The Natural Immune Barrier to Xenotransplantation

Robin L. Geller, Martin A. Turman, Agustin P. Dalmasso, and Jeffrey L. Platt

ABSTRACT

As the shortage of available organs for transplantation becomes critical, many investigators have turned to the possibility of using animals as a source of donor organs. Although there have been several attempts to use organs from closely related species for transplantation into humans, there is relatively little experience in the use of nonprimate animals as clinical donor animals. The major problem in transplants between widely disparate species is hyperacute rejection, a rapid and violent rejection reaction that leads to the destruction of the graft within minutes or hours. Hyperacute rejection appears to be triggered by components of natural immunity, most notably natural antibodies and complement. Recent data suggest that hyperacute rejection may not represent an insurmountable barrier to discordant xenotransplantation. There have recently been several examples of survival of grafts in recipients in the face of antigraft antibodies and an intact complement system referred to as accommodation. Once hyperacute rejection can be averted, it becomes necessary to consider elicited cellular responses. There are a number of issues to be considered in the clinically relevant model of porcine to primate xenografts. These include the size of the responding T-cell repertoire and the extent to which the cell adhesion molecules and cytokines of the donor will be able to stimulate recipient immune responses. Finally, the interactions between T cells and the cells forming the inner layer of blood vessels may have profound effects on the outcome of the graft.

Key Words: Xenotransplantation, hyperacute rejection, natural antibody, T-cell activation

The development of new treatment strategies involving the use of transplanted organs has greatly expanded the need for donor organs. In the case of heart transplantation, the currently available donor pool provides less than one in six organs needed (1). The need for kidneys, livers, pancreata, and lungs magnifies this problem at least severalfold. One solution proposed for what is now a critical shortage of donor organs is the use of animals as donors in lieu of humans, i.e., xenotransplantation.

Clinical xenotransplantation was attempted at various times in the past (2,3). Twenty-eight years ago, Keith Reemtsma and coworkers performed a series of xenografts in which chimpanzee kidneys were transplanted into patients with renal failure; in three cases, the kidneys functioned for a period of months, and in one case, the chimpanzee kidney functioned for 9 months (2). Thomas Starzl et al. performed a series of baboon to human kidney xenografts; survival in most cases exceeded 1 month (4). Despite such results, the use of higher primates as donors for clinical xenotransplantation was not pursued at that time, and with a very few exceptions (5), the use of primate donors has not been taken up more recently because of the risk of transmission to humans of lethal viruses, the relatively limited availability of nonhuman primates, and ethical concerns attendant to the sacrifice of primates. Instead, many now advocate the development of techniques to allow the use of nonprimates such as pigs as a source of donor organs.

There is relatively little experience in the use of nonprimate animals as clinical organ donors (6,7). \textit{Ex vivo} perfusion of xenogeneic livers have provided support for a few patients with hepatic failure (8). On the other hand, kidney (9) and cardiac (10) xeno-
Natural Immune Barrier to Xenotransplantation

grafts have not generally evidenced function. These discouraging results are consistent with the experimental observations that organ xenografts in which the donor and recipient are phylogenetically distant undergo hyperacute rejection, a rapid and violent rejection reaction that leads inexorably to the destruction of the xenograft.

The almost immediate onset of hyperacute rejection strongly favors the view that rejection is mediated by "natural" immunity. As discussed below, the elements of natural immunity that trigger the rejection reaction are most likely "natural" antibodies and complement (2,6,11,12).

Until recently, the risk of hyperacute rejection, ascertained on the basis of the presence of antidonor antibodies in the circulation of the recipient, was thought to pose an absolute barrier to the transplantation of allogeneic or xenogeneic organs (6). It is now apparent, however, that temporary depletion of antidonor antibodies prevents hyperacute rejection and in some cases may even allow survival of the graft after the antibodies have returned to the circulation. This curious phenomenon, which we have termed accommodation (13), has been seen in successful allotransplantation of organs across humoral ABO and HLA barriers and in some cases of transplantation of pig organs into baboons (14–17). It is accomplishment as much as any scientific advance that encourages efforts to adapt xenotransplantation for clinical use.

There is little evidence that hyperacute rejection or vascular rejection can be halted once the process is started. Most efforts targeting the effector mechanisms such as platelets and coagulation have failed to allow prolonged survival of xenogeneic organ grafts (6). Thus, we have focused our research program on approaches that target the recognition events that initiate hyperacute rejection. It is these events, i.e., the mechanisms by which the immune system of the host recognizes the xenograft as foreign, that will be the focus of this review. (Other issues in xenotransplantation have been reviewed by ourselves [13,18–20] and by others [3,6,7,21]).

A MODEL FOR XENO RECOGNITION

We hypothesize that it is the endothelial cells forming the inner layer of small blood vessels in a xenogeneic organ graft that constitute the major target of xenoinmunity. The nearly immediate onset of hyperacute rejection and the fact that it never occurs in isografts suggest that the immune mechanisms involved in the recognition of a xenograft are poised, i.e., natural immune mechanisms and not elicited responses. As suggested by Figure 1, the actual recognition of a xenograft could involve one or more of four processes. The first and "classical" concept is that natural antibodies in the circulation of the recipient attach specifically to antigens in the donor organ (12). This is the mechanism that we believe is responsible for initiating hyperacute rejection of porcine to primate xenografts (6).

Second, either the classical or the alternative pathways of complement could be activated by xenogeneic cell surfaces without the involvement of natural antibodies (Figure 1 [II]). This mechanism probably contributes to the rejection of guinea pig organs by rats (22).

The third mechanism involves complement inhibitory proteins associated with the donor organ. The action of some inhibitory proteins such as decay accelerating factor and CD59 is species specific and thus is impaired on some xenogeneic cell surfaces. The loss of regulation by inhibitory proteins (Figure 1 [III]) could lead to the full activation of the complement cascade and lysis of the cells. The in vitro relevance of this model is yet to be ascertained.

The fourth element concerns natural immunity mediated by natural killer (NK) cells, a unique subset of lymphocytes. Unlike conventional T and B cells, NK cells exist in peripheral blood as functional effector cells without the need for further activation (23). The precise mechanism by which NK cells recognize their targets is not well understood; however, to the extent that they can recognize cells of the xenograft as foreign, they may be capable of mediating early cellular responses to xenografts (Figure 1 [IV]).

THE PATHOGENESIS OF HYPERACUTE REJECTION

The tissue lesions in rejecting xenografts are very similar to those observed in hyperacute rejection as it occurs in allografts. Especially prominent are endothelial cell swelling and detachment, interstitial hem-
orrhage, and vascular occlusion by platelet and fibrin thrombi (24–27). These lesions suggest that the endothelial lining of blood vessels is the main target of the rejection reaction and that the pathophysiology involves global dysfunction of small blood vessels in the graft. We have proposed that the aberrant function of blood vessels in hyperacute rejection might result from endothelial cell "activation" (13). This model is based on work done in several laboratories at the University of Minnesota which formed a group on xenotransplantation (see Acknowledgment).

Under conditions of quiescence, the endothelial lining of blood vessels poses a relatively impermeable barrier to plasma proteins and bloodborne cells and it resists the generation of thrombin. After perturbation of the endothelium by noxious stimuli or by cytokines, the endothelial cells become activated and their functional properties change dramatically (28–34). Activated endothelial cells are adhesive for phagocytes and T cells and lose their barrier function, i.e., the ability to retard the egress of plasma proteins and blood cells. Activated endothelial cells stimulate the generation of fibrin by elaborating substances such as tissue factor and plasminogen activator inhibitor and through the loss of thrombomodulin, a cell surface protein that together with thrombin activates protein C, a circulating anticoagulant. Activated endothelial cells release biologically active substances such as platelet activating factor, which among various biologic properties, promotes adhesion and aggregation of platelets, and cytokines such as interleukin (IL)-1 which mediate proinflammatory changes and T-cell activation. The aberrant physiology of activated endothelial cells might thus contribute to the pathologic changes seen in hyperacute rejection.

In considering the pathogenesis of hyperacute rejection, we have focused on one potential biochemical change in endothelial cells that could underlie several of the pathologic changes, that is, the loss from endothelial cells of heparan sulfate (35). Heparan sulfate proteoglycan promotes the functional integrity of blood vessels by inhibiting the penetration through blood vessel walls of blood cells and plasma proteins (36), it contributes to the structural integrity of blood vessels by cementing endothelial cells to the underlying extracellular matrix, and it mediates the activation and attachment to endothelial surfaces of antithrombin III, a major anticoagulant (37,38), and superoxide dismutase, which clears potentially injurious free oxygen radicals (39,40). Thus, the loss of heparan sulfate from the cells (35). Our recent studies link this event to endothelial cell activation (41, 42).

If the involvement of endothelial cell activation in hyperacute rejection is a useful hypothesis, it is also one that is unproved. In support of the hypothesis, we have recently found that endothelial cells in rejecting xenografts express activation antigens (43, 44) and that glycoaminoglycans are lost very rapidly from rejecting xenografts (45). Yet, Auchincloss has argued that hyperacute rejection would likely occur in the absence of endothelial cell activation (46). Moreover, even if endothelial cell activation is of central pathogenetic import, it is difficult to imagine how all of its manifestations could be effectively countered. Indeed, therapeutic modalities directed at individual manifestations of endothelial cell activation may delay the onset of rejection, but none has allowed clinically significant prolongation of xenograft survival. Thus, regardless of which mechanisms contribute to the ultimate destruction of a xenograft, the optimal point for intervention is more likely to be the point of immune recognition than it is the stage when tissue injury occurs.

NATURAL ANTIBODIES AND THE CELLS THAT SYNTHESIZE THEM

Natural Antibodies: A Definition

All vertebrate animals have natural antibodies the presence of which are not linked to any known sensitizing event (47,48). Natural antibodies have been postulated to provide an initial defense against invasive microorganisms (47,49,50), contribute to the generation of idiotype repertoires (50), help regulate the function of growth factors (51), and aid in removing damaged cells from the blood (52). Some natural antibodies recognize the cells of xenogeneic species (53). One measure of the increasing phylogenetic distance between donor and recipient and thus, perhaps, of susceptibility to hyperacute rejection is the presence in the recipient of natural antibodies directed against cells of the donor (12,54,55). The critical question is whether the xenoreactive antibodies in the circulation of a xenograft recipient only mark phylogenetic distance or whether the antibodies participate in mediating tissue injury.

Involvement of Natural Antibodies in Hyperacute Rejection

There is little doubt that antibodies play the major role in initiating some forms of hyperacute rejection (6,13) (Figure 1, Panel I). We have attempted to determine the importance of human antibodies against pig by characterizing the natural human immune response to pig endothelial cells in vitro and the
primate response to pig organs in vivo. Using cultured porcine endothelial cells as a model for a xenogeneic organ, we showed that human natural antibodies bind to pig endothelial cells (56) and that antibody binding causes complement to be activated (57). Antibody binding and complement activation, in turn, mediate the activation of the endothelial cells. Although both human immunoglobulin (IgG and IgM bind to pig cells, it is IgM that mediates activation of complement (57); if IgM is removed (or deficient) from a serum but complement is intact, the ability of the serum to lyse or to activate endothelial cells is lost (58). These findings suggest that human complement is not activated directly by porcine endothelial cells, and thus, they are consistent with the work of Edwards who demonstrated that human complement is not directly activated by pig erythrocytes (59).

We (60) and others (24,26,61) have shown that pig to primate cardiac xenografts contain deposits of recipient Ig and that graft survival is prolonged by manipulations that deplete xenoreactive Ig from the circulation of the recipient (although other components may be depleted as well) (12,62–64). When, after antibody depletion, the complement system returns to normal, graft rejection does not occur but rather the xenograft functions until antidonor antibodies return to the circulation (13). Perper (61,65), Burdick (66), and coworkers showed that administration to a xenograft recipient of antigrift antibodies causes accelerated rejection of the xenograft.

Although the binding of natural antibodies leading to the activation of complement is clearly one important factor in the rejection process, we have suggested that the attachment of xenoreactive antibodies to certain antigens might perturb the endothelial cell expressing those antigens and thus mediate, independently of complement activation, changes in a graft (18). Thus, the specificity of natural antibodies may be a critical issue in hyperacute rejection.

The Specificity of Human Xenoreactive Natural Antibodies

Until recently, very little was known about the specificity of xenoreactive antibodies. Hammer and others postulated that natural antibodies recognize blood group antigens, perhaps related to the ABO system (53,67). Work by Good et al. using porcine blood cells as targets supports this contention (68). We have found that human natural antibodies recognize predominantly three porcine endothelial cell glycoproteins having molecular sizes of 115, 125, and 135 kd (69); these antigens are also present on porcine platelets. The antigen determinants are located on N-linked substitutions. Expression of a yet-to-be-identified porcine endothelial cell activation antigen is associated with antibody binding to the 135-kd glycoprotein but not with binding to other endothelial cell components (43). This finding is consistent with the idea that the targets of xenoreactive natural antibodies have a role in endothelial cell changes.

The Origin of Natural Antibodies

Two observations suggested to us that humans might share a repertoire of xenoreactive natural antibodies. First, the susceptibility to hyperacute rejection in a given combination of donor and recipient species is either common to nearly all or it is nil among potential donors and recipients (67). Second, xenoreactive natural antibodies directed against the cells of another species are either present in the circulation of many individuals of a given species or they are present in none (53). Although there are several possible explanations, we reasoned from these observations that humans might share a repertoire of xenoreactive natural antibodies. We focused our initial studies on naturally occurring antibodies that are encoded by lg genes with germplasmic sequences (70) and that are shared widely within a species. These natural antibodies are distinct from antibodies elicited by immunization in which the development of the repertoire is associated with Ig gene mutations and somatic rearrangements.

One peculiar characteristic of these broadly shared "natural" antibodies is that they are polyreactive. Polyreactive antibodies bind to a variety of antigens including soluble hormones, nucleic acids, and cytoplasmic proteins (49). Although polyreactive antibodies bind to such "common" ligands as single-strand (ss)DNA and thyroglobulin, each antibody displays a characteristic pattern of reactivities (71–73). Because, as discussed above, xenoreactive antibodies recognize carbohydrate modifications on endothelial cell glycoproteins (69), the "apparent" polyreactivity could be due to the presence of the same or cross-reactive carbohydrate epitopes on a number of different molecules. Conversely, different polyreactive antibodies might recognize the same modification on a given glycoprotein. It was therefore important to demonstrate polyreactivity directly.

We showed that the binding to porcine platelet antigens of human xenoreactive antibodies is blocked significantly by ssDNA and by thyroglobulin (Figure 2) (73). This experiment suggested that the human anti-pig repertoire does include polyreactive antibodies. Consistent with the potential involvement of these antibodies in the rejection of a xenograft, we showed that human antibodies eluted from a rejecting porcine organ would bind to thyroglobulin (74).

Polyreactive antibodies are thought to be made by B1 cells, most of which express CD5 on their surface,
Figure 2. Inhibition by ssDNA and thyroglobulin of binding to porcine antigens of human xenoreactive natural antibodies. Human serum was incubated with increasing amounts of ssDNA and thyroglobulin and was then added to cells precoated with porcine platelet antigens. The binding of IgM (A) and IgG (B) was measured by ELISA and expressed as percentage of binding that occurred in the absence of ssDNA or thyroglobulin.

in addition to the other markers characteristic of B cells (70,75). B1 cells are a unique, self-replenishing subset of B cells; in humans, they comprise 10 to 25% of circulating and splenic B cells (70). There is preferential use of the V{sub}III and V{sub}IV gene families as well as of particular D gene sequences by B1 cells (70).

To investigate the potential origin of xenoreactive natural antibodies, we studied the antibodies in serum from CBA/N mice, which are deficient in B1 cells. As shown in Figure 3, serum from these mice was much less reactive with porcine platelet antigens than was serum from Balb/C mice. We also studied a series of human monoclonal antibodies that were manifestly polyreactive and thus presumed to arise from B1 cells. All of the polyreactive antibodies were xenoreactive, as was demonstrated by their binding to porcine endothelial cells (73). Using one of these antibodies, #103, we developed a series of anti-idiotypic reagents that could be used to probe the human repertoire. The anti-idiotypic antibodies bound to antibodies present in serum from a number of individuals and to a small subset of splenocytes in both human and nonhuman primates (74). This sug-
Figure 3. Reactivity of IgM from normal (Balb/C) and B1-deficient (CBA/N) mice with porcine platelet antigens. Serum from CBA/N or Balb/C mice was tested by ELISA with porcine platelet antigens as a target. O.D., optical density.

Suggested that the xenoreactive repertoire was shared. We then used the anti-idiotypic antibodies to localize polyreactive Ig in pig to primate renal xenografts. The *103 idiotype was found along glomerular and interstitial capillaries in the rejecting kidneys, a location similar to that of IgM (60,74). These results demonstrated that polyreactive natural antibodies are among the xenoreactive natural antibodies that bind to xenogeneic blood vessels and indicate that the repertoire detected by an in vitro assay overlaps the repertoire that reacts with graft.

Although polyreactive natural antibodies are deposited in xenotransplanted organs, their role in the rejection process is unclear. The binding of xenoreactive neutral antibodies may mediate hyperacute rejection, as discussed above. From this hypothesis, it would follow that depletion of polyreactive natural antibodies from the circulation of the recipient would promote graft survival. On the other hand, Cohen and Cooke have suggested that natural antibodies may serve a protective function, preventing autoimmune disease, by masking self-determinants or by modulating the production of autoantibodies (76). A similar role for polyreactive natural antibodies may be postulated in the protection of xenografts.

COMPLEMENT

Antibodies alone are not sufficient for the initiation of hyperacute rejection; the activation of complement is clearly necessary as well. The survival of a discordant xenograft is dramatically prolonged in recipients from which complement is depleted by cobra venom factor (77-80) or when the graft is placed in a recipient who is congenitally deficient in the C5 or C6 components (81,82). In cases where the complement system is intact, complement levels in the serum of the recipient decrease precipitously after perfusion of a discordant xenograft; the decrease is thought to reflect "consumption" of complement in the graft (22,26) because complement proteins accumulate rapidly in such xenografts (26,60,81). Although it is possible that the presence of complement in rejecting xenografts may reflect nonspecific trapping of protein, the expression of C3b and C9 neoantigens, which result from the activation of the complement cascade, suggests that the presence of complement is a manifestation of activation.

Our in vitro studies also support the importance of complement. We have found that, in the activation of porcine endothelial cells by human natural antibodies, some manifestations of endothelial cell activation depend on the activation of complement (35,41,43). We have discussed elsewhere the mechanisms by which complement might mediate tissue injury in a discordant xenograft (19,58). The primary issue we shall address here is whether the mechanism of activation of complement might serve as a recognizable event (Figure 1. Panels II and III).

A model based on the activation of the "classical" pathway of complement is illustrated in Panel I of Figure 1. Hyperacute rejection is begun by the reaction with a graft of cytotoxic antibodies (12,83), and it is antibody binding that triggers complement activation, which in turn mediates tissue injury. Our work suggests that this model most accurately defines the pig to primate situation. Our in vitro studies also demonstrated that it is IgM and not IgG in human serum that caused complement to be activated and that mediated activation of endothelial cells (35,57).
This work was carried further by showing that complement-mediated lysis of porcine endothelial cells by human serum depends on the presence in the serum of IgM; IgM-depleted serum with intact complement does not lyse pig cells. The colocalization of IgM and complement along the endothelial surfaces in rejecting pig-to-primate xenografts is consistent with the findings (60).

Despite the logic of the "classical" model, the primacy of natural antibodies in natural immune recognition of xenogeneic organs has not gone unchallenged (84). Miyagawa et al. (22) and Johnston et al. (86) have shown that hyperacute rejection can occur in some species combinations in the absence of antitragraft antibodies; they favor a model in which complement provides not only the driving force for tissue injury but also the mechanism for xenogeneic recognition (Figure 1, Panel II). Studies of guinea pig to rat and rabbit to newborn pig renal xenografts (80,65) strongly suggest, in our view, that direct activation of complement in a xenogeneic organ might mediate rejection, although in no case can a role for antibody be completely excluded. What is not as yet certain is whether antibody-independent complement activation is initiated by spontaneous formation of the alternative pathway C3 convertase (C3bBb) or whether in some cases complement activation might be initiated by direct binding of C1q, although the former is more likely.

We (86) have suggested a third mechanism for the activation of complement in a xenograft (illustrated in Panel III of Figure 1). Normally, complement activation is regulated by a series of inhibitory proteins such as decay accelerating factor and homologous restriction factor. Because many of these inhibitory proteins are species specific, the activation of the recipient complement cascade in a xenograft might not be subject to negative regulation by these proteins. Thus, complement activation, regardless of the mechanism involved, might be amplified in a xenograft. We and later others (87) have suggested a therapeutic strategy based on this model: complement regulatory proteins of recipient origin might be directly inserted into donor endothelial cell membranes or the proteins might be expressed by developing donor animals having a transgene encoding the recipient proteins. Despite the novelty of this hypothesis, hyperacute rejection cannot require incompatibility of complement regulatory proteins because it occurs in allografts in which there is no incompatibility of these inhibitors.

CELLULAR NATURAL IMMUNITY

NK cells, which comprise 10 to 15% of human peripheral blood lymphocytes, mediate the cellular arm of natural immunity (88). Work from several laboratories suggests that NK cells could contribute to the immediate immune response to a xenograft (Figure 1, Panel IV). NK cells are thought to contribute to host defense against viral infections and to the destruction of tumor cells (references 88 and 89 for reviews). They do so by causing the lysis of target cells; however, unlike cytolytic T cells, NK cells do not require prior sensitization and are able to carry out non-major histocompatibility complex-restricted cytolysis (88). NK cells also mediate a form of cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC), in which antibody-coated target cells are lysed by effector cells bearing Fc receptors for Ig. These characteristics suggest that, analogous to natural antibodies, NK cells may act as a first line of defense against invading organisms. Inverardi et al. have shown that NK cells adhere to xenogeneic endothelial cells and display strong proliferative and cytotoxic responses against xenogeneic but not allogeneic endothelial cells (23). Kotasek et al. have shown that NK cells can cause the lysis of endothelial cells (90). This lysis may be facilitated by antibody-dependent cellular cytotoxicity through the binding of natural antibodies to xenogeneic endothelial cells.
T CELLS AND XENORECOGNITION

Although this review has focused on natural immunity as the immediate barrier to xenotransplantation, it is not unreasonable to imagine that this barrier will someday be breached. In that case, the immune barrier to xenografts will be in the form of elicited immune responses. Bach (91) and Moses and Auchincloss (92) have speculated about the characteristics of cell-mediated immunity that would develop against a xenogeneic organ graft if the humoral response were averted. In the closing paragraphs, as an expression of faith in the future of xenotransplantation, we summarize the issues that we believe will one day dominate the field.

Will an organ xenograft undergo cellular rejection if hyperacute rejection does not occur? Alexandre and we found CD2+ cells infiltrating pig kidney xenografts in monkeys in which the grafts functioned for more than 3 wk (J.L. Platt and G.P.J. Alexandre, unpublished observation). The presence of CD2+ cells is consistent with the infiltration of the graft with either T cells or NK cells, both of which express the CD2 marker.

Given a cellular immune response to a vascularized xenograft, what will be the size of the T-cell repertoire, that is, how many T-cell clones will contribute to the response? In general, the cellular immune system has evolved to recognize "altered self," that is, foreign antigens that are processed and presented in association with MHC antigens expressed on the surface of antigen-presenting cells. This is referred to as "indirect" recognition because the antigen is not seen in its native form but rather as a complex on the surface of antigen-presenting cells. In the rejection of allografts, allogeneic MHC antigens are recognized in this "indirect" manner but they are also recognized "directly." that is, as native structures on the surface of allogeneic cells. One explanation for the direct recognition of allogeneic MHC antigens is that they are only slightly different than "self" MHC and may in fact resemble the "altered self" normally composed of foreign antigens presented in the context of self MHC (93). As a result of both direct and indirect recognition, the repertoire of responder T cells is presumably larger than it would be in the case of indirect recognition alone. The issue, then, is whether the immune system of a xenograft recipient will view the graft as "altered self," thus permitting both direct and indirect recognition, or whether xenogeneic MHC antigens would be sufficiently different as to allow only indirect recognition. Moses and Auchincloss have suggested that the closer that two species are related, the more likely the response will resemble the allograft situation in which both direct and indirect recognition are possible (92). With more divergent combinations, direct recognition may not be possible and, as a result, the responding T-cell repertoire will be smaller (91).

Another salient issue of current interest is the extent to which the cell adhesion molecules and cytokines of the donor will be able to stimulate recipient immune responses (Figure 4). Moses et al. recently demonstrated that the failure of mouse splenocytes to respond to monkey, pig, and human xenoantigens
tested in the mixed lymphocyte reaction could result from multiple "defects" or incompatibilities, including the failure of cell surface molecules to form stable adhesions and the inability of cytokines to regulate the function of xenogeneic responder cells (94). Irwin et al. have shown that mouse CD8 molecules are unable to interact with the α-3 domain of the human MHC antigens (95). The work of Alter and Bach (93) demonstrated that, in the case of the response of human T cells to mouse lymphocytes, a full response, as measured by T-cell proliferation, required the presence of human interleukin-1 (IL-1). However, these models were all based on direct recognition of the xenogeneic antigens.

In considering the response of recipient T cells to a xenogeneic organ graft, it will be crucial to examine the interactions between the T cells and the blood vessels of the graft. Products of T cells and endothelial cells such as cytokines mediate reciprocally the responses of both types of cells. For example, IL-1 and IL-6 from endothelial cells can act as costimulatory signals for T cells (96) and IL-1 produced by T cells can mediate changes in endothelial cells, including increased expression of the cell adhesion molecules I-CAM-1, I-CAM-2, and ELAM-1, and production of IL-1 and IL-6 (97). Another T-cell-derived cytokine, interferon-γ, can induce the up-regulation by endothelial cells of MHC class II molecules and I-CAM (97). Thus, one variable will be the extent to which such reciprocal modulation will occur.

Migration of T cells into a xenograft is likely governed, at least in part, by the integrity of the extracellular matrix of endothelial cells. Naparstek et al. observed that activated human T cells release an enzyme that cleaves heparan sulfate from extracellular matrix (98) and postulated that heparan sulfate degradation would facilitate T-cell infiltration. Our findings that heparan sulfate release from endothelial cells can be mediated by natural antibodies and complement (35) and by neutrophils (99) provides a mechanism for even more rapid influx of blood cells into a graft.

The release of heparan sulfate from endothelial cells might also regulate T-cell and endothelial cell responses. Wrenshall et al. in our laboratory demonstrated that the T-cell proliferative responses are augmented by heparan sulfate (100) and that heparan sulfate causes activation of endothelial cells. These observations may explain one paradox surrounding the response of T cells to xenogeneic stimuli. The numerous incompatibilities that exist in xenogeneic combinations should preclude productive recognition of xenogeneic cells, yet observations in several models document a vigorous cellular immune response to xenografts. We would suggest that the release of heparan sulfate mediated by antibody and complement and/or by neutrophils may enhance the stimulation of responder lymphocytes by activating antigen-presenting cells.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (HL46810 and DK13083), the March of Dimes, and the Minnesota Medical Foundation. J.L. Platt is an Established Investigator of the American Heart Association. The Minnesota program in xenotransplantation involved the laboratories of Fritz H. Bach, R. Morton Bollman III, Arthur Matas, and Gregory Vercellotti, as well as those of the authors.

REFERENCES


48. Hammer C: Preformed natural antibodies (PNAB) and possibilities of modulation of hy-


82. Charrand C, O’Regan S, Robitaille P, Pinto-Blonde M: Delayed rejection of cardiac xenografts in C6-deficient rabbits. Immunology
Natural Immune Barrier to Xenotransplantation

1979;38:245.