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Prolongation of discordant renal xenograft survival by depletion of complement. Comparative effects of systemically administered cobra venom factor and soluble complement receptor type 1 in a guinea-pig to rat model

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Abstract There is an increasing body of evidence to suggest that inhibition of complement activation may be a valuable approach to avert hyperacute rejection. In our study, the guinea-pig to rat discordant kidney xenograft model was adapted for the investigation of renal transplant function and an attempt was made to delay the hyperacute rejection using systemically administered cobra venom factor (CVF) and soluble complement receptor type 1 (sCR1). The saline-treated control recipients experienced a rapid transplant rejection with a xenograft survival averaging 10.5 ± 2.1 min. Administration of a single 60 U/kg i.v. bolus of CVF significantly prolonged renal graft survival to 20.4 ± 2.5 h, and by a single bolus of sCR1 (50 mg/kg) a prolongation of graft survival to 18.8 ± 2.3 h was achieved. The grafts functioned only over periods of 2.5 ± 0.3 and 2.3 ± 0.2 h, respectively. No complications of sCR1 were noted. We concluded that complement inhibition by sCR1 may be an important component in the therapeutic approach aiming at the prevention of hyperacute rejection in human organ transplantation.

Key words Kidney xenograft
Complement · Cobra venom factor
Complement receptor type 1
Guinea-pig to rat model

Introduction

Complement activation is thought to be critical for the hyperacute rejection of xenografts. Hyperacute rejection is initiated when complement is activated by natural antibodies against the vascular endothelium of the transplanted organ or by the endothelium itself. The events in the vasculature of the xenogeneic organ may result in loss of endothelial functional integrity and fibrin deposition. There is an increasing body of evidence to suggest that inhibition of complement activation may be a valuable approach to avert hyperacute rejection [3]. When the guinea-pig heart is transplanted into the rat, it is rejected very rapidly via activation of the so-called alternative complement pathway without the contribution of natural antibody to rejection [6, 7]. Previously, a marked improvement in graft survival has been achieved in this model with cobra venom factor (CVF) used for depletion of complement in the recipients [1]. Recently, the administration of several new reagents blocking the complement alternative pathway such as K76COOH and FUT175, have resulted in prolongation of guinea-pig to rat heart transplant survival [7]. The effects of complement inhibition using systemically administered soluble complement receptor type 1 (sCR1) in the guinea-pig to rat model of hyperacute xenograft rejection have also been

investigated [9]. That study has shown that sCR1 significantly delays hyperacute cardiac rejection. In our study, an attempt was made to interfere with, or at least to delay, the hyperacute rejection of renal xenografts using systemically administered CVF and sCR1. The guinea-pig to rat discordant xenograft model was adapted for the investigation of kidney transplant function, this assay being crucial for evaluation of successful renal xenografting.

Materials and methods

Animals

Male Wistar rats, 12 to 14-weeks of age, and male albino guinea-pigs 4- to 8-weeks of age served as renal xenograft recipients and donors, respectively. Under ether anaesthesia, renal transplantation was performed by a modification of the microvascular technique described by Lee [5]. The termino-lateral anastomoses of the donor aorta patch to the recipient abdominal aorta and of the donor renal vein to the recipient IVC were performed [2]. A ureteral catheter was used for monitoring the diuresis. Renal xenografts were evaluated visually over the first 120 min following the reperfusion, and then by relaparotomy following abdominal closure after cessation of diuresis until rejection. Rejection was confirmed by direct visualization and histoligical examination. Serum samples were collected preoperatively as well as 60 min following xenograft reperfusion and at rejection and stored at −70 °C until examination.

Methods

Recipient rats were randomly allocated to one of the following immunosuppressive regimens:

1. Control group: 3 ml saline ($n = 12$)
2. Cobra venom factor (CVF), 60 U/kg in 3 ml saline ($n = 12$)
3. Soluble complement receptor type 1 (sCR1), 50 mg/kg in 3 ml saline ($n = 12$)

Saline, CVF and sCR1 were administered by intravenous bolus into the recipient IVC, superior to the caval cross-clamp, immediately prior to the xenograft reperfusion.

Saline-treated control recipients were sacrificed at 8–10 min following reperfusion at the first visual sign of rejection for histological study of unmodified xenograft rejection. All CVF- and sCR1-treated recipient rats were evaluated at 120 min following reperfusion by kidney graft biopsy. The time of diuresis was monitored by means of ureteral stents. The surviving animals were sacrificed 24 h after reperfusion and histological examination was performed.

Complement activity was measured by the technique described by Rapp and Borsos [10], and modified by Pruitt [9]. sCR1 was generously provided by Smith, Kline and Beecham Inc., Cambridge, UK. CVF was purchased from Serva Inc., Heidelberg, Germany.

Histological evaluation was performed on biopsy samples collected at the time of rejection or serial sacrifice. The biopsies were fixed in 10% formalin, embedded in paraffin, sectioned, and stained using haematoxylin and eosin. Renal xenografts were evaluated for the presence of necrosis, intravasal thrombosis, interstitial haemorrhage and infiltration.

Statistical analysis was carried out using the log-rank test for comparison of xenograft survival among treatment groups. The SAS statistical package was utilized. A P value of less than 0.05 was considered significant. Values are expressed as mean + SEM.

Results

Xenograft survival

As depicted in Fig. 1 the guinea-pig to Wistar rat kidney xenograft survival differed markedly in the treated and untreated recipients. The saline-treated control recipients ($n = 12$) experienced a rapid transplant rejection with a xenograft survival averaging 10.5 + 2.1 min. Administration of a single 60 U/kg intavenous bolus of CVF ($n = 12$) significantly prolonged renal graft survival to 20.4 + 2.5 h ($P < 0.0001$). No adverse effects of CVF were observed. In the group of 12 recipients treated with a single bolus of 50 mg/kg sCR1 a prolongation of graft survival to 18.8 + 2.3 h ($P < 0.0001$) was achieved. No complications of sCR1 administration were noted.

Diuresis of transplanted organs

The saline-treated guinea-pig to Wistar rat kidney transplants had no function after reperfusion. Despite prolonged transplant survival in the CVF and sCR1 groups, these grafts functioned only over periods of 2.5 + 0.3 h and 2.3 + 0.2 h, respectively. The duration of diuresis in the recipient groups is depicted in Fig. 1.

Complement activity

For each group of recipients treated either with sCR1 (50 mg/kg) or with CVF (60 U/kg), CH50 was signifi-

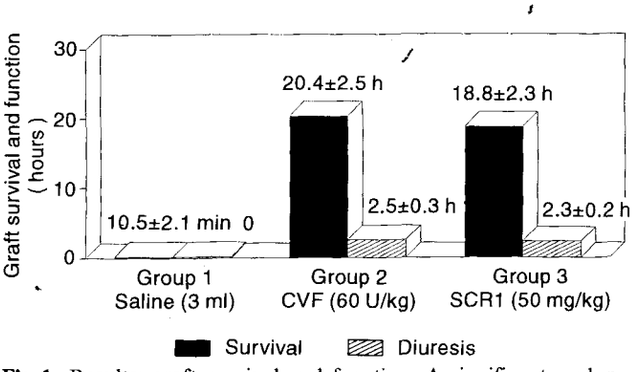

Fig. 1 Results: graft survival and function. A significant prolongation of graft survival from 10.5 + 2.1 min to 20.4 + 2.5 h and 18.8 + 2.3 h with CVF (60 U/kg) and sCR1 (50 mg/kg), respectively was achieved. The grafts functioned over periods of 2.5 + 0.3 and 2.3 + 0.2 h, respectively

cantly reduced 5 min following reperfusion to 5% of its pretreatment activity. CH50 was still markedly inhibited in the CVF-treated rats at the time of rejection, while its level in the group receiving sCR1 was returning slightly toward preoperative values. In the saline-treated control recipients no significant reduction in CH50 levels at the time of rejection.

Histology

In the xenograft recipients in the saline-treated group, platelet aggregates occluding the glomeruli were consistently present at times corresponding to the time of rejection. Rejected xenografts from sCR1, CVF- and saline-treated animals showed significant necrosis, interstitial haemorrhage and platelet aggregates occluding the vessels. In addition, marked infiltration consisting of PMN cells were seen in the sCR1- and CVF-treated transplants.

Discussion

Xenograft models of kidney transplantation

The guinea-pig to rat renal transplantation model used in this study and described elswhere in detail [2], is a good model for discordant xenografting in rodents as the time course of rejection is highly reproducible in this model. Kidney transplants are destroyed by hyperacute vascular rejection within 15 min following revascularization. The mean rejection time for untreated cardiac grafts in the same combination of donor and recipient is also approximately 15 min [4]. However, in contrast to cardiac transplant function, the quantitation of kidney function in this model is more difficult and its reliability remains a matter of debate. In our experience, the monitoring of diuresis by stents inserted into the transplant ureter proved to be highly satisfactory.

Previous studies using CVF

Depletion of the complement system by using CVF has been very efficient in the extension of xenograft survival in the discordant rodent cardiac models. CVF has induced the most durable inhibition of hyperacute vascular rejection in rodents [11]. The cobra C3b causes a massive consumption of complement components by combination with recipient products of the alternative pathway of complement and forming an enzyme leading to exhaustion of these components [8]. Data on efficiency of CVF in coping with the hyperacute rejection of rodent kidney xenografts are not known to date. Our results confirmed the high efficiency of CVF in prolonging renal xenograft survival for up to over 20 h in the guinea-pig to rat renal transplant model.

sCR1 as a new drug for delaying the hyperacute rejection

Using the same animal model, the immunosuppressive activity of the newly developed complement inhibitor sCR1 was assayed. sCR1 administration resulted in a similar survival of xenografts of over 20 h. Moreover, renal function, as measured by diuresis, existed for over 2 h. The histological findings showing prevention of platelet thrombi formation in the glomeruli suggested protective effects of complement inhibition with sCR1 on the hyperacute rejection response. Complement inhibition with sCR1 is similarly efficient in delaying cardiac xenograft rejection and prolonging heart transplant survival and function [9]. We concluded that complement inhibition by sCR1 may be an important component in the therapeutic approach aiming at the prevention of hyperacute vascular rejection in human organ transplantation.

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