Emerging evidence that molecules expressed by mammalian tissue grafts are recognized by the innate immune system

Annette Fox-Marsh and Leonard C. Harrison

Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia

Abstract: The innate immune system existed prior to the emergence of adaptive immunity in sharks and higher vertebrates. Homologues of many mammalian innate immune-system elements such as the toll-like receptors exist in species as distant as Drosophila. Selective pressure has led to the development of highly conserved, soluble, and cell-surface receptors that recognize functionally essential molecules shared by microbial pathogens. It is thought that molecular patterns that exquisitely distinguish pathogenic cells from mammalian cells are recognized. Therefore, it would seem unlikely that innate immune-system elements should recognize mammalian tissues. However, there is increasing evidence to suggest that this is the case and that innate immunity promotes rejection of transplanted mammalian tissues, particularly those from other species (xenografts). Evidence for innate recognition of mammalian grafts, the nature of this recognition, and the bi-directional interactions between innate and adaptive immunity that contribute to graft rejection are discussed in this review, with the emphasis on nonvascular xenografts.


Key Words: PAMP · macrophage · neutrophil · NK cell · complement

OVERVIEW OF COMPONENTS AND FUNCTIONS OF THE INNATE IMMUNE SYSTEM

Physiology: role and organization

The innate immune system serves the extremely important function of rapidly responding to and controlling infection. This is achieved by the widespread and constitutive occurrence of cells and soluble factors that recognize pathogens and elicit antimicrobial compounds and cellular activities, such as phagocytosis. The importance of the innate immune system in this regard is exemplified by the severity and frequency of infection in people with genetic abnormalities in various innate immune-system components [1–5].

It is well established that the innate and adaptive immune systems interact. For instance, B cells bear complement receptors, and T cells activate Møs [6]. There is also increasing evidence to support the suggestion originally made by Janeway [7] that an innate immune response is essential for the generation of an effective, adaptive response. This was based on the sound reasoning that T and B cells with their randomly rearranged receptors cannot discern whether an antigen is associated with a dangerous pathogen. Thus, a reliance on signals from the innate system for effective priming would focus responses on pathogens. Further, it is clear that different pathogens elicit distinct responses. Again, because T and B cells cannot discriminate different pathogens, it was suggested that the innate immune system was responsible for shaping the adaptive response.

Cellular components

The main specialized, cellular components of innate immunity include macrophages (Møs), neutrophils, natural killer (NK) cells, and mast cells. Epithelial cells, generally being the first cells to encounter pathogens, are also important in innate defense. For instance, the process of bacterial attachment to epithelial cells can occur through glycolipid receptors such as toll-like receptor (TLR) 4 leading to interleukin (IL)-8 and IL-6 production and neutrophil recruitment [8, 9].

Soluble components

The innate immune system also encompasses a diverse array of soluble components. Some of these, including the complement molecules, C-reactive protein [10], mannan-binding lectin, and other collectins, are clearly important in pathogen recognition [11–13]. The latter act largely as opsonins for complement and hence cells bearing complement receptors. Others have microbicidal activity including the peptide defensins (also referred to as broad-spectrum antibiotics), lysozyme, phospholipase A2, serprocidins, and iron-binding lactoferrin [14–16].

Molecules involved in recognition: receptors and ligands

The ligands, recognized by innate immune receptors, are referred to as pathogen-associated molecular patterns (PAMPs). In addition to the soluble recognition molecules mentioned, various cell surface-expressed PAMP receptors or pattern-recognition receptors (PRRs) have been identified (reviewed in
ref. [17]). These include the TLRs [18–21], scavenger receptors that binds sialic acid ligands [22], a Mø mannose receptor [12], a β-glycan receptor, a fucose-binding protein, and galactose-specific lectin [23]. The distinctive molecular patterns borne by pathogens are often essential lipid and carbohydrate moieties, presumably because they are major constituents of microbial cell walls. Well-known PAMPs include lipopolysaccharide (LPS), techoic acid, double-stranded RNA, and unmethylated CpG dinucleotides that are common in bacterial DNA [24, 25]. Ligand specificity has been well-described for the mannann-binding lectin that binds microbial cell-surface sugars and activates complement [4]. Mannan-binding lectin contains a carbohydrate-recognition domain that discriminates between self and nonself by preferentially recognizing glucans, lipophosphoglycans, and glycoinositol phospholipids with mannose, glucose, fucose, or α-acethylglucosamine as terminal hexoses [11–13].

Recent studies describing the ligands and receptors involved in innate pathogen recognition demonstrate that the innate immune system has the capacity to discriminate between different pathogens. For Mø-mediated innate immunity, recognition involves cooperation of two classes of innate immune receptors: the phagocytic receptors, such as the mannose receptors, and the TLRs that sample the contents of the vacuole [26]. TLR4 recognizes LPS on gram-negative bacteria [26, 27]. TLR2 can also recognize LPS [28, 29] but appears to be more specialized for recognition of gram-positive bacteria. Peptidoglycan of gram-positive bacteria is recognized by the combination of TLR2 and TLR6, whereas lipopeptide is recognized by TLR2 alone, indicating that TLR2 can discriminate between lipopeptides [30–33]. TLR2 also recognizes yeast [26], gram-negative bacterial glycoproteins yet to be characterized [34], glycosylphosphatidylinositol anchors from the protozoan parasite Trypanosoma cruzi [35], and mycobacterial lipoarabinomannan [36]. TLR5 recognizes flagellin, a virulence factor for gram-positive and -negative bacteria that stimulates innate immunity in organisms as diverse as plants and mammals [37]. TLR9 recognizes CpG DNA [38].

Mode of action

One well-established way in which innate immunity drives adaptive immunity is through the production of cytokines. Thus, in response to many bacteria, dendritic cells and particularly Møs produce IL-12, which induces activation of Th1 helper cell type 1 (Th1) T cells and interferon-γ (IFN-γ) production [39, 40]. For some pathogens, the molecules that induce IL-12 production by Møs have also been identified. These include microbial lipoproteins and bacterial CpG DNA, both acting via TLRs [24, 41]. In turn, TLR binding and signaling, which require the MyD88 adapter molecule [38], are linked to activation of nuclear factor-κB (NF-κB), a transcription factor for an array of cytokines [42]. Various innate stimuli also up-regulate expression of the CD40, CD80, and CD86 costimulation molecules on dendritic cells [17, 38, 43]. Positive-feedback loops also operate within the innate immune system. IL-12, produced by phagocytic cells within a few hours of infection, activates NK cells to produce IFN-γ, which in turn enhances the phagocytic and bactericidal activity of phagocytic cells and their ability to release cytokines including IL-12 [44]. Given that PRRs largely seem to induce IL-12 and Th1-type responses, Th2 responses are suggested to occur by default when microbial constituents are not available and involve increased costimulatory molecule interaction between the T cell and the antigen-presenting cell (APC; reviewed in refs [45, 46]). Support for this suggestion comes from the finding that mice lacking the MyD88 adapter molecule required for signaling through the TLRs have defective Th1- but not Th2-type responses [46]. However, the existence of PAMPs and PRRs, other than the TLRs that actively stimulate Th2-type responses, has not been ruled out.

INNATE IMMUNITY AND TRANSPLANTATION

This review presents various studies, showing that mammalian tissue grafts and mammalian molecules are recognized directly by components of the innate immune system, and focuses on xenografts. Innate recognition of allografts appears to be limited to NK cells or to require tissue damage. The role of innate immunity in rejection is discussed largely in terms of nonvascular xenografts rather than solid organ grafts. Discordant, solid-organ, or vascular xenografts are rejected hyperacutely within minutes because of the presence of preformed antibodies specific to the endothelial cell carbohydrate, Galα(1,3)Gal [47, 48]. This carbohydrate is discussed later. It is clear that the innate immune system, in particular complement, acts in concert with preformed antibodies (Abs) to transform xenograft endothelium into a prothrombic state, such that grafts are rejected hyperacutely through a lack of blood supply [49, 50]. This hyperacute response is so strong, rapid, and hard to prevent that it is difficult to determine the role of innate immunity, independent of preformed antibody. In particular, the independent role of complement cannot be determined, because complement depletion is required to prevent hyperacute rejection. In xenotransplant models in which hyperacute rejection is prevented, delayed xenograft rejection or acute vascular rejection is observed. Again, it is thought that antibody mediates this but in the absence of complement, is the result of endothelial cell activation [51]. It does not require adaptive cellular immunity but may involve cells of the innate immune system, including monocytes and NK cells [52, 53]. Organ allografts are also subject to acute vascular rejection as a result of antibodies specific for human leukocyte antigen (HLA) [51].

Møs and neutrophils

It has been clear for many years that nonvascular xenograft rejection requires T cells, particularly CD4 T cells (reviewed in ref. [54]). However, Møs are the dominant infiltrating cells in xenografts undergoing rejection [55–57], which indicates that they could contribute substantially to rejection. Mø depletion with clodronate-loaded liposomes provided the first evidence that Møs promote T-cell infiltration and rejection of nonvascular fetal pig pancreas (FPP) xenografts [57]. Similar effects of clodronate-mediated Mø depletion on pig-islet graft rejection and T-cell infiltration have been demonstrated recently [58]. Further, granulocyte macrophage-colony stimulating factor
(GM-CSF)-deficient mice have impaired Mø infiltration of xenografts with a concomitant impairment in T-cell infiltration [59]. Although these studies indicate that Møs contribute to rejection and enhance the T-cell response, they do not indicate whether Mø-mediated effects occur in the absence of activation by T cells. This was important to investigate, because we had found that Mø infiltration of FPP grafts in T-cell-deficient, severe combined immunodeficiency (SCID) mice is reduced greatly compared with that in immune-competent mice [60]. Investigation of the response to foreign cells injected intra-peritoneally (i.p.) into T- and B-cell-deficient SCID mice showed that pig xenografts and, to a lesser extent, allografts elicit T-cell-independent neutrophil and Mø responses [61]. Recruitment of both cell types was seen within 1 day, and neutrophils appeared marginally earlier than Møs. Similarly, in immune-competent mice, it was clear that neutrophil and Mø infiltration preceded T-cell infiltration, which started from day 3. Others have demonstrated that human naïve neutrophils [62] and monocytes [63] can activate xenoendothelium in vitro, independently of xenoreactive natural antibodies and complement, indicating direct recognition. Together with studies on NK cells and complement discussed in the sections to follow, these findings indicate that foreign mammalian cells, particularly those from a different species, are recognized by the innate immune system.

The mechanism(s) by which xenogeneic cells stimulate Mø and neutrophil recruitment is unknown. Møs and neutrophils express PRRs, including TLRs 1, 2, 4, and 5 [64], triggering receptor expressed on myeloid cells (TREM-1) [65], complement receptors [66–68], and lectin-type receptors for other carbohydrates [22, 23]. Carbohydrates on the surface of neutrophils (neutrophil-glycoprotein receptors) also bind directly to lectin-like molecules of bacteria in the absence of serum opsonins [69–71]. As with the TLRs, different neutrophil-glycoprotein receptors bind different bacterial species [71]. Neutrophils appear to be the first cells to infiltrate xenografts and may therefore have a role in Mø recruitment. Neutrophils release Mø and T-cell chemoattractants, such as tumor necrosis factor α (TNF-α), macrophage-inflammatory protein-1α (MIP-1α), monokine induced by IFN-γ (Mig), and IFN-inducible protein 10 (IP-10) [72–74]. Neutrophil chemoattractants, IL-8 and KC, followed by Mø chemoattractants, monocyte chemoattractant protein-1 (MCP-1), MIP-1α, and MIP-1β, are detected in skin allografts prior to the induction of alloreactive T cells [75–77]. More recently, defensins and other pre-stored granules in neutrophils have been shown to attract monocytes, dendritic cells, and T cells [78]. Others have similarly suggested that neutrophils respond directly to pathogens and initiate cell-mediated immunity [73]. Supporting a role for neutrophils in initiating infiltration of grafts administration of KC antisera or neutrophil-depleting monoclonal antibody (mAb) RB6.8C5 to allograft recipients within 30 min of cardiac transplantation attenuates intragraft expression of mRNA T-cell chemoattractants IP-10 and Mig and reduces cellular infiltration and rejection [77]. Similarly for xenografts, we recently found that administration of RB6.8C5 significantly reduced Mø infiltration in response to xenogeneic pig cells injected i.p. (unpublished results). Preliminary findings using microarray also indicate increased mRNAs for JE, monocyte chemoattractant protein (MCP)3, MCP5, migration inhibitory factor-related protein-8 (MRP8), CCR1, CCR5, and TNF-receptor II/p80 in FPP xenografts 2 days after transplantation (unpublished results). These results need to be confirmed by RNase protection assay or Northern blotting, and it would also be desirable to demonstrate changes in protein expression.

Cellular or soluble intermediates may be involved in neutrophil and/or Mø recruitment. Complement is able to bind foreign carbohydrates. Complement activation through the release of cytokines and chemokines, especially the anaphylatoxins C3a and C5a, is a major mediator of neutrophil migration [79–81]. Acute phase-response elements such as serum amyloid A are also chemotactic for polymorphonuclear cells and monocytes [82]. The acute-phase response is induced particularly by IL-1, IL-6, and TNF-α, which in turn may be induced by ligation of PRRs on stromal cells or cells of the innate system. Inflammatory mediators such as leukotrienes and prostanoids contribute to cell migration and leakage of serum molecules into inflammatory sites and may also be involved. Finally, local stromal or innate immune-system cells may also initiate inflammation via PRRs and release cytokines and chemokines that directly stimulate neutrophil and/or Mø recruitment.

Complement

Various studies indicate that complement can bind to xenogeneic pig cells in the absence of Ab. First, pig cells are killed by complement-mediated cytotoxicity in the presence of normal and agammaglobulinemic human serum, but killing is abolished by heat-inactivating serum [83]. Second, fetal and adult pig-islet cells bind C3c after incubation with serum, although human immunoglobulin (Ig)M, IgG, and IgA binding was undetectable [84]. This has also been demonstrated in a more direct manner in preliminary flow cytometry and histology studies showing that pig epithelial PK15 cells and FPP exocrine and parenchymal cells are coated with C3 after in vitro incubation with SCID mouse serum (unpublished results). Further studies are required to determine whether this will be the case with other pig cell types and other species combinations to determine the degree of tissue or carbohydrate disparity required for complement binding. Whether Ab-independent binding involves direct interaction of complement with xenogeneic carbohydrates, as with the alternative pathway for complement activation by pathogen, or is mediated by mannan-binding lectin is not known. Nevertheless, we found that complement depletion with the cobra venom factor had no appreciable effect on neutrophil or Mø recruitment in response to xenogeneic cells in SCID mice (unpublished results).

Complement appears to contribute to allograft rejection in some settings. The reduced success of clinical pancreatic islet transplantation compared with whole pancreas transplantation coincides with the demonstration that collagen-isolated islets elicit binding and activation of platelets, binding of leukocytes, and activation of the coagulation and complement systems [85]. These events can be prevented by the addition of complement inhibitor [85]. Therefore, islets transplanted into the liver by injection into the portal vein may well be treated like foreign particles with collagen and other matrix molecules, normally hidden by endothelial cells, now exposed to the host blood. In
contrast to work with xenografts, it is clear that even in this setting, complement activation is not triggered by the allogeneic islets themselves but is secondary to platelet activation thought to occur in response to extracellular matrix proteins including collagens.

**NK cells**

NK cells bear PRRs such as complement receptors [66] and express inhibitory receptors specific for self-major histocompatibility complex (MHC) class I molecules, which has sparked interest in their role in allograft rejection in particular. Inhibitory receptor binding by self-MHC class I molecules inhibits the cytolytic activities of NK cells [86, 87]. NK cells infiltrate organ allografts [88, 89], vascular xenografts [52, 53], and nonvascular xenografts [55, 61]. Similar to neutrophils, MΦs, and complement, it has been shown that NK cells directly recognize oligosaccharides on pig endothelial cells [53, 90, 91]. It appears that this recognition is sufficient to activate in vitro lytic activities of mouse NK cells against pig cells and that pig MHC class I is not able to stimulate inhibitory signals, because pig endothelial cells expressing a mouse MHC I transgene were not lysed [92, 93]. Additionally, NK cells exert nonlytic effects such as induction of endothelial-cell procoagulant activity, which may contribute to acute vascular rejection [53]. Although NK cells appear to inhibit engraftment of allogeneic and especially xenogeneic bone marrow, NK cell depletion alone has had no detectable impact on solid allograft or nonvascular xenograft rejection [55, 88, 94]. However, it has been demonstrated recently that NK cell depletion enhances the effects of MΦ depletion in prolonging pig-islet xenograft survival [58]. Recently, we investigated the effect of NK cell depletion on neutrophil and MΦ responses to i.p. pig cells in SCID mice (unpublished results). Treatment of mice with anti-NK1.1 GM1 to specifically deplete NK cells prevented the usual influx of NK cells in response to pig cells and significantly reduced neutrophil and MΦ infiltration, indicating that the NK cells promote their infiltration. As mentioned in the overview, NK cells respond to IL-12 produced by MΦs, which in turn respond to IFN-γ produced by NK cells. Furthermore, NK cells have been found to be responsible for early chemokine production in allografts [89], providing a mechanism for these results. NK cell depletion in concert with inactivation of CD28-mediated T-cell costimulation prevents rejection of cardiac allografts in mice, whereas these interventions on their own are ineffective [95]. Thus, there is growing evidence that although NK cells cannot reject allografts or nonvascular xenografts single-handedly, they can promote adaptive responses and ultimately rejection, alone or in concert with MΦs.

**IDENTITY OF XENOGENEIC MAMMALIAN MOLECULES RECOGNIZED BY THE INNATE IMMUNE SYSTEM**

We used microarrays to compare fresh FPP with FPP recovered 2 days after transplantation into mice to identify PRRs that might be involved in the innate response to xenografts. Various relevant mRNAs were induced, including a TLR, lipid A-bind-
The local innate response stimulates T-cell infiltration of grafts

We showed that Møs and neutrophils induced by i.p. injection of xenogeneic cells into SCID mice would stimulate T-cell recruitment when purified and transferred to immune-competent mice [61]. As discussed earlier, T-cell infiltration of xenografts is impaired in the absence of Møs, and Møs are able to respond rapidly to grafts as part of an innate response. Thus, it would appear that the innate Mø (± neutrophil) response greatly enhances T-cell infiltration and therefore rejection of xenografts. As depicted in Figure 1, such a relationship between the innate response and T-cell infiltration of inflammatory sites is logical and indeed obligatory. Naïve T cells traffic through lymph nodes, where they will be primed if stimulated by specific antigen delivered and presented by dendritic cells draining the site of infection or inflammation. Once activated, T cells enter the circulation and require signals to extravasate to the site of infection or inflammation to perform function there. It is logical that the innate response that occurs more or less immediately at the site of foreign antigen provides these signals. The capacity of neutrophils and Møs to induce T-cell recruitment is also well-supported by other adoptive-transfer studies and by the ability of these cells to produce T-cell chemoattractant chemokines and cytokines such as IP-10 and TNF-α [73, 74, 105–108]. Møs recruited within 1 day of injecting xenogeneic cells express TNF-α protein such that there is an increase in the absolute number of TNF-α+ Møs at this time [61]. Furthermore, it has been demonstrated that blockade of locally produced chemokines prevents allograft infiltration by alloreactive T cells, although the latter are found in increased numbers in the periphery after transplantation [77, 109]. NK cells may also be an important source of T-cell chemoattractants, particularly for allografts. NK cell infiltration of allografts often occurs before that of T cells [110], and depletion experiments have demonstrated that NK cells are a source of chemokines such as KC, MIP-1α, and MIP-1β expressed early within grafts [89]. Recently, we found that depletion of NK cells with anti-asialo-GM1 also significantly decreased the number of CD8 T cells induced by i.p. pig cells in mice, although the effect was only transient (unpublished results).

Xenograft rejection is far more aggressive and difficult to prevent than allograft rejection [111], which coincides with a stronger innate immune response to xenografts. Thus, innate immunity may contribute to the strength and complexity of the xenograft response by driving and shaping adaptive immunity, as proposed for pathogen responses [7, 17, 112]. However, it is yet to be determined whether innate responses to grafts induce costimulatory signals for activation of adaptive responses.

Preformed Abs stimulate innate immunity

As discussed, preformed Abs reactive with pig cells exist in humans, apes, and Old World monkeys even without prior transplantation of pig tissues [48, 101]. We and others have demonstrated that Ab-transfer accelerates the inflammatory response to xenografts in immune-deficient mice, indicating that preformed Abs may impact the initial innate response to xenografts. Thus, coinjection of hyperimmune serum raised against xenogeneic pig PK15 cells, together with PK15 cells into the peritoneal cavity of SCID mice, increased neutrophil and Mø infiltration compared with injection of PK15 cells alone [113]. Similarly, passive transfer of hyperimmune serum raised against pig-islet-like cell clusters enhanced mononuclear cell infiltration of transplanted islet-like cell clusters [114, 115].

![Fig. 1. Scheme depicting the role of innate immunity in promoting T-cell infiltration of xenografts. Molecular patterns expressed or induced by xenografts are recognized by elements of the innate immune system leading to rapid neutrophil and Mø infiltration. Dendritic cells draining the graft site present xenogeneic antigens to T cells within the local lymph node. T cells then enter the circulation and require signals to extravasate to the graft/inflammatory site. These signals are provided by the innate response, which occurs locally and prior to T-cell activation. Innate immune responses are known to induce chemokine and adhesion-molecule expression on endothelial cells.](image-url)
The innate model for induction of costimulatory signals focuses adaptive responses on pathogens and thus appears far more efficient than the earlier self-nonself model for control of adaptive immunity. The latter did not predict that foreign and harmful antigens would be distinguished from foreign but inert antigens, such that energy would not be wasted on unnecessary adaptive responses. Although, there is much evidence that pathogens trigger the innate immune system to produce costimulatory signals for lymphocyte activation, evidence that adaptive immunity is obligatorily controlled by innate pathogen recognition is somewhat lacking. As suggested by Matzinger [118], this model may not account for the transplant response. Matzinger has also argued that the innate/pathogen model still does not distinguish between harmful and harmless foreign pathogens. However, if innate recognition developed through selective pressure from pathogens, it is likely to be directed toward harmful pathogens. The alternative danger model proposed by Matzinger argues that an immune response is only initiated if an antigen is associated with endogenous signals from cells that are stressed, damaged, or die messily [118, 119]. This model shifts the reliance on exogenous foreign molecules to endogenous danger signals. In light of the identification of PRRs, Matzinger has suggested that PRRs actually recognize endogenous molecules and that bacteria have evolved to recognize and use them for the infectious process. That tolls are involved in development supports this suggestion. A few endogenous ligands for PRRs have been identified so far. The mannose receptor and the scavenger receptors are good examples of receptors that recognize exogenous and endogenous ligands [96, 120].

The investigation of innate and adaptive responses to grafts raises several issues about these models for regulation of costimulation and adaptive immunity. First, xenogeneic mammalian cells, which are neither pathogens nor tissue that has been experienced through evolution, except perhaps as self, stimulate innate immunity. Second and related, adaptive responses to grafts occur in the absence of pathogens, implying that innate immunity is stimulated by nonpathogenic ligands or that costimulation from innate immunity is not required. It is unclear how innate recognition of xenografts fits with the view that the ligand specificity of the innate system developed through pressure from pathogens or with the danger theory, suggesting that innate immunity evolved to recognize endogenous stress or damage signals. Matzinger [118] has suggested that the transplantation response fits better with the danger model—the source of danger being the surgical procedures that result in tissue damage and ischemic cell death. This is supported by findings that the reduced survival of liver allografts from cadaver donors compared with living donors is linked to the occurrence of stressed and ischemic cells that activate components of the innate immune system [reviewed in ref. [110]]. Various studies are described that demonstrate stimulation of NK cells, complement, and other innate components by ischemic mammalian tissues. In conflict, however, are findings using various transplant models. The i.p. model of transplantation does not involve surgical trauma (just a simple injection), such that there is no obvious danger signal. It is also hard to envisage the presence of a danger signal when transplants are rejected upon withdrawal of immunosuppression. Clear evidence of transplant tolerance after withdrawal of immunosuppression is lacking, and in most cases, this leads to T-cell-mediated rejection in humans (reviewed in refs. [121–123]). Further, the danger model does not explain why xenogeneic cells induce greater innate stimulation compared with allogeneic cells, unless the xenogeneic cells in some way undergo more necrotic death or damage host cells. The disparity between xenogeneic and allogeneic cell recognition supports the idea that the innate system recognizes a degree of foreignness. Perhaps there is some overlap between pathogenic and foreign mammalian molecules. Finally, it must be noted that stimulation of an effective, adaptive response to transplanted tissue may occur in the absence of innate immune activation such that neither damage nor pattern recognition is required. As discussed in detail elsewhere (reviewed in ref. [60]), T-cell responses to grafts may be less dependent on costimulatory signals as a result of the unusual situation of direct presentation of an enormous array of graft-derived self-peptides by graft APCs. It would be interesting to examine graft rejection in models in which innate recognition is impaired, such as in MyD88 or TLR knockout mice.

In summary, there are several ways in which responses to grafts could occur in the absence of pathogens. Innate immunity may be stimulated by foreign (exogenous), graft-associated ligands, perhaps because they resemble pathogen-associated molecules; innate immunity may be stimulated by endogenous danger signals induced by the transplantation procedure or in the case of xenografts by toxic substances released by non-physiologic cells; or innate costimulation may not be required for transplant responses.

**CONCLUDING REMARKS**

It has now been demonstrated in various ways that foreign mammalian tissues can stimulate innate immunity. Stimulation may be greatest with the most disparate tissues but can also occur with self-tissues if they are placed in a nonphysiologic environment. Although there is no doubt that innate immune receptors recognize pathogenic molecules, these findings inspire questions regarding the confines and evolution of ligand recognition. Further work is required to identify the molecules that stimulate the innate response to mammalian tissues and determine whether they are endogenous, exogenous, or a combination of both. Innate responses to grafts appear to stimulate T-cell infiltration and may promote other aspects of the adaptive response. This is supported by the increased severity of the rejection response to grafts that induce a greater innate response. The interdependent nature of innate and adaptive immunity is also demonstrated, and adaptive immunity is emerging as an important feedback regulator of innate effector mechanisms. Therefore, therapies that target elements of innate immunity should be investigated as a means of enhancing graft survival.
REFERENCES


11. Fox-Marsh and Harrison.


