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Involvement of the direct and indirect pathways of allorecognition in tolerance induction

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It is generally accepted that there are two pathways of allorecognition, direct and indirect, that together contribute to allograft rejection. Although it has been suggested that the direct pathway predominates during early acute rejection and that the indirect pathway provides a continuous supply of alloantigen responsible for chronic rejection, the true relative contribution of each pathway to the overall rejection process is still not entirely known. It is clear, however, that any strategies designed to achieve the ultimate goal in transplantation, the induction of tolerance, will need to take into account both pathways. This review seeks to explore the involvement of the direct and indirect pathways of allorecognition on a mechanistic level as it relates to the induction of tolerance. A brief historical perspective is included for each pathway as well as a comprehensive review of the mechanisms felt to be active during tolerance induction.

Keywords: direct pathway; indirect pathway; tolerance; transplantation; MHC peptides; allorecognition

1. INTRODUCTION

The principal targets of the immune response to the allograft are the major histocompatibility complex (MHC) molecules present on donor cells. T-cell recognition of these alloantigens is the central and primary event responsible for the initiation of the complex network of cellular and humoral interactions that ultimately leads to allograft rejection (Womer *et al.* 2000*a*; Gould & Auchincloss 1999). Classically, allorecognition was felt to occur solely via the direct pathway, whereby recipient T cells recognize a variety of peptides complexed to intact MHC molecules on the surface of donor antigen-presenting cells (APCs). Evidence for this notion comes from the strong direct stimulation seen in a primary allogeneic mixed lymphocytic reaction (MLR), reflecting the uniquely high precursor frequency of T cells with direct specificity for allogeneic MHC molecules (Liu *et al.* 1993*b*). Originally termed 'cross-priming' to describe the presentation of minor alloantigens by recipient APCs (Bevan 1976), the indirect pathway was later postulated by Lechler *et al.* (Lechler & Batchelor 1982) to include donor MHC and is now recognized as a significant component of the alloresponse. The question remains, however, as to what the relative contribution of each pathway is to the rejection process.

The ultimate goal in transplantation is the induction of transplantation tolerance. Greater understanding of the cellular and molecular mechanisms of the alloimmune response has prompted the development of novel strategies that may one day obviate the need for immunosuppressive medications in human transplantation (Dong *et al.* 1999; Waldmann 1999). The present article seeks to explore the involvement of the direct and indirect pathways of allorecognition on a mechanistic level as it relates to the induction of tolerance. A brief historical perspective is included for each pathway as well as a comprehensive review of the mechanisms felt to be active during tolerance induction. For direct allorecognition, these mechanisms include an attenuated alloresponse as the passenger leucocytes exit the allograft with time post-transplantation, T-cell inactivation induced by the parenchymal cells of the leucocyte-depleted allograft, and activation-associated tolerance upon transit of the donor leucocytes to the lymphoid compartments of the recipient. For indirect allorecognition, oral and intrathymic alloantigen presentation are reviewed, as well as newer *in vitro* peptide studies designed to elucidate the cellular interactions and intracellular events important in tolerance induction. Finally, a brief discussion of possible ways that the direct and indirect pathways may interact with each other to achieve tolerance is included.

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2. DIRECT ALLORECOGNITION

(a) *Overview*

One of the earliest reports of what is now commonly referred to as the direct pathway of allorecognition was made by Snell in 1957, when he suggested that leucocytes associated with the transplanted tissue were the major source of tissue immunogenicity (Snell 1957). By this

assertion, he was simply stating formally the common observation that immunization of a recipient animal with donor spleen or lymph node cells would sensitize a tumour allograft, whereas antigen extracts of the tumour were only weakly immunogenic (Kaliss & Kandutsch 1956). At that time, it was believed that the antigen density or the form of antigen presentation on the surface of leucocytes rendered these transplantation antigens more immunogenic.

Further elucidation of the role allograft leucocytes play in immunogenicity came later with initial studies of T-cell co-stimulation. Using cloned tumour cell lines as the source of allogeneic stimulator cells, Talmage *et al.* (1977) demonstrated that tumours of lymphoreticular origin expressed a stimulating phenotype, whereas epithelial cell line tumours did not. It later became clear that the parenchymal cells of the allograft lacked sufficient co-stimulatory activity to activate recipient T cells. These findings implicated passenger leucocytes as the major immunogenic component of the allograft and provided the impetus for subsequent studies designed to reduce allograft immunogenicity through removal of leucocytes prior to transplantation.

The following sections describe not only how the absence of donor leucocytes in the allograft is associated with tolerance, but also how these cells may lead to peripheral activation and subsequent death of donor-reactive T cells, rendering an allograft often incapable of stimulating the alloresponse necessary for graft rejection.

(b) *The passenger leucocyte theory*

Although reports as early as the 1930s suggested a clinical benefit of organ culture on reducing the immunogenicity of endocrine organs prior to transplantation (Stone *et al.* 1934), without an adequate theoretical base it was not until the 1970s that interest in the area was rekindled. Also unclear from these early experiments was whether the benefit resulted from modulation of antigen expression or, indeed, the postulated loss of passenger leucocytes. To better address this issue, Lafferty *et al.* studied the effect of organ culture on the survival of thyroid allografts in mice (Lafferty *et al.* 1976). Prolongation of allograft survival was demonstrated with increasing time in culture, and allografts showed normal function with no evidence of chronic rejection. Moreover, cultured allografts could be rejected if the host's immune system was stimulated with viable donor leucocytes, and hosts with these functioning allografts were not tolerant of donor tissues as demonstrated by the ability to reject a second uncultured allograft from the same donor.

Though these elegant experiments seemed to confirm the passenger leucocyte theory, there were still some suggestions that the allograft vascular endothelium might provide the major stimulus for allograft immunity. Vascular endothelium degenerates during organ culture (Parr *et al.* 1980), and its destruction could account for the reduction in tissue immunogenicity achieved with organ culture. Subsequent studies utilized cyclophosphamide treatment of donor animals, which causes a profound drop in the capacity of spleen cells to stimulate allogeneic T cells but which has no obvious effect on thyroid endothelium. After such treatment alone, 30% of thyroid allografts functioned normally, suggesting that the donor endothelium was not a major source of tissue immunogenicity (Lafferty & Woolnough 1977).

Batchelor further clarified the role of passenger leucocytes in a series of experiments using rat renal allografts. It was demonstrated that long-surviving, passively enhanced MHC-incompatible renal allografts, when transplanted from a primary to a secondary recipient of the same genotype, did not elicit T-cell alloimmunity in the secondary recipient (Batchelor *et al.* 1979). Restoration of immunogenicity to these allografts was later demonstrated by the addition of donor-strain dendritic cells (DCs) (Lechler & Batchelor 1982).

(c) *T-cell inactivation by allograft parenchymal cells*

Following alloantigen recognition by the direct pathway, an intense infiltration of lymphokine-secreting alloreactive T cells occurs in the graft. Interferon (IFN)-γ induces the expression of MHC class II on endothelial and epithelial cells, conferring the ability to present antigen to $CD4^+$ T helper (Th) cells (Bal *et al.* 1990; Fuggle *et al.* 1986; Steinhoff *et al.* 1988). As passenger leucocytes leave the graft with time post-transplantation, these endothelial and epithelial cells remain as the only donor MHC class-II-expressing cells. The consequences of antigen recognition by such a mechanism, however, are still not entirely clear. Several studies have sought to determine the fate of these T cells, with results ranging from deletion of naive T cells, silencing and/or switching to a Th2 phenotype in antigen-specific T-cell clones, and anergy in memory cells, with failure to secrete interleukin (IL) 2 (Lo *et al.* 1989; Gaspari *et al.* 1988; Burkly *et al.* 1989, 1990; Lombardi *et al.* 1997; Marelli-Berg *et al.* 1997). Moreover, addition of recombinant IL-2 restores the response of these putative anergic T cells to alloantigen (Braun *et al.* 1993*a*). Still other studies have demonstrated lack of proliferation of resting T cells that could be overcome by the addition of B7, suggesting that non-professional APCs lack effective co-stimulatory ability (Marelli-Berg *et al.* 1996). These observations raise the question as to whether T cells that have been recently activated *in vivo* exhibit B7-independent proliferation or whether a B7-independent phenotype might arise in T cells as a consequence of repetitive antigenic stimulation (Marelli-Berg *et al.* 1999).

More recently, the differential outcome of endothelial and epithelial cell antigen presentation was demonstrated by Marelli-Berg *et al.* (1999). T-cell clones that require B7-mediated co-stimulation to be activated were unable to proliferate to antigen presented by endothelial cells but could proliferate on subsequent re-challenge with antigen presented by professional APCs. Antigen presentation by epithelial cells, however, induced permanent non-responsiveness. These findings are relevant when considered in the context of a possible physiological role of endothelial and epithelial cells as APCs. In an immune response, the purpose of the endothelial cell may be to facilitate the entry of activated T cells into the tissue without altering its functional behaviour or activation status. Once in the tissue, antigen-specific T cells could either interact with recruited monocyte and macrophage APCs to be reactivated or, alternatively, with the parenchymal cells to be functionally inactivated. In the transplant setting, once donor leucocytes have left the

allograft, antigen recognition via parenchymal cells may lead to a form of donor-specific tolerance in T cells previously activated by the direct pathway.

(d) *Activation-associated tolerance*

Early observations that liver transplants in certain porcine (Calne *et al.* 1969), mouse (Qian *et al.* 1994), and rat (Kamada *et al.* 1981) strains are accepted across the MHC barrier have furthered our understanding of the role the passenger leucocyte may play in the induction of tolerance after its exit from the allograft. In the rat strain combination PVG→DA, lymphocytes of immunized animals are 1000 times more potent than those of non-immunized animals in effecting graft destruction when transferred to irradiated hosts (Hall *et al.* 1978), with rapid rejection of skin, heart and kidney allografts of the donor strain. Liver allografts, however, not only fail to reject but render the recipient tolerant to subsequent solid organ or skin grafts of the donor strain (Kamada *et al.* 1981), with the ability to reverse ongoing rejection of heart allografts with greater efficacy than cyclosporine (Kamada & Wight 1984). Donor irradiation prior to transplantation, which reduces liver passenger leucocytes and hence the number of cells available for migration to recipient tissues, abrogates such tolerance (Sun *et al.* 1995). These findings stimulated interest in donor leucocytes and subsequent T-cell activation via the direct pathway as a possible mechanism of tolerance.

In a series of experiments using the PVG→DA strain combination, Bishop *et al.* (1996) have demonstrated an early upregulation of IL-2 and IFN-γ in tolerant lymphoid tissues compared with non-tolerant strain combinations. The donor irradiation, as previously mentioned, significantly reduced the number of donor leucocytes in these tissues and, in addition, partially prevented the superinduction of these two cytokines. Likewise, treatment of donor animals with corticosteroids at the time of transplantation, which suppresses upregulation of IL-2 and IFN-γ (Arya *et al.* 1984), reduced the subsequent survival of skin grafts of the donor strain. Parking of the irradiated liver in a normal PVG animal restored tolerance and reconstituted the number of leucocytes in lymphoid tissues. Although IL-2 and IFN-γ are generally regarded as promoters of immune responses such as rejection, these findings are consistent with other lines of evidence supporting activation-associated tolerance, where high levels of antigen induce antigen-reactive cells to proliferate rapidly but then to die or to become unresponsive (Webb *et al.* 1990; Von Boehmer 1993; Moskophidis *et al.* 1993; Rocha & Von Boehmer 1991; Critchfield *et al.* 1994). Admittedly, IL-2 and IFN-γ may merely represent markers of immune activation, with tolerance resulting from the activated cells themselves rather than the cytokines that they produce.

To characterize further the donor leucocytes felt responsible for induction of tolerance in the PVG→DA liver transplantation model, the same group studied the effect of intravenous injection of varied donor leucocyte types (Sun *et al.* 1996). Both liver and spleen leucocytes successfully reconstituted tolerance to irradiated livers but not to donor hearts. Deletion of T cells but not B cells or monocytes from this inoculum reduced survival time, implying a necessity for the T-cell subset. To determine whether the larger mass of the liver compared with the heart might be responsible for the tolerance induction, two hearts and two kidneys were transplanted. These combined organs were accepted, indicating a requirement for a large amount of donor tissue, in association with donor leucocytes, for activation-associated tolerance.

Although these reports do not adequately explain why livers are rejected in some strain combinations and not in others, a model for activation-associated tolerance is suggested, whereby low doses of antigen fail to stimulate a sufficient alloresponse and high doses of antigen result in deletion of alloreactive clones (Bishop *et al.* 1997). Studies demonstrating that injection of IL-2 after transplantation prevents tolerance in liver allografts would appear to contradict this model (Tu *et al.* 1997), implying that insufficient IL-2 production rather than overstimulation of T cells is responsible for the tolerance. However, it is possible that this large cohort of activated T cells requires more IL-2 than can be produced endogenously, leading to their exhaustion and subsequent deletion. Administration of exogenous IL-2 might fulfil their requirement for IL-2, thereby promoting their proliferation and maturation to effector cells with the capability of destroying the transplanted liver. Support for this hypothesis is provided by a recent study demonstrating a reduction of apoptotic activity and enhanced cytotoxic T-lymphocyte (CTL) activity in mouse liver grafts after the administration of IL-2 (Qian *et al.* 1997). These findings have important clinical implications, considering recent studies that suggest treatment with cyclosporine prevents the induction of activation-associated tolerance (Li *et al.* 1999; Wells *et al.* 1999).

3. INDIRECT ALLORECOGNITION

(a) *Overview*

Early studies documenting the presence of soluble, intact, donor MHC molecules at the site of the graft suggested that such circulating molecules could represent a source of donor proteins for presentation by recipient APCs (Charlton & Zmijewski 1970). Although the indirect pathway of allorecognition is the physiological mechanism of an immune response, it was not until the 1980s that this pathway was actually considered to contribute to the destruction of allografts. Lechler *et al.* noted that while retransplantation of passenger leucocyte-depleted rat kidney allografts did not elicit significant alloresponses, with time post-transplant, elevated serum urea levels and other signs of rejection were detected (Lechler & Batchelor 1982). In 1986, Sherwood *et al.* (1986) demonstrated that adoptive transfer of T-cell-depleted syngeneic splenocytes primed to donor antigen caused accelerated donor skin rejection, proving that alloantigen presentation by host APCs could be a potent route of allosensitization (Sherwood *et al.* 1986).

In vitro studies have confirmed the presentation of donor MHC peptides by recipient APCs for both class I (Chen *et al.* 1990; Essaket *et al.* 1990) and class II (De Koster *et al.* 1989) peptides. Likewise, T-cell recognition of MHC class I and II antigens in the context of self-MHC class I molecules has also clearly been shown to occur (Shinohara *et al.* 1986; Song *et al.* 1988; Kievits & Ivanyi 1991). Evidence from elution studies demonstrates that processing and presentation of MHC-derived

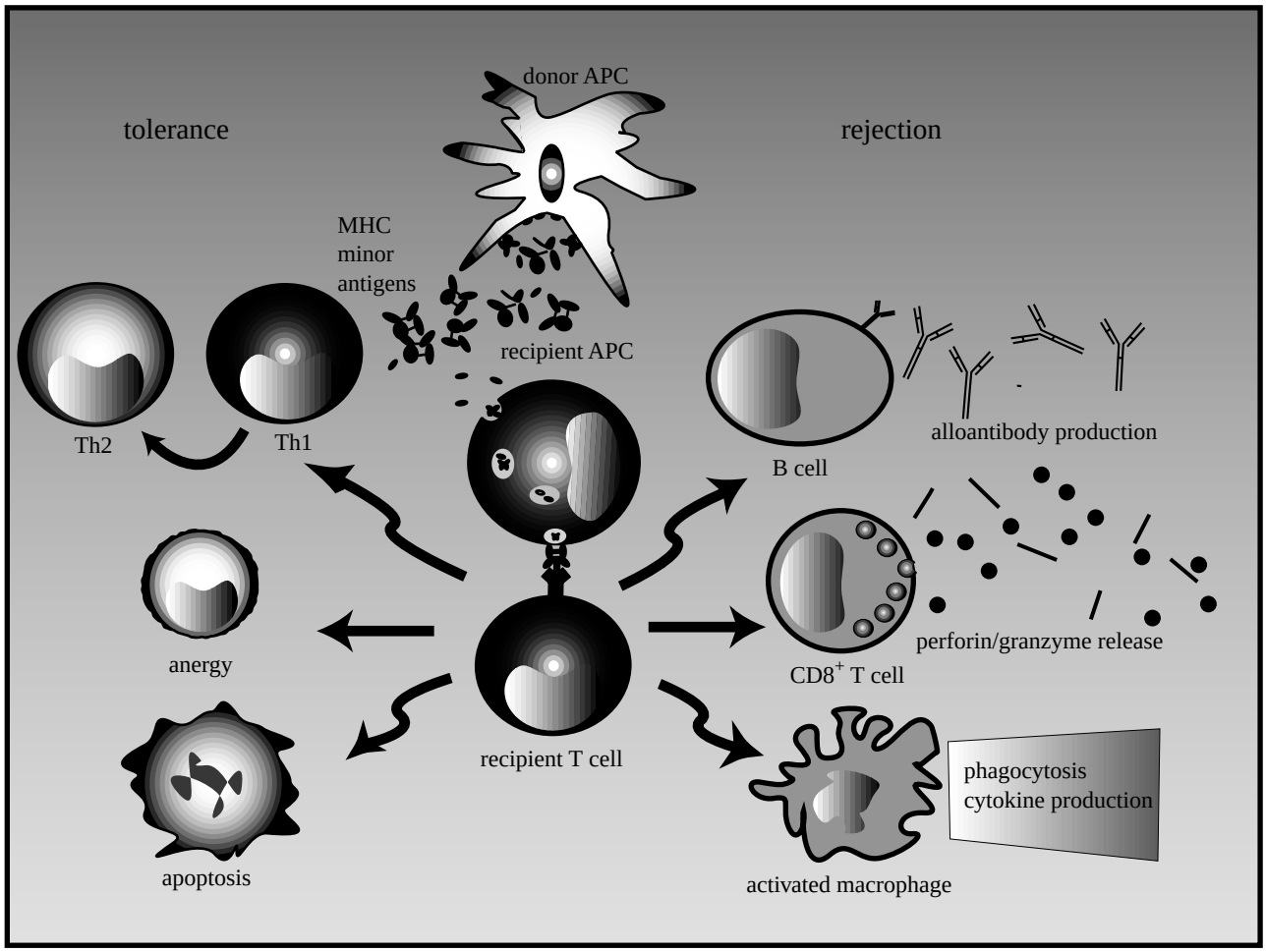

Figure 1. Dual role of indirect allorecognition in allograft rejection and tolerance. During indirect allorecognition, donor major histocompatibility molecules (MHC) and minor antigens are shed from donor antigen-presenting cells (APCs) to be processed by recipient APCs. These peptides are then presented to recipient $CD4^+$ T cells, which have the dual role of either activating the effector mechanisms of allograft rejection or the various regulatory processes associated with tolerance.

peptides are common physiological events *in vivo* (Chicz *et al.* 1992; Hunt *et al.* 1992). Subsequent studies have clearly demonstrated that during mouse (Benichou *et al.* 1992; Auchincloss *et al.* 1993), rat (Fangmann *et al.* 1992; Watschinger *et al.* 1994; Gallon *et al.* 1995) and human (Liu *et al.* 1992) allograft rejection, donor MHC molecules are processed and presented as peptides by recipient APCs. *In vivo* models (Lee *et al.* 1994) have confirmed *in vitro* data (Golding & Singer 1984) that self-restricted T helper cells can provide help for CTL induction during allograft rejection. Likewise, the indirect pathway is critical for alloantibody production (Steele *et al.* 1996), consistent with the normal physiological mechanism. Finally, self-restricted MHC class II allopeptide-specific T helper cell clones can transfer delayed type hypersensitivity (DTH) responses to naive animals *in vivo* (Chen *et al.* 1996; Waaga *et al.* 1998). These studies confirm that the indirect pathway is active in allograft rejection and can support the effector mechanisms necessary for allograft rejection.

In the light of recent evidence suggesting that indirect allorecognition may be the dominant pathway mediating chronic rejection (Braun *et al.* 1993*b*; Hornick *et al.* 1998; Ciubotariu *et al.* 1998; Vella *et al.* 1997*a*; Womer *et al.* 2000*b*), any efforts aimed at the induction of transplantation tolerance in humans must, therefore, take into account this pathway. Recent evidence suggests that rejection by the indirect pathway (but not the direct pathway) may be suppressed by Th1 to Th2 immune deviation (Li *et al.* 1998), a state easily achieved in certain animal models. Moreover, indirect allorecognition may be an absolute requirement for tolerance induction via co-stimulatory blockade (Yamada *et al.* 2001). Others have used the indirect pathway as a tool to induce tolerance through autologous bone marrow transfection with allogeneic antigens, with resulting donor-specific hyporesponsiveness (Fraser *et al.* 1995). Thus, it is clear that the indirect pathway may play a dual role (rejection versus tolerance) depending on whether presentation of donor peptides leads to activation of effector or regulatory cells (figure 1).

(b) *Peptide-based immunotherapy*

Recent insights into the principles of indirect antigen recognition have provided the basis for peptide-based immune strategies to modulate T-cell alloresponses *in vivo*. Initial studies in animal (Benichou *et al.* 1994; Watschinger *et al.* 1994) and human (Liu *et al.* 1993*a*) models of allograft rejection suggested that indirect responses are directed to a single or only a few dominant determinants on the donor MHC antigen, although in

animal models, these responses vary among responder haplotypes (Gallon *et al.* 1995). This limitation of indirect T-cell alloresponses to discrete sites on the alloantigen suggested that selective immune interventions might interfere with the rejection process. However, subsequent studies have confirmed the phenomenon of epitope spreading with loss of alloresponses to previously dominant determinants and restoration of immunogenicity to previously cryptic epitopes (Liu *et al.* 1996; Vella *et al.* 1997*b*; Ciubotariu *et al.* 1998). Despite these recent findings, there is ample evidence to suggest that selective peptide immunotherapy may allow the induction of tolerance to the indirect pathway.

The following sections review studies using synthetic allopeptides as a tool for the better understanding of the role indirect allorecognition plays in tolerance induction. Specifically, antigen presentation by oral and intrathymic routes is discussed, as well as newer approaches aimed at determining the inter- and intracellular processes important in tolerance induction by the indirect pathway.

(i) *Oral allopeptide presentation*

Early efforts examined the tolerogenicity of orally administered synthetic class II MHC allopeptides in the rat (Sayegh *et al.* 1992). Administration of mixtures of immunodominant allopeptides significantly reduced DTH responses to the synthetic peptides and to allogeneic splenocytes in animals previously immunized with these mixtures. Furthermore, orally administered peptides from non-polymorphic regions of class I MHC effected long-term heart allograft survival (Nisco *et al.* 1994). Subsequent studies confirmed that intestinal DCs can indeed process orally administered allopeptides and, in addition, can prime naive T cells and stimulate primed T cells *in vitro* (Liu & MacPherson 1993). T and B cells from the same intestinal environment are, however, quite inert. It appears that antigen presentation by this method results in a Th2 environment, which is responsible for the down-regulation of the immune response and induction of tolerance (Khoury *et al.* 1992). Such an environment induces tolerance to antigens other than those presented orally, a phenomenon termed 'antigen-driven by-stander suppression' (Miller *et al.* 1991), and may explain how non-polymorphic peptides can prevent allorejection.

(ii) *Intrathymic allopeptide presentation*

Another strategy that has received major attention in the induction of tolerance has been the intrathymic injection of allopeptides. The thymus plays a critical role in the ability of the immune system to discriminate between self- and non-self-antigens (Dong *et al.* 1999). In search of a new strategy for re-educating the adult thymus to recognize alloantigen as self, it was reasoned that the effectiveness of the T-cell receptor (TCR)–MHC–peptide interactions necessary for self–non-self discrimination might be influenced by the presence of allopeptide in the thymus. Initial studies showed that intrathymic injection of mixtures of immunodominant class II allopeptides induced tolerance to rat cardiac allografts in a class II MHC disparate strain combination (Sayegh *et al.* 1993). Similar results were seen using a single immunodominant class I MHC allopeptide (Shirwan *et al.* 1995). Subsequent studies designed to elucidate the mechanisms of such tolerance induction showed that thymic recognition of allopeptides induces T-cell anergy (Sayegh *et al.* 1994), with suppression of Th1 cytokines in lymphoid compartments (Oluwole *et al.* 1999) and an intragraft state of immune deviation to Th2 cytokine gene expression (Shirwan *et al.* 1998).

More recent studies have demonstrated that single class I MHC immunodominant allopeptide-pulsed bone-marrow-derived DCs, which have the capacity *in vitro* to present allopeptide to naive and allopeptide-primed syngeneic cells, induce tolerance to rat cardiac allografts (Garrovillo *et al.* 1999) and islet cell transplants (Ali *et al.* 2000) when injected intrathymically. These findings suggest that thymic DCs acquire, process and present injected allopeptide to the developing T cells during the induction phase of acquired thymic tolerance and provide evidence for the indirect pathway of allorecognition in the thymus as the effector mechanism of such tolerance. Still not entirely explained is how a single immunodominant allopeptide is able to induce tolerance to an entire allograft. Speculations include the recent phenomenon of linked recognition (Davies *et al.* 1996) or epitope suppression (Wong *et al.* 1997), whereby a single allogeneic MHC molecule induces acceptance of an allograft, provided that a particular MHC molecule is among the numerous MHC disparities in the allograft. These observations may explain the clinical reports of improved cardiac and renal allograft survival in recipients of one HLA-DR antigen-matched pretransplant blood transfusion over recipients of unmatched transfusions or in recipients who received no transfusion (Lagaaij *et al.* 1989).

(iii) *Newer approaches*

Although the original concept of peptide immunotherapy held that antigen recognition at the MHC–TCR interface was inhibited via physical blockade, it is now clear that the mechanisms responsible for such effects are much more complex than once believed. There is now substantial evidence that the response of the T cell is not an 'all or none' response, but rather a qualitative one that may be altered by subtle changes in the sequence of the MHC-bound peptide (Kersh & Allen 1996). Such plasticity in T-cell recognition can allow the activation of T cells by peptides that are unrelated in sequence. Peptides that differ by as little as a single amino acid may alter the pattern of phosphorylation events involved in T-cell intracellular signalling, resulting in an altered activation state (Sloan-Lancaster *et al.* 1994). Recent *in vitro* studies have sought to determine the effect of selective peptide therapy on MHC–TCR interactions and the subsequent cascade of intracellular events responsible for T-cell activation and differentiation. Moreover, newer molecular modelling and chemical optimization techniques may allow the design of peptides with enhanced immunomodulatory effects and improved resistance to protease degradation (Murphy & Krensky 1999).

Boytim *et al.* (1998) demonstrated that the synthetic peptide, HLA-DQα^{*}03011(65-79), inhibits anti-CD3 monoclonal antibody, mitogen and alloantigen-induced T-cell proliferation in an allele-independent manner. Cell cycle analysis showed that the peptide prevented DNA replication by blocking the G1 to S transition, suggesting

that the peptide may work in a manner similar to rapamycin. These speculations were confirmed by finding that the peptide inhibited cyclin-dependent kinase 2 via a block in the degradation of the inhibitory protein p27. The effects, however, unlike rapamycin, are not mediated through binding to FK-506-binding protein since the peptide did not compete for binding with this drug.

Murphy *et al.* (1995) studied the immunomodulating effects of the synthetic peptide HLA-DQα*0101(62-77). The sequence was found to be an efficient inhibitor of the rat, mouse and human MLR, indicating that the effect was not strain-, allele- or species-dependent. Furthermore, the peptide inhibited CTL generation but not preformed effector CTLs, suggesting that the inhibitory effect was targeted at Th-cell function. As HLA-DQα*0101(62-77) has no effect on mitogen-induced proliferation, but does inhibit superantigen proliferation, the peptide appears to interfere with MHC–TCR interactions. Subsequent studies showed that the immunomodulatory effect is mediated through the induction of apoptosis (Murphy *et al.* 1999). Although HLA-DQα*0101(62-77) and HLA-DQα*03011(65-79) are derived from the same region but different alleles, the mechanisms of action appear to be remarkably different.

Zechel *et al.* (1996) synthesized a peptide with a sequence derived from MHC class II-associated invariant chain peptide (CLIP), which is essential for proper loading of exogenous peptide on MHC. This peptide inhibited antigen-specific T-cell responses *in vitro* and *in vivo* following immunization, presumably through interruption of antigenic peptide loading on MHC. Other *in vitro* studies have looked at synthetic peptides corresponding to the human leucocyte antigen (HLA) sequences with which CD4 and CD8 are thought to interact, with results showing varying immunomodulatory effects (Clayberger *et al.* 1994). These novel studies are not only important in their ability to inhibit the alloimmune response, but also in their potential to highlight molecules and pathways that may represent future targets for immunotherapy.

4. INTERACTIONS BETWEEN DIRECT AND INDIRECT ALLORECOGNITION

Although the primary MLR reflects the strength of the initial alloresponse initiated by direct allorecognition, it is clear from the data presented that this pathway may also be associated with the induction of donor-specific tolerance *in vivo*. There is also evidence that the direct pathway may act in concert with the indirect pathway to achieve such tolerance. For example, studies have also demonstrated tolerance induction through intrathymic injection of peptide-pulsed *donor* DCs (Garrovillo *et al.* 1999). Although these findings may indicate that the direct pathway of allorecognition is active in the thymus, it is also possible that peptides shed into the thymus by these donor DCs are taken up and presented by host DCs to the thymocytes by the indirect pathway. Donor APCs may, therefore, function primarily as vehicles for transporting donor antigen to recipient lymph nodes, rather than as direct stimulators of the immune response.

Other theories include an inhibition of the indirect pathway by the direct pathway to explain reports of tolerance to class-I-mismatched skin grafts after class-II-MHC matched kidney transplantation (Pescovitz *et al.* 1984). Such a theory may explain the benefit seen in human transplantation with HLA-DR matching and is supported by recent *in vitro* T-cell clone work by Frasca *et al.* (1998) from a renal transplant patient with class I MHC mismatching but similar HLA-DR matching with the donor (DRβ1*1502 and *1501, respectively). All of these T-cell clones, with indirect allospecificity to the donor class I MHC, proliferated in response to stimulator cells expressing the mismatched class I antigen/recipient HLA-DR, with only three out of ten responding to APCs expressing the class I mismatched antigen/donor HLA-DR. These results may simply imply that recipient APCs were responsible for priming and maintaining T cells with indirect specificity. Alternatively, these cells may have been rendered tolerant *in vivo* as a result of antigen presentation by the parenchymal cells of the transplanted kidney, similar to the mechanisms described previously. It could, therefore, be argued that sharing of an identical HLA-DR allele favours the induction of tolerance by the indirect pathway.

5. CONCLUSIONS

Sufficient evidence exists to implicate the involvement of both the direct and indirect pathways of allorecognition in the induction of tolerance. The hypothesis that direct allorecognition can inhibit indirect alloresponses is obviously far from proven. However, such a shift from conventional thinking may aid in the design of future strategies to promote long-term allograft acceptance. Rather than developing strategies aimed at diminishing direct recognition, perhaps attempts should be made to encourage direct responses in ways that enhance their ability to inhibit the crucial indirect recognition of donor MHC peptides.

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