Direct and indirect allograft recognition: pathways dictating graft rejection mechanisms

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Purpose of review
The T cell-dependent recognition of allogeneic tissues and organs is complicated by the fact that both donor and host antigen-presenting cells can present donor antigens to host T cells. As such, these pathways result in T cells that can be restricted to either donor ('direct') or host ('indirect') major histocompatibility complex (MHC). These pathways are well recognized, but how these distinct patterns actually dictate allograft recognition is less clear. Thus, the purpose of the review is to summarize results from preclinical animal models in an attempt to clarify the distinct forms of allograft rejection dictated by these recognition pathways.

Recent findings
$CD4^+$ and $CD8^+$ donor MHC-restricted T cells are sufficient to reject allografts by a T-cell receptor-mediated direct ('cognate') interaction using a defined array of effector molecules. Conversely, 'noncognate' host MHC-restricted $CD4^+$ T cells must interact with intermediate host-type antigen-presenting cells and so greatly amplify the response by triggering antibody and inflammatory responses.

Summary
Importantly, 'cognate' $CD4^+$ and $CD8^+$ T cells have strikingly similar requirements for rejection, suggesting that this effector mechanism is dictated by the nature of allograft recognition rather than by T-cell subset. Conversely, 'noncognate' allograft recognition drives an increasingly appreciated role for inciting innate immunity in mediating allograft injury.

Keywords
allograft recognition, innate immunity, rejection mechanisms, T lymphocytes

INTRODUCTION

This review focuses on T cell-dependent allograft rejection because of the central contribution of antigen-specific T-cell recognition in marshaling allograft immunity. Although the innate immune system plays an important role in many forms of rejection, T cells are generally the rate-limiting step for conventional allograft rejection as illustrated by indefinite allograft acceptance in nearly all forms of alpha/beta T cell receptor-deficient animal models. The notable exception is the case of bone marrow transplantation in which natural killer (NK) cells are sufficient to eliminate allogeneic bone marrow stem cells independently of T cells. Despite the importance of classical adaptive recognition by T cells in transplantation, the innate immune system should be not viewed as an inert participant in initiating the immune response. In addition to the well appreciated role of a variety of nonspecific injury and pathogen-related signals in the activation and maturation of myeloid lineage antigen-presenting cells (APCs), very intriguing recent results indicate that a presumable primitive self/nonself recognition system plays an important role in the early maturation of host monocytes to active dendritic cells [1▪▪]. Thus, like the germline-encoded family of NK cell activating and inhibitory receptors that discriminate between a variety of MHC alleles, monocytes also appear to contribute to such self/nonself discrimination in the setting of transplantation.

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KEY POINTS

- Allografts sensitize host T-cell immunity that is both host and donor MHC restricted.
- T cells that are donor MHC restricted are capable of a cognate, TCR-mediated interaction with graft cells whereas host MHC-restricted T cells mount a noncognate response to the graft by requiring an interaction with intermediate host-type APCs.
- Both $CD4^+$ and $CD8^+$ T cells recognizing the graft via a cognate interaction can mediate rejection by similar effector mechanisms requiring a combination of IFNγ production and cytolytic mediators.
- A noncognate form of graft recognition is the predominate pathway inciting both B cell-dependent humoral immunity and inflammation by host innate myeloid lineage cells.

General pathways of allograft recognition – direct, semidirect, and indirect

Key to the following discussion is the nature of recognition of alloantigens by the recipient and the role of this process in ultimately shaping the specific graft rejection mechanism. Unlike immune responses to conventional antigens, our understanding of allograft immunity is confounded by two broad forms of donor antigen recognition that are defined by the source of APC-presenting cells: 'direct' presentation (donor APC dependent) in which donor-derived cells display donor major histocompatibility complex (MHC) molecules to the recipient and 'indirect' presentation (host APC dependent) in which donor-derived antigens are acquired by recipient APCs that process and present these peptides to the host. It is essential to note that the consequence of direct donor presentation is that T cells are restricted by 'donor' MHC molecules whereas indirect donor presentation results in T cells that are 'recipient' MHC restricted. Importantly, a third form of donor presentation is the 'semidirect' presentation (or 'cross-dressing') in which donor membrane components are fused with recipient APCs, and thus present intact donor MHC molecules to the host [2,3]. Although this pathway may involve host APCs, it nevertheless activates T cells, mostly like those induced by the direct pathway, therefore generating effector T cells that are donor MHC restricted. Direct T cells specific for native allogeneic MHC:peptide complexes account for the vast majority of the high frequency of alloreactive T cells in unprimed animals (roughly 1–5% of naïve T cells), while the corresponding initial frequency of the indirect pathway is assumed to be lower. However, the actual magnitude of the initial indirect response *in vivo* appears to be much greater than anticipated [4]. This is likely because of an early response of NK cells that can rapidly eliminate nonself-bearing allogeneic APCs [5,6], thus potentially blunting the direct response and diverting donor antigens in favor of the indirect, host APC-dependent response [7].

'Cognate' allograft rejection – a mechanism defined by the nature of donor recognition rather than the T-cell subset

The MHC restriction of activated, alloreactive T cells forms a fundamental distinction that corresponds to two 'classes' of rejection mechanisms defined above by the nature of T-cell receptor recognition relative to the allograft. Specifically, 'cognate' T cells as those that are donor MHC restricted and are capable of a direct, contact and donor MHC-dependent interaction with donor target cells. Conversely, 'noncognate' T cells as those restricted by host MHC molecules. These cells cannot engage graft cells via their T-cell receptor (TCR) but rather require an interaction with an intermediate host MHC-expressing cell.

To date, the pathway involving cognate T-cell interaction appears to be a fairly well delineated response in which T-cell engagement of the donor cell triggers a rather limited array of effector mechanisms necessary to perform donor cell killing. Importantly, results suggest that there are two key rate-limiting components of cognate T cell-mediated rejection: the production of the proinflammatory cytokine IFN-γ and the alternative use of cytolytic mediators perforin and/or FasL (CD95L). For example, initial experiments showed that $CD8^+$ T cells required IFN-γ to mediate islet allograft rejection [8]. This was followed by findings indicating that these cells had the additional requirement for the alternative use of perforin or FasL (CD95L) and donor MHC class I expression [9]. Neither individual perforin nor FasL deficiency alone had a dramatic impact on acute rejection. Taken together, we hypothesize that these requirements for both cytokine production and cytolytic activity by $CD8^+$ T cells is consistent with a 'two-hit' model of rejection in which IFN-γ 'conditions' the target cell by increasing expression of molecules such as MHC class I and FasL, thereby rendering the target sensitive to the subsequent lethal events triggered by perforin/granzymes and/or FasL. Moreover, this type of rejection would be expected to have a high degree of target specificity, as was noted in classic skin transplant studies in which the hallmark feature of the cognate rejection mechanism was the cell

selectivity of the response [10]. It is important to note, however, that the requirements for $CD8^+$ T cell-mediated rejection may not be the same for all tissues and organs.

Another, and an arguably under-recognized form of cognate allograft rejection is that mediated by $CD4^+$ T cells. Given their broad role in orchestrating the adaptive immune response, $CD4^+$ T cells tend to be underestimated in their role as direct effectors of graft rejection [11,12] and tumor immunity [13]. Several years ago, we found that $CD4^+$ T cells were both necessary and sufficient to mediate acute cardiac allograft rejection and that this response required donor and not host MHC class II expression [11,12]. These findings strongly suggested a direct (cognate) form of allograft rejection. Interestingly, the requirements for this type of $CD4^+$ T cell-mediated cardiac allograft rejection almost exactly mirrors those for $CD8^+$ T cell-mediated islet rejection described above. Initial results indicated that $CD4^+$-mediated rejection required IFN-γ receptor expression by the cardiac allograft target [14]. Additional findings then showed that $CD4^+$ T cells also required cytolytic function involving alternative use of perforin and/or FasL [15]. Thus, $CD4^+$ T cells also could mediate primary acute allograft rejection by a contact-dependent, cytotoxic mechanism that is classically associated with $CD8^+$ T cells. Taken together, we would posit that the mechanism of cognate T cell-mediated rejection is defined by the nature of allograft recognition rather than by the T cell subset involved. Specifically, both $CD8^+$ and $CD4^+$ T cells appear capable of similar forms of contact-dependent rejection provided that the corresponding donor MHC I and class II target molecules, respectively, are expressed by the allograft.

There is a very interesting situation that blurs the distinction between 'direct' and 'indirect' alloreactivity involving $CD8^+$ T cells. There is limited but intriguing evidence for host MHC-restricted 'indirect' (or cross-primed) $CD8^+$ T cells in a noncognate 'type' response. Notably, there is an unusual circumstance in which host MHC class I-restricted $CD8^+$ T cells can trigger the rejection of cellular (skin) but not primarily vascularized solid organ (cardiac) allografts [16]. Valujskikh *et al.* [16] found that $CD8^+$ T cells specific for the male H-Y antigen presented by self MHC class I molecules could also reject male skin grafts from MHC-unrelated donors. Because skin grafts are revascularized by a comprised of a significant proportion of host-derived endothelium, this is a special case in which much of the graft-associated vascular could present the H-Y antigen 'directly' to self MHC-restricted T cells, serving essentially as targets of a cognate rejection mechanism. Consistent with this interpretation was the finding that the same H-Y reactive $CD8^+$ T cells could not reject cardiac allografts that are comprised almost entirely of donor-type vasculature. As such, this scenario clearly blurs the distinction between direct versus indirect allograft recognition by $CD8^+$ T cells. Nonetheless, this example illustrates a key principal when considering how a given recognition pathway ultimately impacts allograft rejection. Namely, how are donor antigens recognized, and what is the consequence of that recognition to the transplant? In the case of indirect, alloreactive $CD8^+$ T cells, effector cells can actually mediate a contact-dependent, cognate-like interaction that injures the allograft only if the donor expresses the appropriate target (i.e., host type vasculature).

Non-cognate allograft rejection: a key driver of antibody and innate mechanisms of rejection

A major component of the allograft response is composed of T cells recognizing donor antigens via the indirect, or host MHC-restricted pathway. Although these indirect T cells may comprise only a minority of initial alloreactive precursor cells, they appear to rapidly respond *in vivo* [4] and can have a profound biological impact on allograft survival. As defined above, such host MHC-restricted cells cannot mediate a direct, TCR-mediated engagement with donor cells. As such, aspects of this response have remained ambiguous, especially in regards to the impact of these noncognate T cells on allograft injury and how such cells ultimately contribute to allograft rejection. A key component, however, is that this noncognate interaction requires a host MHC-expressing intermediate cell that presents donor-derived antigens. This being the case, we propose that the impact of noncognate, donor antigen-specific T cells on the allograft is largely dictated by the type of host APC that is presenting the donor antigens. Current evidence points to a predominant role for host MHC class II-restricted $CD4^+$ T cells in marshaling two forms of antidonor, noncognate immunity: one adaptive (antibody production) and the other innate (inflammation by myeloid lineage cells).

Firstly, antidonor antibody responses are a clearly a primary consequence of the indirect pathway by this noncognate form of immunity. Specifically, MHC class II-restricted $CD4^+$ T cells, presumably follicular helper cells interacting with host B cells presenting donor-derived antigens (i.e., the host APC) is the prototypical means of initiating the formation of donor-specific antibodies.

However, the consequence of this antibody response to the allograft is multifaceted. That is, antibodies participate in accentuating allograft injury in a variety of ways. Antibodies can have an adjuvant-like effect of enhancing ongoing T-cell reactivity to alloantigens by acting as opsonins [17]. More recent evidence suggests that a blended response of alloreactive $CD4^+$ T cells in addition to antibodies can result in renal allograft rejection in mice [18]. Moreover, antibodies can interact with other innate cells, such as macrophages [19▪] and NK cells [20] by 'arming' them to inflict acute and chronic antibody-mediated rejection. This is significant because even though the original antibody response requires a noncognate $CD4^+$ T cell, the impact of the ensuing antibody response can result in amplification of the response through innate immune cells, possibly without the ongoing requirement of the original inciting $CD4^+$ T cell. Thus, this form of allograft injury would be $CD4^+$ T-cell dependent, but not necessarily T cell mediated. For example, NK cells in concert with donor-specific antibodies can inflict ongoing chronic heart allograft rejection in the absence of any adaptive (T cell) response [20].

Alternatively, another important consequence of noncognate $CD4^+$ T-cell alloantigen reactivity is inflammation because of direction interaction with other innate cells, notably macrophages. Although this type of response normally occurs concurrently with other forms of reactivity (such as cognate, allograft reactive T cells), some studies indicate that this type of donor recognition can be sufficient to trigger acute allograft rejection. For example, autoreactive $CD4^+$ T cells specific for islet-specific antigens can cause acute destruction of islet allografts, even in the absence of antidonor antibodies or donor MHC class II expression [21]. Importantly, this type of noncognate event requires the intermediate host macrophage presenting processed islet-derived autoantigens to trigger rejection [22]. Again, although this response is $CD4^+$ T-cell dependent, it does not appear to be directly T-cell mediated but rather is the result of secondary licensing of macrophages resulting in an unclear form of tissue injury. This type of rejection can also occur by noncognate alloreactive TCR transgenic $CD4^+$ T cells recognizing donor MHC class I-derived peptides presented by host MHC class II-expressing cells [23]. More recent studies also highlight a role for $CD4^+$ T cells activating host macrophages that in turn can mediate allograft rejection [6]. In this study, once $CD4^+$ T cells had primed macrophage activation, these innate cells were then able to adoptively transfer allograft rejection in the absence of T cells, suggesting that macrophages themselves may have unexpected mechanisms for self/nonself discrimination.

A related dilemma especially pertinent to a noncognate form of rejection is how the response is limited to prevent injury of adjacent self-tissues, especially in the case of generalized inflammatory injury triggered by innate cells such as activated macrophages. Interestingly, macrophages armed with donor-specific antibodies via Fc receptor binding may play an important role in guiding the cellular specificity of rejection. In addition, more recent studies indicate that programmed death-1 and, importantly, self-MHC expression play important roles in preventing injury to adjacent tissues [24▪]. It is intriguing to consider that the same self/nonself recognition system recently described for monocytes [1▪▪] could conceivably play a role in preventing injury to self-tissues during what has long been considered to be a nonspecific innate immune response. That is, it appears likely that innate cells show a greater degree of cellular discrimination during rejection that has been previously considered [6]. Thus, we must reevaluate the notion that innate cells simply trigger 'nonspecific' local inflammation during allograft injury.

CONCLUSION

The primary model described in this review is that T cells have two primary recognition patterns relative to the allograft: cognate and noncognate. Cognate T cells are capable of a direct, TCR-mediated interaction with allograft cells and appear to require a limited range of effector molecules. Notably, both $CD4^+$ and $CD8^+$ T cells are capable of this contact-dependent form of T cell-mediated rejection. Conversely, noncognate T cells require an interaction with other host MHC-expressing APCs to interact with allograft-derived antigens, and thus are presumed to be the key mediators orchestrating the response of both B lymphocytes and other innate cells in mediating inflammatory tissue injury. Because these varied responses occur simultaneously and may interact and change with time, it is an ongoing challenge to ascribe the precise role of these individual cell types within these recognition pathways when mediating acute and chronic allograft rejection. Finally, the greatest plasticity in directing the course of allograft immunity appears to lie within the $CD4^+$ T-cell-dependent indirect response. On the one hand, indirect $CD4^+$ T cells interacting with B cells and activated macrophages can enhance allograft immunity as described above. Alternatively, this same pathway also plays a key role for promoting allograft tolerance [25–28]. Clearly, increased understanding of the key events

involved in T cell–APC interactions that promote allograft tolerance versus immunity will continue to be major goal of the transplant field.

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Conflicts of interest

There are no conflicts of interest.

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

1. Oberbarnscheidt MH, Zeng Q, Li Q, *et al.* Nonself recognition by monocytes ■■ initiates allograft rejection. J Clin Invest 2014; 124:3579–3589.

A very intriguing study that reveals a previously unrecognized capacity by monocytes for self/nonself discrimination that can contribute to allograft immunity.

2. Tsang JY, Chai JG, Lechler RI. Antigen presentation by mouse CD4+ T cells involving acquired MHC class II:peptide complexes: another mechanism to limit clonal expansion? Blood 2003; 101:2704–2710.
3. Herrera OB, Golshayan D, Tibbott R, *et al.* A novel pathway of alloantigen presentation by dendritic cells. J Immunol 2004; 173:4828–4837.
4. Brennan TV, Jaigirdar A, Hoang V, *et al.* Preferential priming of alloreactive T cells with indirect reactivity. Am J Transplant 2008; 9:709–718.
5. Yu G, Xu X, Vu MD, *et al.* NK cells promote transplant tolerance by killing donor antigen-presenting cells. J Exp Med 2006; 203:1851–1858.
6. Liu W, Xiao X, Demirci G, *et al.* Innate NK cells and macrophages recognize and reject allogeneic nonself in vivo via different mechanisms. J Immunol 2012; 188:2703–2711.
7. Garrod KR, Liu FC, Forrest LE, *et al.* NK cell patrolling and elimination of donor-derived dendritic cells favor indirect alloreactivity. J Immunol 2010; 184:2329–2336.
8. Diamond AS, Gill RG. An essential contribution by IFN-g to $CD8^+$ T cell-mediated rejection of pancreatic islet allografts. J Immunol 2000; 2000:247–255.
9. Sleater M, Diamond AS, Gill RG. Islet allograft rejection by contact-dependent CD8+ T cells: perforin and FasL play alternate but obligatory roles. Am J Transplant 2007; 7:1927–1933.
10. Rosenberg AS, Singer A. Evidence that the effector mechanism of skin allograft rejection is antigen-specific. Proc Natl Acad Sci U S A 1988; 85:7739–7742.
11. Pietra BA, Wiseman A, Bolwerk A, *et al.* CD4 T cell-mediated cardiac allograft rejection requires donor but not host MHC class II. J Clin Invest 2000; 106:1003–1010.
12. Grazia TJ, Pietra BA, Johnson ZA, *et al.* A two-step model of acute CD4 T-cell mediated cardiac allograft rejection. J Immunol 2004; 172:7451–7458.
13. Perez-Diez A, Joncker NT, Choi K, *et al.* CD4 cells can be more efficient at tumor rejection than CD8 cells. Blood 2007; 109:5346–5354.
14. Wiseman A, Pietra BA, Kelly BP, *et al.* Donor IFN-gamma receptors are critical for acute CD4+ T cell-mediated cardiac allograft rejection. J Immunol 2001; 167:5457–5463.
15. Grazia TJ, Plenter RJ, Weber SM, *et al.* Acute cardiac allograft rejection by directly cytotoxic CD4 T cells: parallel requirements for Fas and perforin. Transplantation 2010; 89:33–39.
16. Valujskikh A, Lantz O, Celli S, *et al.* Cross-primed CD8+ T cells mediate graft rejection via a distinct effector pathway. Nat Immunol 2002; 3:844–851.
17. Burns AM, Chong AS. Alloantibodies prevent the induction of transplantation tolerance by enhancing alloreactive T cell priming. J Immunol 2011; 186:214–221.
18. Gaughan A, Wang J, Pelletier RP, *et al.* Key role for CD4 T cells during mixed antibody-mediated rejection of renal allografts. Am J Transplant 2014; 14:284–294.
19. Abe T, Su CA, Iida S, *et al.* Graft-derived CCL2 increases graft injury during ■ antibody-mediated rejection of cardiac allografts. Am J Transplant 2014; 14:1753–1764.

An important new model for studying antibody-mediated rejection in a mouse solid organ transplant model.

20. Hirohashi T, Chase CM, Della Pelle P, *et al.* A novel pathway of chronic allograft rejection mediated by NK cells and alloantibody. Am J Transplant 2012; 12:313–321.
21. Kupfer TM, Crawford ML, Pham K, Gill RG. MHC-mismatched islet allografts are vulnerable to autoimmune recognition in vivo. J Immunol 2005; 175:2309–2316.
22. Calderon B, Suri A, Unanue ER. In CD4+ T-cell-induced diabetes, macrophages are the final effector cells that mediate islet b-cell killing. Am J Pathol 2006; 169:2137–2147.
23. Honjo K, Xu XY, Bucy RP. CD4+ T-cell receptor transgenic T cells alone can reject vascularized heart transplants through the indirect pathway of alloantigen recognition. Transplantation 2004; 77:452–455.
24. Thangavelu G, Gill RG, Boon L, *et al.* Control of in vivo collateral damage ■ generated by T cell immunity. J Immunol 2013; 191:1686–1691.

The study addresses a long-standing dilemma of how adjacent tissues are protected from bystander injury during the course of allograft rejection.

25. Tsang JY, Tanriver Y, Jiang S, *et al.* Conferring indirect allospecificity on CD4+CD25+ Tregs by TCR gene transfer favors transplantation tolerance in mice. J Clin Invest 2008; 118:3619–3628.
26. Okumi M, Fishbein JM, Griesemer AD, *et al.* Role of persistence of antigen and indirect recognition in the maintenance of tolerance to renal allografts. Transplantation 2008; 85:270–280.
27. Luo X, Pothoven KL, McCarthy D, *et al.* ECDI-fixed allogeneic splenocytes induce donor-specific tolerance for long-term survival of islet transplants via two distinct mechanisms. Proc Natl Acad Sci U S A 2008; 105:14527–14532.
28. Ding Q, Yeung M, Camirand G, *et al.* Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. J Clin Invest 2011; 121:3645–3656.