, in vitro reactivity, and genetic specificity of tolerance. *J. Exp. Med.* **162** (1), 231–244.
156. Alard, P., Matriano, J.A., Socarras, S., Ortega, M.A. and Streilein, J.W. (1995). Detection of donor-derived cells by polymerase chain reaction in neonatally tolerant mice. Microchimerism fails to predict tolerance. *Transplantation* **60** (10), 1125–1130.
157. Hayashi, H., LeGuern, C., Sachs, D.H. and Sykes, M. (1996). Long-term engraftment of preculture post-5-fluorouracil allogeneic marrow in mice conditioned with a nonmyeloablative regimen: relevance for a gene therapy approach to tolerance induction. *Transplant. Immunol.* **4** (1), 86–90.
158. Sykes, M. (1996). Chimerism and central tolerance. *Curr. Opin. Immunol.* **8** (5), 694–703.
159. Sykes, M., Szot, G.L., Swenson, K.A. and Pearson, D.A. (1997). Induction of high levels of allogeneic hematopoietic reconstitution and donor-specific tolerance without myelosuppressive conditioning. *Nature Medicine* **3** (7), 783–787.
160. Billingham, R. and Brent, L. (1956). Quantitative studies on transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease. *Phil. Trans. R. Soc. Lond. (Biol.)* **242**, 439–477.
161. Russell, P.S. (1962). Modification of runt disease in mice by various means. *Transplantation: Ciba Foundation Symposium* (eds. Wolstenholme, C.E.W., Cameron, M.P., London, J., Churchill, A). Little Brown, Boston.
162. Jankowski, R.A. and Ildstad, S.T. (1997). Chimerism and tolerance: from freemartin cattle and neonatal mice to humans. *Hum. Immunol.* **52** (2), 155–161.
163. Kaufman, C.L., Colson, Y.L., Wren, S.M., Watkins, S., Simmons, R.L. and Ildstad, S.T. (1994). Phenotypic characterization of a novel bone marrow-derived cell that facilitates engraftment of allogeneic bone marrow stem cells. *Blood* **84** (8), 2436–2446.
164. Gaines, B.A., Colson, Y.L., Kaufman, C.L. and Ildstad, S. (1996). Facilitating cells enable engraftment of purified fetal liver stem cells in allogeneic recipients. *Exp. Hematol.* **24** (8), 902–913.
165. Ricordi, C., Karatzas, T., Selvaggi, G. *et al.* (1995). Multiple bone marrow infusions to enhance acceptance of allografts from the same donor. *Ann. NY Acad Sci.* **770**, 345–350.
166. Ricordi, C., Ildstad, S.T., Demetris, A.J., Abou el-Ezz, A.Y., Murase, N. and Starzl, T.E. (1992). Donor dendritic cell repopulation in recipients after rat-to-mouse bone-marrow transplantation [letter]. *Lancet* **339** (8809), 1610–1611.
167. Sharabi, Y., Abraham, V.S., Sykes, M. and Sachs, D.H. (1992). Mixed allogeneic chimeras prepared by a non-myeloablative regimen: requirement for chimerism to maintain tolerance. *Bone Marrow Transplant.* **9** (3), 191–197.
168. Khan, A., Tomita, Y. and Sykes, M. (1996). Thymic dependence of loss of tolerance in mixed allogeneic bone marrow chimeras after depletion of donor antigen. Peripheral mechanisms do not contribute to maintenance of tolerance. *Transplantation* **62** (3), 380–387.
169. Touraine, J.L., Roncarolo, M.G., Plotnicky, H., Bachetta, R. and Spits, H. (1993). T lymphocytes from human chimeras do recognize antigen in the context of allogeneic determinants of the major histocompatibility complex. *Immunol. Lett.* **39** (1), 9–12.

170. Roncarolo, M.G., Bacchetta, R., Bigler, M., Touraine, J.L., de Vries, J.E. and Spits, H. (1991). A SCID patient reconstituted with HLA-incompatible fetal stem cells as a model for studying transplantation tolerance. *Blood Cells* **17** (2), 391–402.
171. Roncarolo, M.G., Yssel, H., Touraine, J.L. *et al.* (1988). Antigen recognition by MHC-incompatible cells of a human mismatched chimera. *J. Exp. Med.* **168** (6), 2139–2152.
172. Orloff, M.S., DeMara, E.M., Coppage, M.L. *et al.* (1995). Prevention of chronic rejection and graft arteriosclerosis by tolerance induction. *Transplantation* **59** (2), 282–288.
173. Colson, Y.L., Zadach, K., Nalesnik, M. and Ildstad, S.T. (1995). Mixed allogeneic chimerism in the rat. Donor-specific transplantation tolerance without chronic rejection for primarily vascularized cardiac allografts. *Transplantation* **60** (9), 971–980.
174. Tomita, Y., Khan, A. and Sykes, M. (1994). Role of intrathymic clonal deletion and peripheral anergy in transplantation tolerance induced by bone marrow transplantation in mice conditioned with a nonmyeloablative regimen. *J. Immunol.* **153** (3), 1087–1098.
175. Thomas, J., Carver, M., Cunningham, P., Park, K., Gonder, J. and Thomas, F. (1987). Promotion of incompatible allograft acceptance in rhesus monkeys given posttransplant antithymocyte globulin and donor bone marrow. I. In vivo parameters and immunohistologic evidence suggesting microchimerism. *Transplantation* **43** (3), 332–338.
176. Anderson, G., Moore, N.C., Owen, J.J. and Jenkinson, E.J. (1996). Cellular interactions in thymocyte development. *Annu. Rev. Immunol.* **14**, 73–99.
177. DeKoning, J, DiMolfetto, L, Reilly, C., Wei, Q., Havran, W.L. and Lo, D. (1997). Thymic cortical epithelium is sufficient for the development of mature T cells in relB-deficient mice. *J. Immunol.* **158** (6), 2558–2566.
178. Lo, D., Reilly, C.R., Burkly, L.C., DeKoning, J., Laufer, T.M. and Glimcher, L.H. (1997). Thymic stromal cell specialization and the T-cell receptor repertoire. *Immunol. Res.* **16** (1), 3–14.
179. Markowitz, J.S., Auchincloss, H. Jr., Grusby, M.J. and Glimcher, L.H. (1993). Class II-positive hematopoietic cells cannot mediate positive selection of $CD4^+$ T lymphocytes in class II-deficient mice. *Proc. Natl Acad. Sci. USA* **90** (7), 2779–2783.
180. Kappler, J.W., Roehm, N. and Marrack. P. (1987). T cell tolerance by clonal elimination in the thymus. *Cell* **49** (2), 273–280.
181. Zepp, F., Cussler, K., Mannhardt, W., Schofer, O. and Schulte-Wissermann, H. (1987). Intrathymic tolerance induction: determination of tolerance to class II major histocompatibility complex antigens in maturing T lymphocytes by a bone marrow-derived non-lymphoid thymus cell. *Scand. J. Immunol.* **26** (6), 589–601.
182. Hengartner, H., Odermatt, B., Schneider, R. *et al.* (1988). Deletion of self-reactive T cells before entry into the thymus medulla. *Nature* **336** (6197), 388–390.
183. Speiser, D.E., Lees, R.K., Hengartner, H., Zinkernagel, R. MacDonald, H.R. (1989). Positive and negative selection of T cell receptor V beta domains controlled by distinct cell populations in the thymus. *J. Exp. Med.* **170** (6), 2165–2170.
184. Tanaka, Y., Mamalaki, C., Stockinger, B. and Kioussis, D. (1993). In vitro negative selection of alpha beta T cell receptor transgenic thymocytes by conditionally immortalized thymic cortical epithelial cell lines and dendritic cells. *Eur. J. Immunol.* **23** (10), 2614–2621.
185. Ferrero, I., Anjuere, F., MacDonald, H.R. and Ardavin, C. (1997). In vitro negative selection of viral superantigen-reactive thymocytes by thymic dendritic cells. *Blood* **90** (5), 1943–1951.
186. Naspetti, M., Aurrand-Lions, M., DeKoning, J. *et al.* (1997). Thymocytes and RelB-dependent medullary epithelial cells provide growth-promoting and organization signals, respectively, to thymic medullary stromal cells. *Eur. J. Immunol.* **27** (6), 1392–1397.
187. Roncarolo, M.G., Yssel, H., Touraine, J.L., Betuel, H., De Vries, J.E. and Spits, H. (1988). Autoreactive T cell clones specific for class I and class II HLA antigens isolated from a human chimera. *J. Exp. Med.* **167** (5), 1523–1534.
188. Yagihashi, A., Takahashi, S., Murase, N., Starzl, T.E. and Iwaki, Y. (1995). A monoclonal anti-body (L21-6) recognizing an invariant chain expressed on the cell surface in rats with the exception of the BN ($RT1^n$): a study of tissue and strain distribution. *Transplant. Proc.* **27**, 1519–1521.
189. van Schilfgaarde, R., Hermans, P., Terpstra, J.L. and van Breda Vriesman, P.J. (1980). Role of mobile passenger lymphocytes in the rejection of renal and cardiac allografts in the rat. A passenger lymphocyte-mediated graft-versus-host reaction amplifies the host response. *Transplantation* **29** (3), 209–213.

190. Nemlander, A., Soots, A., von Willebrand, E., Husberg, B. and Hayry, P. (1982). Redistribution of renal allograft-responding leukocytes during rejection. II. Kinetics and specificity. *J. Exp. Med.* **156** (4), 1087–1100.
191. Thomson, A.W., Lu, L., Wan, Y., Qian, S., Larsen, C.P. and Starzl, T.E. (1995). Identification of donor-derived dendritic cell progenitors in bone marrow of spontaneously tolerant liver allograft recipients. *Transplantation* **60** (12), 1555–1559.
192. Thomson, A.W., Lu, L., Murase, N., Demetris, A.J., Rao, A.S. and Starzl, T.E. (1995). Microchimerism, dendritic cell progenitors and transplantation tolerance. *Stem Cells* **13** (6), 622–639.
193. Starzl, T.E., Demetris, A.J., Murase, N., Thomson, A.W., Trucco, M. and Ricordi, C. (1993). Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation. *Immunol. Today* **14** (6), 326–332.
194. Adams, D.H. and Hutchinson, I.V. (1997). Microchimerism and graft tolerance: cause or effect? [comment]. *Lancet* **349** (9062), 1336–1337.
195. Burlingham, W.J., Grailer, A.P., Fechner, J.H. Jr., *et al.* (1995). Microchimerism linked to cytotoxic T lymphocyte functional unresponsiveness (clonal anergy) in a tolerant renal transplant recipient. *Transplantation* **59** (8), 1147–1155.
196. Caillat-Zucman, S., Legendre, C., Suberbielle, C. *et al.* (1994). Microchimerism frequency two to thirty years after cadaveric kidney transplantation. *Hum. Immunol.* **41** (1), 91–95.
197. Reinsmoen, N.L., McSherry, C., Chavers, B., Hertz, M.I. and Matas, A.J. (1995). Correlation of donor antigen-specific hyporeactivity with allogeneic microchimerism in kidney and lung recipients. *Pediatr. Nephrol.* **9** (Suppl.), S35–39.
198. Schlitt, H.J., Hundrieser, J., Hisanaga, M. *et al.* (1994). Patterns of donor-type microchimerism after heart transplantation. *Lancet* **343** (8911), 1469–1471.
199. Schlitt, H.J., Hundrieser, J., Ringe, B. and Pichlmayr, R. (1994). Donor-type microchimerism associated with graft rejection eight years after liver transplantation [letter]. *N. Engl. J. Med.* **330** (9), 646–647.
200. Sivasai, K.S., Alevy, Y.G., Duffy, B.F. *et al.* (1997). Peripheral blood microchimerism in human liver and renal transplant recipients: rejection despite donor-specific chimerism. *Transplantation* **64** (3), 427–432.
201. Suberbielle, C., Caillat-Zucman, S., Legendre, C. *et al.* (1994). Peripheral microchimerism in long-term cadaveric-kidney allograft recipients. *Lancet* **343** (8911), 1468–1469.
202. Matzinger, P. and Guerder, S. (1989). Does T-cell tolerance require a dedicated antigen-presenting cell? *Nature* **338** (6210), 74–76.
203. Zhang, L.I., Martin, D.R., Fung-Leung, W.P., Teh, H.S. and Miller, R.G. (1992). Peripheral deletion of mature $CD8^+$ antigen-specific T cells after in vivo exposure to male antigen. *J. Immunol.* **148** (12), 3740–3745.
204. Fu, F., Li, Y., Qian, S. *et al.* (1996). Costimulatory molecule-deficient dendritic cell progenitors (MHC class II^+, $CD80^{dim}$, $CD86^-$) prolong cardiac allograft survival in nonimmunosuppressed recipients. *Transplantation* **62** (5), 659–665.
205. Finkelman, F.D., Lees, A., Birnbaum, R., Gause, W.C. and Morris, S.C. (1996). Dendritic cells can present antigen in vivo in a tolerogenic or immunogenic fashion. *J. Immunol.* **157** (4), 1406–1414.
206. Inaba, K., Pack, M., Inaba, M., Sakuta, H., Isdell, F. and Steinman, R.M. (1997). High levels of a major histocompatibility complex II-self peptide complex on dendritic cells from the T cell areas of lymph nodes. *J. Exp. Med.* **186** (5), 665–672.
207. Saleem, M., Sawyer, G.J., Schofield, R.A., Seymour, N.D., Gustafsson, K. and Fabre, J.W. (1997). Discordant expression of major histocompatibility complex class II antigens invariant chain in interstitial dendritic cells. Implications for self-tolerance and immunity. *Transplantation* **63** (8), 1134–1138.
208. Kauffman, S.A. (1993). *The Origins of Order: Self-Organization and Selection in Evolution.* Oxford University Press, Oxford.
209. Hill, A.V., Yates, S.N., Allsopp, C.E. *et al.* (1994). Human leukocyte antigens and natural selection by malaria. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **346** (1317), 379–385.
210. Klein, J. and O'Huigin, C. (1994). MHC polymorphism and parasites. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **346** (1317), 351–357; discussion, 357–358.
211. Coutinho, A. (1989). Beyond clonal selection and network. *Immunol. Rev.* **110**, 63–87.

212. Chen-Woan, M., Delaney, C.P., Fournier, V. *et al.* (1995). A new protocol for the propagation of dendritic cells from rat bone marrow using recombinant GM-CSF, and their quantification using the mAb OX-62. *J. Immunol. Methods* **178** (2), 157–171.
213. Chen-Woan, M., Delaney, C.P., Fournier, V. *et al.* (1996). In vitro characterization of rat bone marrow-derived dendritic cells and their precursors. *J. Leukocyte Biol.* **59** (2), 196–207.
214. Beschorner, W.E. and Armas, O.A. (1991). Loss of medullary dendritic cells in the thymus after cyclosporine and irradiation. *Cell Immunol.* **132** (2), 505–514.

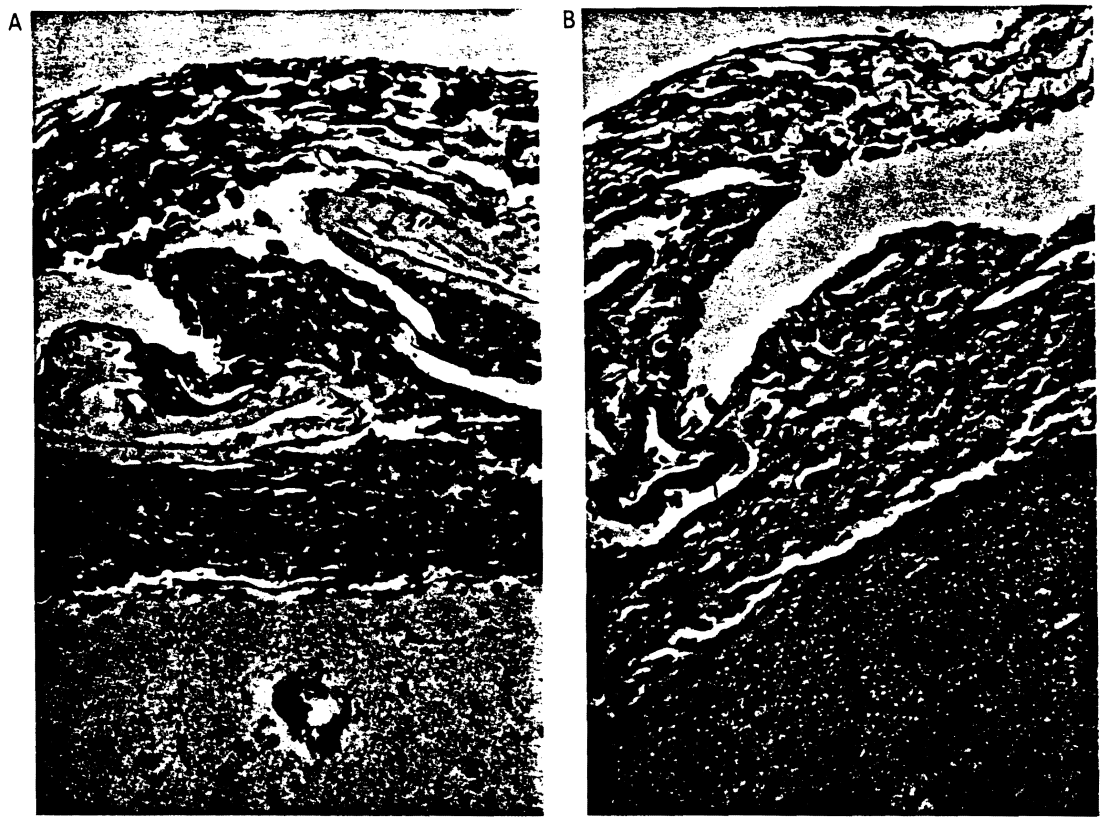

Plate 19.4. Juvenile xanthogranuloma family. (A) 12-year-old girl had meningitic involvement. (B) Fascin immunostain is strongly positive on JXG cells.

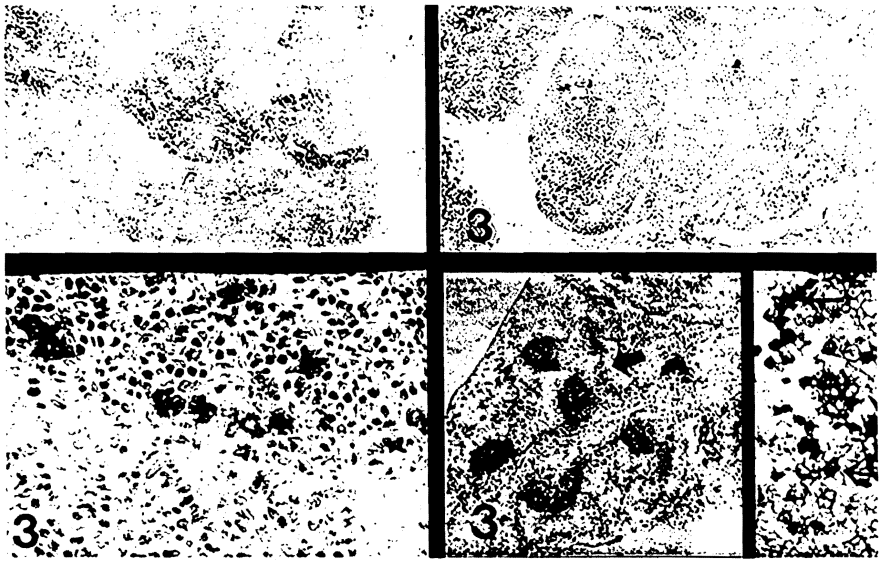

Plate 20.1. Identification of donor cells in the recipient spleen and thymus. Series of photomicrographs taken from sections of the recipient spleen (all except upper right frame) and thymus (upper right frame), 3 days after transplantation of a rat liver allograft. The recipients were treated with immunosuppression and all of the tissues are stained with a monoclonal antibody that reacts with MHC class II antigens of the donor, but not that of the recipient [188]. Note the presence of donor cells in the marginal zone and B cell follicles in the spleen in the upper left, lower left and lower right frames. Donor MHC class II^+ cells are also detected in the thymic medulla (upper right). In the lower right frames, the tissue is also stained for BrdU, which labels cells synthesizing DNA. Note that the presence of donor cells in the recipient spleen is associated with proliferation of both cell populations [63].

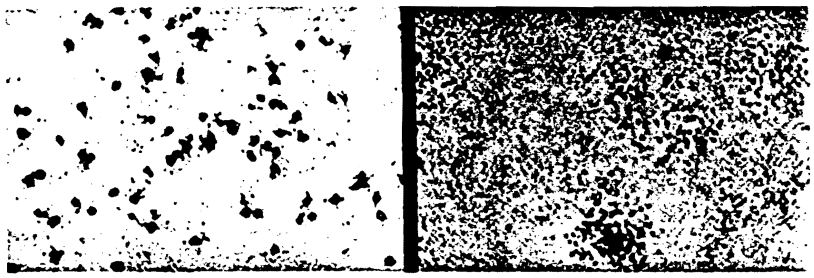

Plate 20.2. Donor CFU-C in recipient bone marrow. Photomicrographs of a colony-forming unit-culture (CFU-C) assay using a fibrin clot system. The cultured cells were obtained from the bone marrow of a recipient rat, 10 days after liver transplantation, and stained for donor MHC class II antigens. Note the presence of donor hematopoietic colonies on the right. On the left, note the presence of myelomono-cytic cells, some of which have a characteristic dendritic morphology, typical of rat dendritic cells [212, 213].

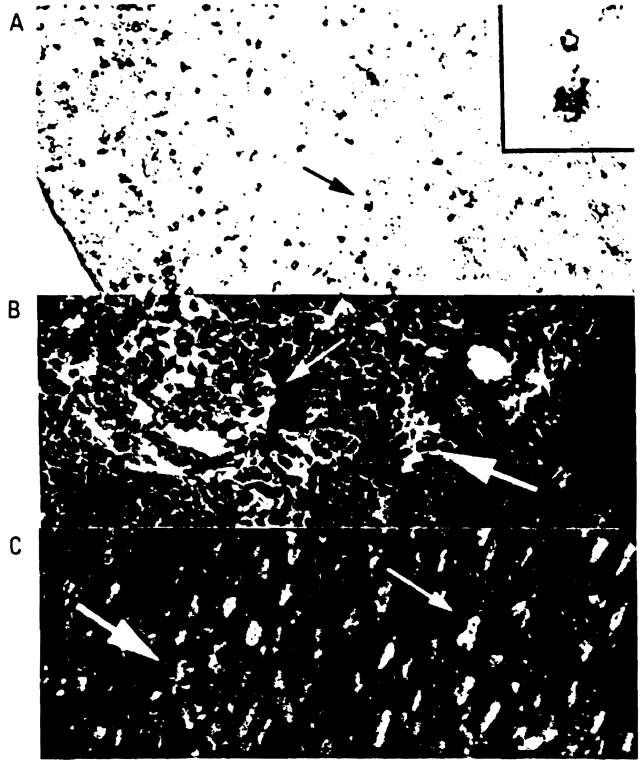

Plate 20.3. Donor dendritic cells within recipient thymus. Thymus of a rat liver allograft recipient, 30 days after transplantation. The tissue is double stained for donor MHC class II-positive cells (rust color) and ED2 (blue), which highlights tissue macrophages. Note the presence of the donor dendritic-shaped cell in the recipient thymus (arrow and inset). The 'shrunken' medulla in this recipient can be attributed to the transient 2-week course of FK506, which is known to cause damage to the thymic medulla [214]. It may also create a milieu conducive to the recruitment of donor DC progenitors [3, 214]. (B) Donor DC can also be detected in the spleen. This is a section of recipient spleen, obtained 30 days after liver transplantation and double stained for donor MHC class II (red) and OX62, an integrin, known to be expressed by rat DC [213]. Note the presence of the yellow, doubly labeled donor DC. (C) Challenge donor heart allograft 100 days after transplantation in a recipient rendered tolerant to the donor by a previous liver allograft. This section is double stained for donor MHC class II (red) and OX62. Note the red, single positive MHC class II^+ donor interstitial cell (large arrow) and yellow, double positive donor DC. Even though this recipient received no immunosuppression, donor DC persisted in the challenge allograft and it was resistant to chronic rejection [120].