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Production of proinflammatory cytokines and adhesion molecules in ex-vivo xenogeneic kidney perfusion

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Abstract Xenogeneic transplantation of solid organs is limited due to hyperacute rejection. In concordant systems, the mechanisms of rejection can be studied due to cross-reactivity of mediators with anti-human monoclonal antibodies. The aim of this study was to obtain information about the kinetics of proinflammatory cytokines and production of soluble adhesion molecules in the acute phase of reperfusion, eight kidneys from rhesus monkeys were perfused ex-vivo with human blood (group B/0) for 1 hour in a closed system. Blood levels of IL-1b, IL-6, TNFα, soluble ICAM, and E-selectin were measured using an ELISA technique under steady-state conditions. Cytokine levels rose significantly within the 60-min interval (IL-1b, 6.1 ± 2.6–$161.1 + 98.5$ pg/ml; IL-6, 30.2 ± 7.7–274.2 ± 75.8 pg/ml; TNFα, 544.2 ± 363.6–1651.0 ± 25.7 pg/ml; $P < 0.05$). Immediately after the beginning of reperfusion, soluble ICAM-1 and selectin levels were abnormally high and rose constantly throughout the observation period, reaching significance at 60 min. High levels of proinflammatory cytokines may lead to an induction of adhesion molecules, thus, upregulating the leukocyte-endothelial interaction in a complement-independent mechanism. Specific pretreatment with monoclonal antibodies against ICAM-1, LFA-1, or other soluble mediators may be useful in down-regulating hyperacute rejection in trans-species transplantation.

Key words Xenotransplantation
Ex-vivo hemoperfusion
Kidney transplantation

Introduction

Serious shortage of human donor organs has led to a continuing interest in xenotransplantation of solid organs including kidneys. Many factors have been found to contribute to hyperacute rejection in trans-species transplantation. However, the relevance of proinflammatory cytokines and adhesion molecules and their implication for hyperacute rejection are not precisely known [1]. Due to species specificity, the interaction of soluble mediators of leukocyte and endothelial activation can only be observed in closely related (concordant) systems when antigeneic cross-reactivity exists. Thus, rhesus monkey mediators can be monitored by the use of anti-human monoclonal antibodies. Ex-vivo hemoperfusion of rhesus kidneys with human blood represents an established model for xenotransplantation under standard laboratory conditions [2, 3]. In concordant systems (e.g. rhesus-man), hyperacute rejection occurs to a lesser degree, and is reflected by a lower profile of plasma prostanoids

within the 1st hour of reperfusion [3]. The role of preformed natural antibodies (pNAB) and complement activation of the classical or alternative pathway has been discussed previously [4].

High levels of proinflammatory cytokines may lead to induction of adhesion molecules. Thus, ICAM-1 expression is found in acute kidney allograft rejection [5, 6], and acts as an inductor of leukocyte-endothelial interaction mediated by LFA-1. In humans, soluble ICAM-1 [7] and proinflammatory cytokines [8] have been found in acute liver allograft rejection, underlying the importance of these key mediators in immune functions after transplantation. Therefore, we postulated that the expression and release of adhesion molecules might contribute to the process of microvascular thrombosis in xenotransplantation. The aim of this study was to analyze, in an ex-vivo hemoperfusion model, the production of proinflammatory cytokines and adhesion molecules (ICAM-1, ELAM) in order to obtain further information about the kinetics of mediator release in concordant xenograft rejection.

Materials and methods

Perfusion experiment

Eight rhesus monkeys served as multiple organ donors. Under general anesthesia with fentanyl and halothane, they were perfused via an aortic catheter using at least 3 l University of Wisconsin (UW) solution. At the end of perfusion, the kidneys were rapidly removed and stored at 4 °C for 1 h and then reperfused for 60 min with pooled fresh heparinized human blood of group B or 0 in a specially constructed perfusion chamber using an oxygenator and a pulsatile roller pump. The hematocrit was kept constant at 30% throughout the whole experiment. During reperfusion, pH, pO_2, pCO_2, urine production, blood flow, and temperature were monitored.

Laboratory investigations

At defined time points (1, 5, 15, 30, and 60 min), serum samples were taken and Na, K, osmolality, and creatinine were determined IL-1b, IL-6, TNF-alpha, sICAM-1, and s-E-selectin were measured using the ELISA technique.

Results (Fig. 1)

Kidney function was maintained for at least 60 min, and was confirmed by the plasma/urine osmolality ratio. Plasma osmolality, serum electrolyte levels, blood gases, and pH values in the perfusate remained unchanged or were corrected during the experiment. However, beginning at 30 min, there was a marked decrease in blood flow due to increasing perfusion pressure, which was paralleled by a decrease in urine production; 90–120 min postreperfusion there were signs of graft thrombosis. Cytokine production increased at 30 min and continued to rise up to 60 min. Blood levels of ICAM-1 and E-selection were abnormally high in the early reperfusion phase, with a constant rise during the observation period, whereas IL-2 receptor levels showed no significant changes (data not shown).

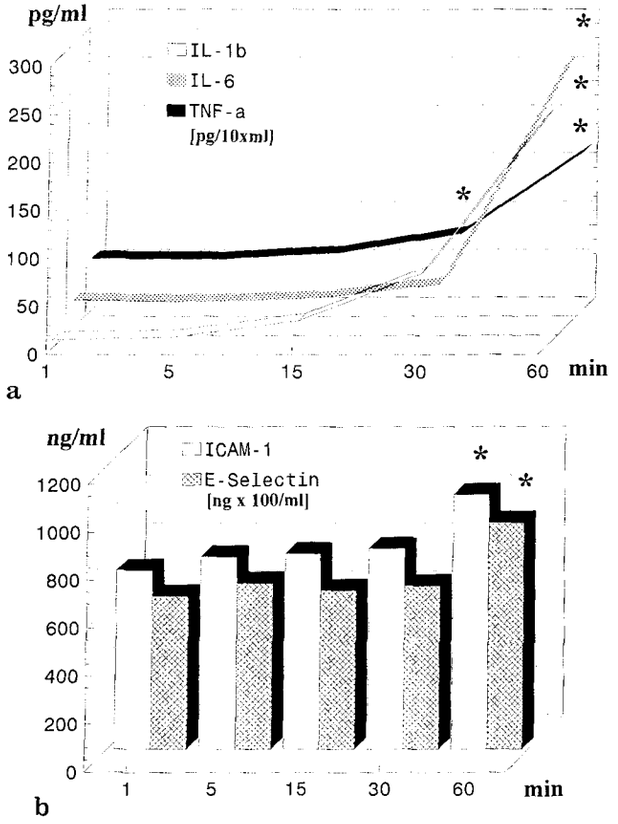

Fig. 1 **a** Proinflammatory cytokine and **b** soluble adhesion molecule production during the 60-min reperfusion period

Discussion

As shown by our experiments, a vigorous activation of leukocytes, macrophages, and endothelial cells occurred during concordant xenogeneic ex-vivo reperfusion. In this transplantation model, the mechanisms of rejection were studied without any immunosuppressive therapy or pretreatment of the perfused blood, such as heating to remove complement. The observed levels of soluble

mediators of immune activation may only represent a small window to the complicated interactions of leukocytes and endothelial cells, that, as a final consequence, lead to microvascular thrombosis and final graft rejection. It is known that high levels of proinflammatory cytokines, especially TNF, may upregulate the expression of adhesion molecules and, thus, augment a preexisting leukocyte-endothelial interaction that may occur at reperfusion. Studies have shown that anti-TNF antibodies in combination with TGF-β (an effective immunosuppressive substance) are able to prevent rejection of islet xenografts in mice [9]. Whether this is also possible when solid organs are transplanted is unknown.

The source of circulating ICAM-1 in our experiments was unclear, since a period of 60 min is too short for de-novo synthesis by endothelial or tubular cells; immunohistological studies are necessary and under way to answer this question. In humans, de-novo synthesis of adhesion molecules may act as early predictive markers of rejection [10]. Monoclonal antibodies against ICAM-1 are able to prolong allograft survival in monkeys [11], which supports the theory that adhesion molecules play a role in acute rejection. In humans, no such studies are available.

In conclusion, our study showed that hyperacute xenogeneic rejection leads to an activation of the perfused blood as well as the perfused organ within 1 h of reperfusion. Further experiments that combine the elimination of complement activity with the pretreatment of neutralizing monoclonal antibodies against cytokines, adhesion molecules, or LFA-1 [12], or a combination of these, are necessary before xenotransplantation of kidneys can be successfully performed in humans.

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