The role of inflammatory reactions in xenotransplantation

Leonard Makowka, Frances Chapman, Donald Cramer, Linda Sher, Louis Podesta, Todd Howard and Thomas E. Starzl

Departments of Surgery and Pathology, University of Pittsburgh, the Veterans Administration Medical Center, Pittsburgh, PA and the Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA

The pathophysiological events leading to hyperacute rejection (HAR) consist of a non-specific effector cascade which invokes most, if not all, of the classical mediator systems of the acute inflammatory process. This response is generally thought to be humorally mediated and has been reported to occur in ABO-incompatible patients [1, 2], in those previously sensitized by a transplant [3], as a result of multiple blood transfusions [4], secondary to pregnancy [5] or in xenotransplantation [6]. The pathogenesis of HAR for the kidney and the heart has been extensively investigated and fairly well established. The causal event is a specific antibody-mediated injury (IgG and IgM) to the vascular endothelium [7], which is propagated by secondary vasoconstriction [8], the recruitment of polymorphonuclear leukocytes [9], and aggregated platelets [10, 11], then followed by intravascular coagulation [10–12]. The combination of vasoconstriction and the platelet-leukocyte-plugs leads to occlusion of the small arterioles and capillaries with resultant ischemic necrosis of the organ.

Humorally mediated HAR due to preformed antidonor antibodies also represents the mechanism of rejection in discordant xenografts. Support for this hypothesis has included the fact that heterospecific antibodies were often demonstrable by preoperative in vitro testing of the recipient serum [13] and that the physicochemical removal of immunoglobulins or the inactivation of complement in the recipient [14] often improved xenograft survival. The process of xenograft rejection is pathophysiologically very similar, if not identical, to that observed in ABO blood group major incompatibility or in the presence of serum cytotoxic antigraft antibodies [13,15]. In phyloge-
netically closer species (concordant), graft rejection appears more cellular in nature [6]. Therefore HAR represents a specific and defined, but incompletely understood, entity which can be elicited in different transplant situations and in different organs. At present, HAR cannot be managed with any significant consistency and, although it represents the greatest threat to kidney transplants, it is increasingly becoming an important consideration in cardiac and hepatic transplantation.

Further characterization of the mechanism(s) involved in, and the management of, HAR are clearly indicated. Firstly, patients with wide ranging antibodies who have become non-candidates for renal transplantation could again be considered. Secondly, and of major importance for the future of organ transplantation, is xenotransplantation which remains an important potential solution to the problem of insufficient donor supply, especially in pediatric patients. While not yet a clinical reality, xenotransplantation is the focus of intensive laboratory research.

Many of the mechanisms involved in the rejection of xenografts have been elucidated and a number of therapeutic approaches are under evaluation. This chapter will present an overview of the field and will describe some of the newer concepts in the pathophysiology and potential management direction of hyperacute (xenograft) rejection.

Pathophysiology of hyperacute rejection: Failure of the microcirculation

**Antigen-antibody reaction**

- Endothelial injury
- Activation of phospholipase A2
- Arachidonic acid cascade
- Thromboxane synthetase
- Thromboxane TxA2 (vasoconstrictor, proaggregatory)
- Organic peroxides
- Leukotrienes
- Adhesion, vasoconstriction, endothelial sticking
- Activation of PMN's

- Platelet - Leukocyte - Erythrocyte plug
- Occlusion of microvasculature
- NADPH oxidase
- O2

**Fig. 1.** Schematic illustration of the pathophysiology of hyperacute rejection.
Importance of the inflammatory process in hyperacute rejection

Hyperacute rejection fundamentally represents a devascularization in which small blood vessels become plugged with coagulation products and formed blood elements. While controversy remains over which antibodies are to blame, and under what circumstances immediate graft dysfunction occurs, hyperacute rejection must be regarded as the product of a complex immune/inflammatory reaction (Figs. 1 and 2).

The acute inflammatory mediators responsible for tissue injury are derived either from plasma proteins or from various types of cells, including polymorphonuclear leukocytes (PMNs), platelets, monocytes, macrophages, lymphocytes and endothelial cells. The four classic components of the inflammatory response (the complement, coagulation, fibrinolysis and the kallikrein-kinin systems) may generate inflammatory plasma proteins responsible for some of the key pathophysiological processes seen in HAR. These four protein systems interact with one another during the activation process and, furthermore, interact with and activate the cellular elements. In fact, it has been the insight recently gained into the cell-derived mediators which has allowed a fuller explanation and understanding of the interrelationships of the various pathophysiological processes which are further outlined below. Following the bind-

![Diagram]

Fig. 2. Schematic illustration depicting the final events of the immune inflammatory response following antigen-antibody interaction. Continued from Fig. 1.
ing of antigrift antibody to antigen on the endothelium, all the events that occur thereafter are immunologically non-specific, namely, activation of clotting, fibrin deposition, fibrinolysis, capillary leaking and the influx of inflammatory cells with their own release of mediators and consequent tissue injury. Thus, the process of hyperacute rejection, like that of organ ischemia and necrosis, may involve most of the components of a typical inflammatory response [16]. Obstruction of the small arteries by the platelet-neutrophil plugs results in cellular ischemia, which in turn may lead to activation of the complement system by the alternate pathway. The release of the vasoactive substances C3a and C5a results in enhanced inflammation with neutrophil margination and migration to the damaged tissue. This results in neutrophil activation, with a burst of oxygen consumption and the production of oxygen-derived free radicals, for example, via membrane bound NAD(O)H oxidase and release of lysosomal enzyme [16]. The activation of neutrophils leads to an increase in phospholipase A2 activity which hydrolyses arachidonic acid from membrane phospholipids [16]. A cascade is initiated which produces a large number of biologically active lipid products including the prostaglandins, leukotrienes and thromboxanes. Two of the more important pathophysiological lipid mediators include leukotriene B1, a potent neutrophil chemoattractant and thromboxane A2, a potent vasoconstrictor and platelet pro-aggregator.

Acetyl glycerol ether phosphorylcholine (AGEPC), also known as platelet-activating factor (PAF), is a mediator of many inflammatory reactions [17, 18] and represents the most recently described and novel class of lipid autocoids [19]. Moreover, PAF can evoke almost all the well-known cardinal signs of acute inflammation and probably plays a central role in the induction of tissue injury [25]. The biological effects of PAF include: (1) platelet and neutrophil aggregation and activation; (2) increase in vascular permeability; (3) smooth muscle contraction; and (4) the release of numerous vasoactive and potent biological mediators. Through these properties, PAF influences the microvasculature of all organs and therefore must be considered a key participant in the disruption of microvascular flow. There is considerable evidence for the candidacy of PAF as a central and key biological mediator in the pathogenesis of humorally mediated hyperacute allograft rejection [20–22].

As depicted in Figs. 1 and 2, the inflammatory reactions involved in HAR are remarkably complex. Furthermore, they are resilient and redundant in their organization, so that the same set of functions can be executed through many alternative backup systems. Consequently, even if the potent biological activities of one specific inflammatory mediator could be completely blocked by highly specific antagonists, the combined release of other inflammatory and biologically active agents could easily replace the functions of the missing agent and would be sufficient to mediate an unwanted inflammatory response.
Experimental manipulation of hyperacute rejection

Experimental concordant xenotransplantation
Concordant xenotransplantation is performed between two species that are closely related immunologically, e.g., two different species of subhuman primates or mouse and rat. The mechanism of rejection is primarily cellular in nature and is similar to classic acute allograft rejection. Therapeutic experimental studies have, for the most part, focused predominantly on the use of immunosuppressive regimens. Experimental models of various concordant combinations have been reported. Significant prolongation of cardiac survival in a hamster-rat combination was achieved with the addition of cyclosporine (CsA) [23]. However, virtually toxic levels of this drug were required for an effect, suggesting that its use alone may not be appropriate in a clinical setting. Knechtle evaluated total lymphoid irradiation along with CsA in hamster-rat and rabbit-rat combinations [24]. Highly significant prolongation of graft survival ( > 100 days) was evident in the first combination; however they were unable to prolong survival of rabbit-rat cardiac xenografts. Here again, the choice of donor-recipient combinations was critical. While both species combinations are considered concordant, the hamster-rat combination is phylogenetically more closely related than the latter.

Hardy and colleagues reported that peritransplant immunosuppression with a combination of palladium-109, hematoporphyrin and anti-lymphocyte globulin prolonged cardiac xenograft (hamster-rat) survival 10-fold [25]. Histological examination of the rejected hearts from treated animals demonstrated minimal lymphocyte infiltration or myocardial fiber destruction with patent coronary arteries. These findings were characteristic of the primarily cellular nature of this response, in contrast to control animals which consistently exhibited the classic features of hyperacute rejection.

Histopathological studies in these concordant models demonstrated significant acute cellular and chronic rejection, with a suggestion of a humoral component. Thus, although the immunological reaction in concordant cardiac xenotransplantation appears to be primarily an aggressive cellular rejection, it is suggested that the humoral and perhaps inflammatory component is significant and that it must be addressed in the management scheme.

Experimental discordant xenotransplantation
Discordant cardiac xenotransplantation demonstrates an entirely different immunological phenomenon. Species differences are sufficiently great that preformed heterospecific antibodies are present in the recipient. Rejection occurs within minutes and is humorally mediated. The severity and course of the rejection process, and the resultant pathology resemble the hyperacute rejection seen clinically in situations of ABO mismatch or hypersensitization. Although first described in renal transplantation [1–6, 26], HAR can also be seen in liver [27] and cardiac [28] transplantation. Experi-
mental discordant xenotransplantation has been utilized to further characterize the pathological lesions observed in HAR as well as the mechanism(s) involved. Conventional immunosuppression has generally proven ineffective.

In a highly discordant combination of guinea pig-rat cardiac graft survival time in untreated controls is 16 min. Treatment with cyclosporine, cyclophosphamide or splenectomy combined with a 1.5 plasma column exchange, resulted in significantly increased graft survival [29]. However, when each treatment regimen was used alone, no beneficial effect was observed. Short-term prolongation of discordant xenograft survival has also been reported following recipient pretreatment with cobra venom factor (CVF) or antiplatelet agents in combination with plasma modifications [30], as well as multiple administrations of CVF alone. Further, Shons reported that pretreatment with aspirin (80 mg/kg) extended pig-dog renal xenograft survival from 5 to 21 min [30]. Immediate graft dysfunction has also been prevented by prophylactic total-body heparinization in hypersensitized dogs. The effect of anti-platelet agents (aspirin) has also been studied in the guinea pig-rat combination, however, successful prolongation could not be achieved [31].

Prolongation of xenografts has been reported by transplanting successive organs from the same donor [32, 33]. Presumably protection of the final graft was accomplished by antibodies absorbed by the initial graft. In addition, mitigation of the xenograft reaction has also been described following removal of immunoglobulins by plasmapheresis [34].

Microscopic evaluation of discordant cardiac xenografts are characterized by capillary congestion and injury, massive extra vascular hemorrhage, interstitial edema, ischemic myocardial contracture and lack of interstitial white blood cell infiltration [35]. Thus, one of the motivations for studying discordant xenotransplantation is to further elucidate the mechanism(s) and pathophysiology of hyperacute rejection as well as to investigate various experimental manipulations that might abrogate this process.

When one considers the multiple inflammatory effectors and events involved in the pathogenesis of hyperacute rejection, it is not surprising that a successful therapeutic approach has not as yet been identified. There have, however, been significant advances involving the early events of the immunological cascade with less success in the late, end-stage events.

Advances in the management of hyperacute rejection in xenotransplantation

Newer approaches and progress in the abrogation of hyperacute rejection have resulted from studies on two of the more recently recognized and characterized effector arms of the secondary response, namely PAF and the arachidonic acid cascade.

In vitro studies have demonstrated that PAF is released when endothelial cells are incubated with antibody to cell surface angiotensin-converting enzyme [20]. This has important implications in the biology of the body and carries [21].

With the roles of the effector arms of hyperacute rejection now clearly defined, PAF was the first to be recognized and has been shown to have significant effects on the generation and propagation of proinflammatory events.

In a pig rat xenograft model, treatment with 4 mg of PAF receptor antagonist in cat-rabbits followed by 4 mg of conversion of arterial blood pressure (ABP) to 8.23 vs. 8.44 mm Hg. Histological examination revealed significant tubular dilatation and atrophy of the renal cortex.

In conclusion, hyperacute rejection was abrogated in all four of the animals treated with PAF.

Further studies have demonstrated that PAF plays a critical role in the pathogenesis of hyperacute rejection, and that its blockade may represent a novel therapeutic strategy for its prevention.
important implications for hyperacute rejection, in which the specific initiating event is a transplantation antigen-antibody interaction on the endothelial surface. In a model of hyperacute renal allograft rejection in sensitized rabbits, PAF was identified in the blood effluent from the transplanted kidney, following immediate antibody and complement fixation to the endothelium of glomerular and peritubular capillaries [21]. No PAF could be detected in the rabbits without clinical or histological signs of hyperacute rejection (i.e., there was no PAF released in syngeneic controls of renal allografts transplanted into unsensitized recipients).

With the availability of specific receptor antagonists of PAF, further insight into the role of PAF in the pathogenesis of HAR is now possible. The potential for a new method of controlling the effector cascade of HAR by pharmacological intervention can now also be explored.

In a model of ex vivo rabbit heart perfusion with transplantation alloantibodies, PAF was released in the absence of inflammatory cells [27]. The addition of a PAF receptor antagonist, SRI 63-072 (Sandoz Company), prevented the reduction of coronary flow by 70% within 2-4 min after its addition to the perfusate. Heart action was prolonged after the addition of SRI 63-072, however, eventual hyperacute rejection was not prevented. Implications that PAF may also contribute to cell-mediated cardiac allograft rejection have also been reported by Foegh et al. A slight but significant prolongation of rat heterotopic heart allograft was achieved following administration of a receptor-specific antagonist of PAF, SRI 63-441, when combined with prostanoids [36].

In a pig-dog renal xenograft model, we have previously reported an improvement in graft survival and function following the administration of a receptor-specific antagonist of PAF, SRI 63-441, when combined with prostanoids [37]. Similarly, in a cat-rabbit renal transplant model [38], we demonstrated that recipient pretreatment with 4 mg kg SRI 63-441, given intravenously prior to revascularization, significantly prolonged xenograft survival compared to vehicle-only treated controls (133.3 ± 8.23 vs. 84.3 ± 5.26 min. P < 0.002).

Histological studies of kidneys from SRI 63-441 treated rabbits showed only mild tubular vacuolization at one hour, compared to overt ATN in the control kidneys. At 1 1/2 hours both kidneys were similar, with at most 3% glomerular thrombosis and ATN.

While organ graft survival and function was extended in these studies, hyperacute rejection was not prevented. The need for a polypharmaceutical approach for the treatment of hyperacute rejection is clearly indicated.

Further evidence supporting the role of PAF in hyperacute rejection [3, 8] has been generated through studies in our laboratory utilizing a well-established model of hyperacute rejection in rats [39]. Inbred Lewis strain (RT1b) recipients were sensitized by four successive skin grafts from ACI strain (RT1a) donors. Control (vehicle only) animals consistently rejected donor cardiac grafts in a hyperacute fashion (216 ± 40.0 min. n = 74). Treatment of sensitized rats with a single intravenous bolus of SRI
Administration of 5 or 10 mg/kg failed to demonstrate significant prolongation of graft survival. At a dosage of 20 mg kg, three out of eight animals died with pulsating grafts suggesting some level of drug toxicity. Significant prolongation of cardiac allograft survival was achieved, however, when animals were treated with 15 mg/kg of SRI 63-441 (2475 ± 560 min, n = 20, P < 0.001). In fact, nine of these hearts functioned for 2–5 days following transplantation. The combination of a potent immunosuppressant, FK506 [40] and SRI 63-441 treatment in this model of hyperacute cardiac rejection resulted in remarkable prolongation of graft survival in healthy animals. The survival, for the most part, far exceeded the allograft survival in nonsensitized recipients (6.3 ± 0.5 days) and, in several cases, graft survival either approached or exceeded one month. These studies provide further insight into the prominent role of PAF in hyperacute rejection, its release from transplanted organs and its subsequent recruitment of inflammatory cells resulting in graft failure.

Experimental studies aimed at blocking HAR in its earliest stage, thereby preventing the initiation of the immunological event, is an area of current interest which may have significant implications. Its principle involves the ability of the Staphylococcus A exotoxin to bind selectively the Fc receptor of IgG and IgM [41]. By attaching the Staph-A protein to a sepharose column, it is possible to run plasma over the column and subsequently absorb the immunoglobulin [42]. Instrumentation (Citem-10, DuPont, Excorim) has been developed with two Staph-A columns, in parallel; as plasma is run over one column, the other saturated column is eluted free of bound antibody. By immunoadsorbing enough plasma volume, IgG and IgM levels can be decreased ten-fold (unpublished data). In theory, sufficient immunodepletion of preformed antibodies might eliminate the endothelial antigen-antibody interaction, and thus abrogate hyperacute rejection by preventing the initiation of the secondary cascade. Preliminary work with Staph-A immunodepletion in a pig-dog xenograft model has been performed in our laboratory [43], demonstrating some prolongation of graft survival (unpublished data). Experiments along a similar line, using total plasma exchange in a guinea pig-rat cardiac xenograft model have demonstrated even longer graft survival [29].

Summary

Since the original description of hyperacute rejection of the kidney, numerous experimental and clinical studies have attempted to define the precise mechanism(s) of this process. While our interpretation of HAR has advanced, one must recognize this phenomenon as multifactorial and the product of a complex immune inflammatory reaction.

The non-specific effector inflammatory cascade that ensues once an antigen-antibody reaction has initiated hyperacute rejection serves as the target for therapeutic intervention been consid.

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intervention by various modalities. Classically, the platelet-coagulation system has been considered to be the most important and has received the greatest attention. These therapeutic modalities have included heparin, aspirin, dextran, citrate, defibrinating agents, cobra venom factor, induction of thrombocytopenia by antibodies, and various prostaglandins [28-30]. All these approaches have been either too toxic or the results too inconclusive to be accepted for generalized use.

The difficulty in overcoming the inflammatory process in hyperacute rejection and in explaining the discrepancies in therapeutic results rest not only in the complexity of the biological reactions, but also in their resiliency and redundancy. That is, there are numerous alternative mediator pathways that can back up the same set of functions. Thus, the inhibition of one arm of an inflammatory reaction can easily be replaced by the release of other inflammatory autocoids with complete mediation of the disease process.

Conclusions

Future therapeutic approaches in the treatment of hyperacute rejection rely on a better understanding of the basic pathophysiological processes of inflammation. It is very likely that a combination of approaches, affecting both the immunological and non-immunological components of this reaction will be required to achieve complete or significant elimination of hyperacute rejection. Perhaps the combination of Staph-A immunodepletion, PAF inhibition with eicosanoid-mediated vasodilatation, and potent new immunosuppressive regimens with such agents as FK 506 will be effective. At this point, the potential for success in xenotransplantation appears significant.

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References


