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Pathways of major histocompatibility complex allorecognition

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Abstract

Purpose of review—Here, we review the pathways of allorecognition and their potential relevance to the balance between regulatory and effector responses following transplantation.

Recent findings—Transplantation between nonidentical members of the same species elicits an immune response that manifests as graft rejection or persistence. Presentation of foreign antigen to recipient T cells can occur via three nonmutually exclusive routes, the direct, indirect and semi-direct pathways. Allospecific T cells can have effector or regulatory functions, and the relative proportions of the two populations activated following alloantigen presentation are two of the factors that determine the clinical outcome. Regulatory T cells have been the subject of significant research, and there is now greater understanding of their recruitment and function in the context of allorecognition.

Summary—A greater understanding of the mechanisms underlying allorecognition may be fundamental to appreciating how these different populations are recruited and could in turn inform novel strategies for immunomodulation.

Keywords

allorecognition; direct; human; indirect; mouse; regulatory T cells; semi-direct; transplantation

Introduction

Allorecognition is the term used to define immunological recognition of histoincompatible antigens between genetically disparate individuals within the same species. In the context of transplantation, the consequence of allorecognition is the initiation of an adaptive immune response with recruitment of allospecific T cells. This response is known as the alloresponse. In a nontolerant recipient and in the absence of immunosuppressive drugs, the consequence of an alloresponse is invariably rejection of transplanted tissue. During pregnancy, however, in which semi-allogeneic foetal tissues are present, and in transplant recipients that have developed tolerance to donor antigens, allorecognition does not result in rejection, indicating that effector immune responses are controlled by mechanisms of developing tolerance. The relative magnitude of these opposing responses recruited through allorecognition determines the outcome following clinical transplantation.

Central to both effector and regulatory responses are allospecific T cells, some of which have predominantly inflammatory and some of which regulatory phenotypes. Here, we review the pathways of alloantigen presentation and their relevance to activating effector

and regulatory T cells. Given the long-term adverse effects associated with clinical immunosuppression, including increased mortality from infectious diseases and malignancy, an increasing understanding of the mechanisms of allorecognition may afford novel targets for immunomodulation in the context of transplantation that may obviate some of the current requirements for long-term immunosuppression.

To date, three nonmutually exclusive and concurrent mechanisms of allorecognition have been demonstrated (Fig. 1 [1]): the direct [2], indirect [3] and semi-direct [4] pathways that differ in the origin of antigen-presenting cells (APCs), kinetics and contribution to the alloresponse over time.

Direct allorecognition

The direct pathway is the mechanism by which recipient T cells recognize determinants on intact donor major histocompatibility complex (MHC) molecule-peptide complexes displayed on the surface of transplanted cells [2] (Fig. 1a) without the requirement for antigen processing by recipient APCs. The primacy of direct allorecognition is emphasized by the uniquely high frequency of T cell reactivity against alloantigens [5,6] compared with other nominal antigens [7].

The direct response can most readily be demonstrated *in vitro* by the mixed lymphocyte reaction in which only direct allopresentation can occur and *in vivo* by transplanted *Rag*^{-/-} MHC class II^{-/-} mice reconstituted with syngeneic CD4⁺ T cells. These mice lack CD8⁺ T cells and the capacity to present antigen via the indirect pathway (see below) but have the ability to reject cardiac allografts, demonstrating that direct pathway CD4 cells are sufficient to mediate graft rejection [8].

That donor dendritic cells are the cells that primarily trigger the recipient immune response via the direct pathway is suggested by observations that depletion of donor dendritic cells by an intermediate parking strategy leads to loss of immunogenicity that is only restored following addition of dendritic cells of donor strain [3]. Under the influence of proinflammatory signals engendered by the transplantation procedure, donor dendritic cells traffic to secondary lymphoid tissues of the recipient [6,9] and initiate direct responses at these sites. Indeed, responses to engrafted tissues can be greatly reduced in animals lacking secondary lymphoid tissues [10,11].

Thymic education of T cells ensures the selective survival of those lymphocytes capable of recognizing self-MHC. As a result, the mature T cell repertoire is biased towards recognition of foreign peptides restricted by self-MHC [12]. The high frequency of direct antidonor alloreactivity within the T cell repertoire [13,14] is, therefore, counterintuitive; this apparent paradox is explained by significant T cell receptor (TCR) cross-reactivity (between self and allogeneic MHC-peptide complexes) [15-17]. There are at least two theories that further delineate the molecular characteristics of the high frequency of direct alloreactivity, the 'high determinant density' and the 'multiple binary complex' models that differ on whether alloreactive T cells directly recognize polymorphisms in allogeneic MHC or presented peptide in the MHC peptide-binding groove (reviewed in Ref. [17]). In practice, it is probable that both mechanisms contribute to direct allorecognition, the overall contribution of each being related to the site and magnitude of the structural differences in MHC molecules between responder and stimulator cells.

Indirect allorecognition

The indirect pathway refers to recognition of processed peptides of allogeneic histocompatibility antigens presented by self-MHC in a self-restricted manner [3,18] (Fig.

1b) and is akin to recognition of nominal antigens. Indirect alloantigen presentation (in the context of self-MHC class II) invariably results in alloresponses that are dominated by CD4⁺ T cells. As T cell help for B cells to class switch and differentiate into antibody secreting plasma cells is provided by CD4⁺ T cells that recognize peptides derived from antigens internalized by B cell surface immunoglobulins, the presence of class-switched alloantibodies is indicative of help provided by indirect pathway T cells [19,20].

In mice, presentation of peptides from allogeneic MHC by self-MHC can be inferred by the demonstration that dendritic cells of H-2A^b recipients (not expressing the H-2E antigen) injected with H-2^k B cells (expressing H-2E) can be isolated from draining lymph nodes and stained positively with an antibody specific for complexes of H-2A^b occupied by peptides of H-2E [21] and that CD8-depleted or MHC class I-deficient recipients of MHC class II-negative skin grafts (presenting foreign MHC class I via self-MHC class II to CD4⁺ cells) rapidly reject their transplants [22]. Furthermore, immunization of animals with peptides of allogeneic MHC (by definition able to elicit only indirect rather than direct responses) results in vigorous allograft rejection [23] whereas intrathymic injection of similar peptides down-modulates the indirect response sufficiently to prolong survival of subsequent allografts of the same MHC type [24].

In humans, there is ample evidence for the involvement of this pathway in graft rejection [25-28], including in-vitro detection of amplified indirect responses in recipients of heart, kidney and liver allografts with the clinical features of chronic rejection [25,27,29].

The requirement for antigen processing in the indirect pathway, despite considerable amplification of this response through epitope spreading, naturally correlates with slower responses than those engendered via the direct pathway. Additionally, the lower frequency of T cells in the normal repertoire with indirect, compared with direct, allospecificity [30] suggests that the direct response dominates the early posttransplant period, whereas the indirect pathway plays a role in long(er)-term alloantigen presentation when passenger (donor) APCs have been exhausted [13,31-33]. Although there is significant evidence in support of this assertion [29,33,34], it is important to note that, in the absence of direct responses, the indirect pathway alone can also result in rapid acute graft rejection [22].

Semi-direct allorecognition

The traditional dogma of cross-talk between CD4⁺ and CD8⁺ T cells during the generation of an immune response relies on a 'three-cell' or 'linked' model, whereby both CD4⁺ and CD8⁺ T cells are activated by the same APCs [35]. In the context of transplantation, observations of cross-talk between the direct and indirect pathways (that CD4⁺ T cells with indirect allospecificity can amplify [36] or regulate [37] directly allospecific CD8⁺ T cells), in contrast, represent an apparent paradox to this model, as it would appear that direct pathway CD8⁺ cells and indirect pathway CD4⁺ T cells are activated through different (donor vs. recipient) APCs and, by definition, appear to necessitate a 'four-cell' or 'unlinked' model (Fig. 2).

Immunological cells have the capacity to exchange surface molecules (reviewed in Ref. [38•]). Specifically, dendritic cells are able to acquire intact MHC-peptide complexes from other dendritic cells and endothelial cells and to present them to alloreactive T cells [4,39]. This observation helps to resolve the four-cell problem and is the basis of the semi-direct pathway of allorecognition [40], which proposes that recipient APCs acquire intact allogeneic MHC-peptide complexes through MHC transfer (and stimulate CD8⁺ T cells through the direct pathway), as well as presenting peptides of allogeneic histocompatibility antigens from phagocytosed necrotic cell material (which are internalized, processed and presented by self-MHC class II to indirect pathway CD4⁺ T cells). In this way, both

allospecific CD4⁺ and CD8⁺ cells are activated by the same APC and linked help or linked suppression can occur via a three-cell model (Fig. 1c). The mode of acquisition of intact MHC can be by direct cell-to-cell contact [4,41,42] or via release and uptake of small vesicles ('exosomes') [43,44].

Although there is no direct evidence for an in-vivo role of this pathway in the context of allograft rejection, it does provide an alternative explanation for other previously published observations. For example, embryonic thymic epithelium, which naturally lacks bone marrow-derived APC, can still be acutely rejected in the absence of the indirect pathway [45], presumably through semi-direct presentation of allogeneic MHC acquired by recipient dendritic cells (to otherwise directly allospecific T cells). Similarly, reports of MHC class II-deficient recipients (i.e. lacking the indirect pathway) rejecting costimulation-deficient (CD80^{-/-}CD86^{-/-}) allogeneic cardiac grafts as rapidly as wild-type transplants [46] could be attributed to semi-direct presentation of MHC from the graft rather than to trans costimulation. An additional implication of the semi-direct pathway is that it could lead to sustained stimulation of directly reactive allospecific T cells even after donor APCs have been exhausted. In the context of viral infection, it is also possible that presentation of antigen via the semi-direct pathway might ensure a more faithful delivery of antigen to lymph node-resident T cells than would be the case through antigen processing and presentation alone.

Allorecognition and regulatory T cells

Allorecognition through any of the pathways described above, particularly in an environment that favours inflammation (as would be the case following transplantation) in which APCs are activated to express co-stimulatory molecules and migrate to secondary lymphoid tissue (and possibly enhance MHC transfer [4]), leads to activation of allospecific T cells. The nature of those T cells, their interaction with each other, the graft and the recipient's immune system and microenvironment determine the clinical outcome.

Many cells with regulatory properties have been described in both mouse and humans. These include interleukin-10-secreting T-regulatory 1 (Tr1) cells [47], transforming growth factor (TGF)- β -secreting Th3 cells [48], Qa-1-restricted CD8⁺ cells [49], CD8⁺CD28⁻ T cells [50], CD8⁺CD122⁺ T cells [51], CD3⁺CD4⁻CD8⁻ cells [52] and naturally occurring CD4⁺CD25^{hi} T cells [53]. Given the development of severe autoimmune diseases in both humans and mice in the absence of CD4⁺CD25^{hi} cells [54,55], these cells are considered to be the most important naturally occurring regulatory T cells identified to date and have been the focus of attention in the literature (they will be referred to as T_{regs} in this article).

The presence of T_{regs} in tolerant grafts suggests that they play a role in persistence of transplanted organs [56,57], an assertion that is supported by adoptive transfer experiments that demonstrate their capacity to mediate tolerance to engrafted tissue [58,59]. Positive selection of naturally occurring T_{regs} in the thymus, on the basis of reactivity to self-MHC [60,61], ensures a high frequency of self-specificity [62,63] in this population and, as with other self-MHC-restricted T cells, significant alloreactivity [64].

The question is whether the pathways of allorecognition discussed above participate in the induction of tolerance by presentation to T_{regs} with specificity for antigens on the allograft. The presence of allospecificity among T_{regs} and their accumulation within tolerated grafts [57] is certainly suggestive and there is also evidence to support this assertion. A significant barrier to successful pregnancy, for instance, is foetal carriage of paternally derived histocompatibility antigens (50% of foetal alloantigens are paternal in origin). Nevertheless, in contrast to partially matched transplanted allografts, foetal tissues are tolerated [65] despite the persistence of maternal T cells alloreactive to paternal antigens throughout

pregnancy [65]. This situation is the result of a complex interaction between foeto-maternal immune systems (reviewed in Ref. [66]) that includes a significant increase in the proportion of T_{regs} , both locally (uterine) and systemically (spleen and lymph node) [67]. Indeed, the role of T_{regs} cannot be underestimated as T_{reg} deficiency leads to termination of pregnancy between genetically disparate, but not genetically identical, parents [67] and their increment is temporally related to the frequent amelioration of many human (maternal) autoimmune diseases during pregnancy [68,69].

In the context of experimental transplantation, the allospecific T_{regs} of anti-bm12 (ABM) mice [transgenic for a TCR specific for an intact class II molecule (I-A^{bm12})] can completely suppress rejection of cardiac allografts that bear their cognate, but not third-party, antigens [70]. These observations suggest that recognition of cognate antigens on transplanted tissues by allospecific T_{regs} confers a potent capacity to promote tolerance. Indeed, real-time studies in autoimmune models show that stable associations between T_{regs} and dendritic cells precede inhibition of helper T cells [71], suggesting that the APC, and cognate specificity, is central to the in-vivo function of T_{regs} .

Induction of tolerance to fully allogeneic grafts by recruitment of regulatory ($CD4^+$) T cells can also be achieved by administration of allogeneic splenocytes or MHC molecules via the mucosal (intratracheal, intranasal or oral) route [72-75]. Absence of administered (labelled) splenocytes from local lymph nodes (and the similarity of tolerance achieved when administering peptides of MHC instead of splenocytes) in these experiments suggests presentation via the indirect (or, indeed, semi-direct) pathway [73]. Although indirect antidonor allospecificity is a feature of T_{regs} that mediate transplantation tolerance in experimental animals [37,76-78] and there are reports of similar populations in human renal allograft recipients [79], T_{regs} cannot be exclusively dependent on the indirect pathway of presentation, however, as they are capable of suppressing the in-vitro mixed lymphocyte reaction in which only direct allopresentation is available. Recent data resolve some of this dilemma by showing that T_{regs} have both direct and indirect allospecificity *in vivo*, but that their regulatory function is several orders of magnitude more pronounced in allograft responses driven by the indirect pathway [80]. In addition, peripherally inducible (thymically independent) T_{regs} [81], although by no means fully characterized, might be dependent on indirect allopresentation for their generation [82].

Similarly, human data in stable renal transplant recipients demonstrate that T_{regs} do not significantly contribute to direct pathway hyporesponsiveness [83] and have a suppressive effect predominantly on the indirect antidonor alloresponse [84]. This fact is not surprising given the three-cell model described above but, as a result, expanded populations of allospecific T_{regs} [64] would not be expected to alter significantly the clinical response to the graft in the short term following transplantation without additional suppression of the direct pathway. Fortunately, the immunosuppressive agent rapamycin is nontoxic to T_{regs} [85] and transplantation with this agent could pharmacologically inhibit the early (direct) response while preserving infused/induced T_{regs} that have the capacity to suppress the indirect response and improve longer term graft outcome.

Conclusion

Alloantigen barriers prevent successful transplantation between nontolerant individuals of the same species through at least three nonmutually exclusive pathways. Recipient direct pathway T cells can recognize intact MHC-peptide complexes directly on the surface of donor cells whereas trafficking of recipient APCs through transplanted tissues leads to acquisition of dead and necrotic material and presentation of processed peptides of histocompatibility antigens to recipient T cells in a manner equivalent to well characterized

immune responses to micro-organisms (the indirect response). A third pathway, the semi-direct, resolves the four-cell dilemma through presentation of both intact donor MHC (acquired by membrane transfer or the exosomal route) and peptides of processed donor histocompatibility antigens (acquired through phagocytosis) by recipient APCs.

The consequence of allopresentation is the activation of allospecific T cells, some of which will have effector function and mediate graft rejection and others that have regulatory function and will attempt to prevent graft rejection. The relative proportions of these two populations are two of the factors that determine the clinical outcome. From the therapeutic perspective, the predominantly indirect allospecificity of T_{regs} and their principally suppressive effect on the indirect alloresponse makes it unlikely that their adoptive transfer in the early post-transplant period will have significant effect on tolerance induction; other methods of inhibiting rejection at this time point are, therefore, required. What is clear is that a greater understanding of the mechanisms of allorecognition and the methods by which cells with an effector phenotype and cells with a regulatory phenotype are recruited will offer insights into how to alter the balance between these two populations and provide novel targets for immunomodulatory intervention.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 453–476).

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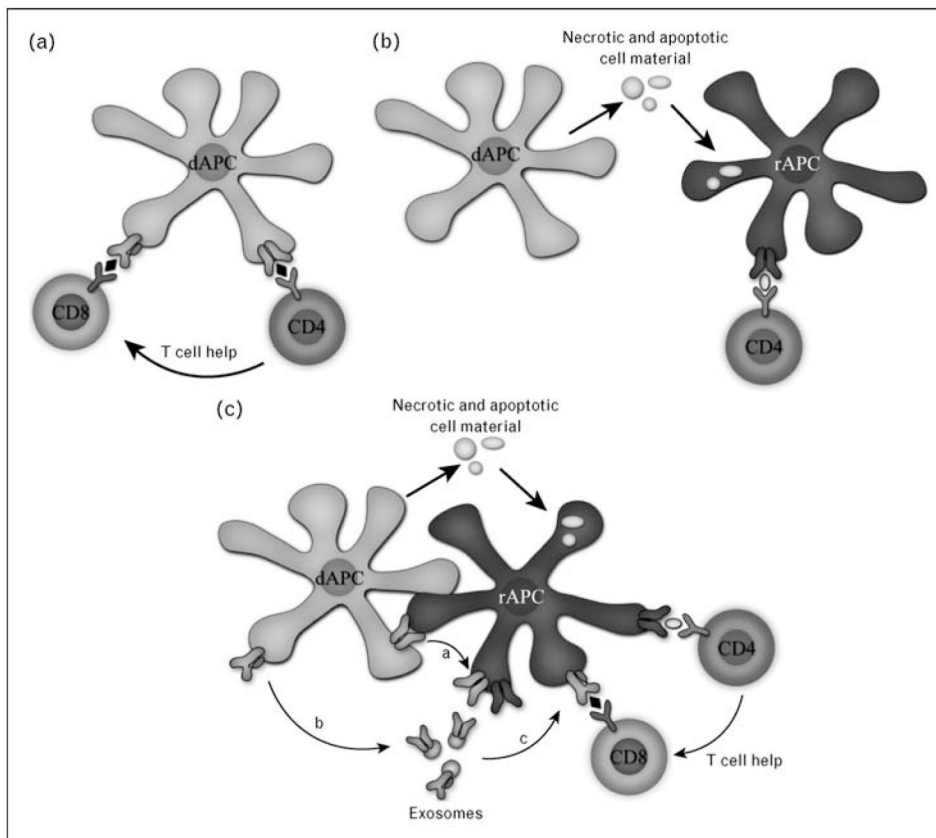


Figure 1. Direct, indirect and semi-direct pathways of allorecognition

(a) Direct pathway. Recognition of intact foreign MHC on donor APC primes CD4⁺ and CD8⁺ recipient T cells. CD4⁺ cells then provide T cell help for the effector function of CD8⁺ cells. (b) Indirect pathway. Recipient APCs traffic through transplanted organs, phagocytose allogeneic MHC shed from foreign cells through cell necrosis and apoptosis and present the processed peptides in the context of self-MHC class II to MHC class II-restricted CD4⁺ T cells. (c) Semi-direct pathway. Cell-to-cell contact between donor and recipient APC may transfer intact membrane components including intact allo-MHC (a). Likewise, donor APC can release small vesicles, known as ‘exosomes’ containing intact MHC (b), which fuse with the membrane of recipient APCs (c). Recipient APCs, now chimeric for MHC, stimulate direct pathway CD4 and CD8 responses through intact foreign MHC and indirect responses through processing and presentation of peptides of foreign MHC acquired from necrotic and apoptotic cell material. Given that the same APC stimulates both CD4 and CD8 cells, linked help can occur. APC, antigen-presenting cells; dAPC, donor APC; MHC, major histocompatibility complex; rAPC, recipient APC. Modified from [1].

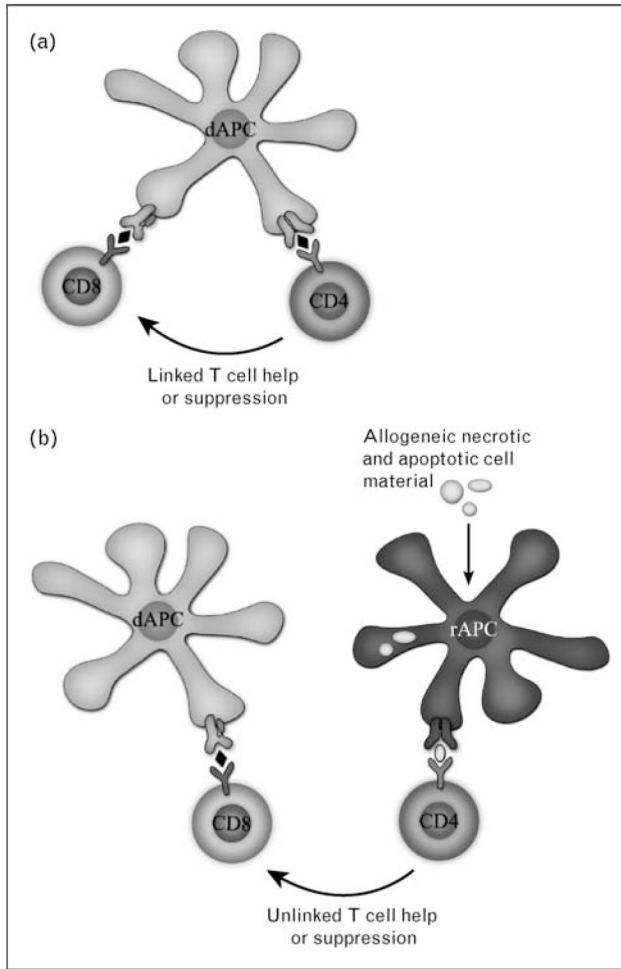


Figure 2. The three and four cell models of T cell cross-talk

(a) The three-cell model of T cell cross-talk. The traditional dogma of CD4⁺ T cell help to or suppression of a CD8⁺ T cell stipulates that both T cells should be primed by the same APC, thereby resulting in ‘linked’ help or suppression. (b) The four-cell problem. To explain the observation that recipient CD8⁺ T cells stimulated through the direct pathway by donor APC can receive T cell help or suppression from CD4⁺ T cells activated via the indirect pathway by recipient dendritic cells, it is necessary to invoke a four-cell or ‘unlinked’ model. APC, antigen-presenting cells; dAPC, donor APC; rAPC, recipient APC. Modified from [1].