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Rolf Linke
Michael Diefenbeck
Rainer Friedrich
Daniel Seehofer
Claus Hammer

Monitoring of microhemodynamic changes during ex vivo xenogeneic liver perfusion using intravital microscopy

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R. Linke (✉) · M. Diefenbeck
R. Friedrich · D. Seehofer · C. Hammer
Institute for Surgical Research,
Klinikum Grosshadern, Marchioninistr. 15,
D-81366 Munich, Germany
Fax: + 49 89 70 95 88 97

Abstract The main targets of xenogeneic rejection mechanisms are the endothelial cells of the graft. Their activation and the consequent alteration of the organ's microcirculation lead to the destruction of the xenograft. Microhemodynamic changes occurring during this process are still poorly characterized. The aim of this study was to analyze the microcirculation during xenogeneic ex vivo hemoperfusion of rat livers and to monitor the impact of treatment strategies using intravital fluorescence microscopy. In contrast to the isogeneic control group, blood flow almost completely stopped within the first minutes of xenoperfusion. Simultaneously, perfusion pressure increased and bile production was reduced. Acetylsalicylate (Aspisol) and the platelet-activating factor antagonist WEB 2170 improved the microcirculation and function of the xenoperfused liver. The combination showed a synergistic effect. After apheresis of preformed xenogeneic antibodies, the parameters measured were comparable with those seen in isogeneic experiments. Complement degradation with cobra venom factor revealed a minor improvement in perfusion. A rapid, extensive, and irreversible leukocyte accumulation in terminal portal vessels was observed in all xenogeneic experiments. Blood counts of the perfusate confirmed the early trapping of leukocytes and platelets in the xenoperfused liver, indicating nonimmunological, cellular involvement in this rejection process.

Key words Xenotransplantation, liver, intravital microscopy · Liver transplantation, xenografting, hemoperfusion, intravital microscopy · Intravital microscopy, liver transplantation, xenografting

Introduction

Animal organs, especially those of pigs, could one day compensate for the increasing shortage of allografts for humans. The mechanisms underlying hyperacute xenogeneic rejection after discordant xenotransplantation remain a central topic of research. Investigations using different combinations of species indicate that donor endothelial cells (EC) play a key role in the discordant xenogeneic rejection process. EC are activated by xenoreactive natural antibodies and/or the complement system [2, 11, 22]. Activated EC change their physiological behavior with regard to vascular integrity and cell interaction by switching their anticoagulant surface to a coagulant condition, which leads to a complete breakdown of the microcirculation of the graft [23, 31]. Specific inhibition of these humoral factors, particularly the complement cascade, using cobra venom factor (CVF) or the transgenic expression of complement regulatory proteins by ECs of xenogeneic organs seems to prevent the initial step of the rejection process and to prolong graft function [8, 25]. In comparison with the rejection process after allotransplantation, where alterations in microhemodynamic parameters are well docu-

mented [18, 19, 26, 27], the microcirculatory changes occurring during xenogeneic graft rejection and the efficacy of treatment have not been reported. Strategies in different animal models, e.g., studying the influence of acetylsalicylate [1] and platelet-activating factor antagonist [16] on platelet function, or the inhibition of the complement system using CVF [33], or the removal of antibodies [13], have resulted in only limited prolongation of graft survival.

The aim of the present study was to analyze microcirculatory changes occurring during xenogeneic liver perfusion and to quantify the efficacy of treatment strategies. We used a modification of the well-established isolated rat liver perfusion system [20] and applied intravital microscopy (IVM) for 45 min while livers were xenoperfused with human blood.

Materials and methods

Animal preparation

All experiments were performed in accordance with the German legislation on the protection of animals. Livers of Spraque-Dawley (SD) rats weighing 250–300 g (Charles River, Germany) were mobilized in situ under intraperitoneal pentobarbital anesthesia (60 mg/kg). The common bile duct, the portal vein (as the influx), and the thoracic inferior vena cava (as the drain) were cannulated. Both the abdominal vena cava and aorta were ligated and the rats anticoagulated with 200 IU heparin. During flushing of the livers with 30 ml of 25 °C lactated Ringer's solution at a hydrostatic pressure of 12 cm, rats were euthanized with an overdose of pentobarbital.

Preparation of perfusion medium

For isogeneic control perfusion, blood was taken by puncturing the aorta of SD rats under pentobarbital anesthesia. Human blood from two volunteers (blood group 0, Rh-negative) was taken for xenoperfusion. The blood was anticoagulated with heparin (100 IU/ml) and diluted with 6% iso oncotic hydroxyethyl starch (Fresenius, Germany) to a hematocrit (Hct) of 30% to provide an optimal combination of blood flow and oxygen transport capacity while maintaining physiological perfusion pressures in the system used [29].

Perfusion system and experimental design

The in situ livers were hemoperfused for 45 min at a constant physiological flow rate of 1.15 ml/min per gram. The closed perfusion circuit consisted of a reservoir, a peristaltic roller pump (IPSS 8, Ismatec, Germany), silicone tubes (ID 2 mm), a bubble trap, a blood filter (60 µm, Millipore, Ireland), and a membrane oxygenator with thin-walled silicone tubing (ID 1 mm, wall thickness 0.2 mm, Detakta, Norderstedt, Germany) coiled around a warming core. Temperature and pH of the perfusate were kept in physiological ranges and the perfusion pressure was monitored continously with a pressure transducer (Ohmeda DTxx, Germany) and monitor (Siemens Sirecust 304, Germany). The priming volume of the whole system was 10 ml, and the starting volume in the reservoir was 50 ml.

To investigate the efficacy of different treatment strategies during rat liver hemoperfusion, the following groups ($n = 6$) were analyzed:

Control groups administered a perfusate consisting of:

1. isogeneic rat blood (ISO), Hct 30%
2. xenogeneic human blood (XENO) Hct 30%

Experimental groups, perfusate consisting of human blood, Hct 30%, in addition to:

3. acetylsalicylate (Aspisol; 0.1 mg/ml[1], Bayer, Leverkusen, Germany; ASP)
4. Platelet-activating factor antagonist (WEB 2170, 0.033 mg/ml[1], Boehringer Ingelheim, Germany; WEB)
5. a combination of ASP and WEB (COMB)
6. cobra venom factor (1 µg/ml[1]; CVF), a gift of Dr. D. White, Cambridge, England
7. removal of antibodies (IgG and IgM) using sepharose apherese columns coated with polyclonal antihuman IgG/IgM antibodies (Therasorp, Germany; ABR).

Intravital microscopy (IVM)

For IVM, blood was stained with fluorescein sodium (4.5 µg/ml, Sigma, Germany) to enhance the contrast of plasma. Rhodamine 6G (0.6 µg/ml, Sigma) was used to identify leukocytes and platelets. The mobilized right liver lobe was investigated during perfusion using a fluorescence microscope (Leica, Germany) with epi-illumination. A water immersion objective and different filter blocks were employed to distinguish the distinct fluorescent dyes. The microscopic pictures were transferred via a CCD camera (Pieper Fk 6990, Switzerland) to a monitor (Sony Trinitron PVM 2043, Japan) and recorded with an SVHS video recording system (Panasonic 7350, Japan). A video timer (VTG-33, FOR-A, Japan) placed between the camera and monitor allowed for a time-related interpretation of the video sequences. During the observation period of 45 min, 10–12 liver fields, 10–12 postsinusoidal venules, and 5–10 terminal portal venules were randomly selected and recorded for 50 s (magnification × 800 on the monitor). It is known that the microscopic investigation of individual liver lobules for 30–60 s does not result in deterioration of the microcirculation due to light exposure [37]. After 45 min of perfusion, the lobe was scanned transversally and 30–50 acini were recorded at a lower magnification (magnification × 400 on the monitor) to determine the acinar perfusion.

Off-line video analysis

To quantify sinusoidal perfusion failure during hemoperfusion, the sinusoidal perfusion rate was calculated as the number of perfused sinusoids per total number of sinusoids. To quantify the perfusion status of the whole liver, taking into consideration the distribution of perfusion failure, an acinar perfusion index was calculated. A total of 30–50 acini were categorized according to hepatocellular uptake of fluorescent dyes and sinusoidal flow as: not perfused (no staining, no flow), irregularly perfused (patchy staining, flow only in parts of the acinus), and regularly perfused (homogeneous stain-

[1] The dosage used was determined with the help of previous experiments [1, 8, 10] in preliminary dose finding experiments.

Table 1 Perfusion pressure during ex vivo liver perfusion as mean ± SEM in mmHg

Group (treatment)	Time (min)				
	0	5	15	30	45
ISO	11.9 ± 0.9	10.9 ± 1.0*	10.3 ± 1.4*	09.8 ± 1.2	09.7 ± 1.0
XENO	11.5 ± 2.7	20.9 ± 3.1**	19.2 ± 3.9**	12.2 ± 2.2	11.2 ± 2.6
ASP	10.5 ± 2.4	13.9 ± 2.0*	13.4 ± 1.4*	10.5 ± 1.7	09.0 ± 1.9
WEB	09.4 ± 1.3	14.9 ±2.0*, **	14.3 ± 1.6**	12.2 ± 1.4	09.4 ± 1.4
COMB	11.4 ± 0.8	15.3 ± 0.8*, **	11.4 ± 0.7*	09.5 ± 0.9	08.1 ± 1.1
CVF	09.0 ± 0.8	17.7 ± 1.6**	14.4 ± 1.3**	12.4 ± 1.0	11.9 ± 0.9
ABR	08.3 ± 1.0	09.6 ± 1.4*	12.0 ± 3.1*	10.7 ± 2.9	09.2 ± 2.4

* $P < 0.05$ vs XENO perfusion group; ** $P < 0.05$ vs ISO control group

ing and flow). The index of acinar perfusion was calculated using the formula: Index (acinar perfusion) = [N (regular perfused) + 0.5 N (irregular perfused)]/N (total).

Additional parameters

Bile flow, as an indicator of liver function, was measured before the start of the experiment (basal bile flow) and at 15-min intervals during perfusion, calculated as flow (µl) per gram of liver per minute.

Blood samples for hematological (Hct, Hb, WBC, RBC, and platelets) and biochemical (AST, LDH, potassium, sodium, and calcium) analysis were taken at given time intervals (0, 5, 15, 30, and 45 min). Serum complement activity after CVF treatment was tested with the CH-50 method. After incubation of blood with 1 µl/ml CVF for 2 h, the complement activity was completely abolished without any signs of hemolysis. Preformed natural antibodies (PXNA) were quantified by agglutination of rat erythrocytes, as described elsewhere [7]. Apheresis of PXNA resulted in a decrease of 95% of total IgM and of 85% of total IgG [34]. At the end of liver perfusion, specimens were fixed in 7% formalin and embedded in paraffin. Sections (3–4 µm) were stained with hematoxylin and eosin. Histological findings were graded according to the degree of damage: grade 0, no damage; grade 1, portal and sinusoidal hyperemia; grade 2, parenchymal lesions; grade 3, complete necrosis.

Statistical analysis

The data are expressed as means ± SEM. Normality was tested with the Kolmogorov-Smirnov test. All data were analyzed with the one-way ANOVA and the all pairwise multiple comparison method Student-Newman-Keuls test, using Sigmastat 1.0. A difference was regarded as significant when the P value was less than 5%.

Results

Portal perfusion pressure

The portal pressure of the isogeneic control group at a constant blood flow of 1.15 ml/min per gram ranged from 11 (± 0.9) mmHg at the beginning and 9.7 (± 1.0) mmHg at the end of perfusion. In contrast, there was a significant increase in pressure in the xenogeneic control group (XENO) in the first 5 min to 20.9 (± 3.1) mmHg, followed by a slow decrease to an almost normal value of 11.2 (± 2.6) mmHg at 45 min. In livers treated with WEB (0.033 mg/kg) or ASP (0.1 mg/kg), the portal pressure was reduced significantly at the 5 and 15 min observation times. The best portal pressure was seen in the combined group with 11.4 (± 0.7) mmHg at 15 min. After removal of antibodies, almost normal values of between 8.3 (± 1.0) and 12 (± 3.1) mmHg were maintained throughout the entire perfusion. Portal pressure after CVF treatment remained unexpectedly high: 17.7 (± 1.6) and 14.4 (± 1.3) mmHg at 5 and 15 min of the observation period, respectively (Table 1).

Bile production

Bile production in the ISO group during the first 15 min of perfusion reached 100 (± 7)% of basal bile production and dropped slightly to 87.3 (± 2.7)% at 30 min and to 76.4 (± 4.5)% at 45 min. In comparison, bile production in the XENO group remained at a significantly lower level: 63.1 (± 3.6)% at the beginning and 48.6 (± 3.6)% at the end of the perfusion period. In the group treated with WEB or ASP, bile production was raised significantly, almost to values seen in the ISO group. The best results were seen in the combined group: 91.3 (± 5.2)% of basal bile production at the beginning and 67.8 (± 2.6)% at the end of the experiment. After apheresis of antibodies, no significant differences with respect to the ISO group in the first 15 min of perfusion could be observed. A more marked decrease to 60.9 (± 4.7)% was seen during the subsequent observation time. Bile production after CVF treatment was the lowest of all groups, with 43.6 (± 5.8)% after 15 min of perfusion; however, it went back up to 68.9 (± 2.6)% by the end of perfusion (Table 2).

Aspartate-aminotransferase (AST)

A marked increase of 406.8 (± 53.7)% in AST was observed during xenogeneic perfusion, while values during

Table 2 Bile production during ex vivo liver perfusion as mean ± SEM in percent of basal bile production in the intact animal

Group (treatment)	Time interval (min)		
	0–15	16–30	31–45
ISO	100 ± 07.0*	87 ± 02.7*	76 ± 04.5*
XENO	063 ± 03.6**	48 ± 02.7**	49 ± 03.6**
ASP	081 ± 06.4*, **	71 ± 05.6*, **	51 ± 03.7**
WEB	080 ± 12.0*	71 ± 11.0*	68 ± 10.0
COMB	091 ± 05.2*	82 ± 02.6*, **	68 ± 02.6*
CVF	044 ± 05.8*, **	55 ± 15.0**	69 ± 02.7*
ABR	097 ± 07.8*	66 ± 04.7*, **	61 ± 04.7*, **

* $P < 0.05$ vs XENO perfusion group; ** $P < 0.05$ vs ISO control group

isogeneic perfusion remained almost stable. Under all treatments, the increase in AST was reduced, but values remained at higher levels than in the ISO group (Table 3).

Leukocytes and platelets during perfusion

During the first 15 min of perfusion, a major reduction in the number of leukocytes (81.2%) and of platelets (88.7%) in the perfusate was observed. This trapping of cells in the liver could not be influenced by any treatment used. In contrast, only a slight drop in cell counts was seen during isogeneic perfusion, the number of leukocytes falling by 33.3% and platelets by 13.1%.

Quantification of microcirculation

The calculation of an index of acinar perfusion as an indicator of the perfusion status of the livers showed the best correlation with the homogeneous isogeneic perfusion group (0.91) and the worst with the xenogeneic group (0.48) within the first minutes of perfusion. This indicated an almost complete breakdown in microcirculation with a lack of homogeneity of perfusion. Perfusion after modification of the perfusate with CVF, antibody removal, or a combination of ASP and WEB improved significantly (Table 4). Evaluation of the perfusion of single sinusoids indicated homogeneous perfusion in the isogeneic group with 94% of the sinusoids being perfused but a markedly reduced perfusion of 67% of the sinusoids in the xenogeneic group. Where sinusoidal perfusion failed, apheresis of antibodies improved it to 81% and combined treatment with ASP and WEB resulted in 82.5% perfused sinusoids. With CVF 74.5% of all observed sinusoids were perfused with a less homogeneous perfusion, indicating a weaker effect (Fig. 1).

A massive accumulation of leukocytes in the terminal portal vessels was observed in the first 15 min of perfusion in all xenogeneic groups. Their quantification was not possible due to the periportal overload of fluorochrome, caused by sticking leukocyte and platelet aggregates. As a result of the periportal trapping of leukocytes during xenogeneic liver perfusion, fewer postsinusoidal leukocytes were observed in the xenogeneic groups (Table 5).

Histology

The damage to the livers detected in the histological sections was of grade 0–1 in all groups, with no obvious differences between the groups.

Discussion

The prerequisite for a vital xenograft is homogeneous hemoperfusion, on which its function is based. To investigate rejection mechanisms and the efficacy of therapeutic strategies, it is essential to have a method that allows the quantification of microhemodynamic changes. Techniques for studying hepatic microcirculation in small animals, e.g., the microsphere method, indicator dilution methods, hydrogen clearance, reflectance spectrophotometry, and laser Doppler flowmetry, are described elsewhere [37]. Yet, these techniques are limited in their spatial resolution and particularly in their ability to register dynamic changes. In contrast, intravital microscopy is a tool that permits the repetitive and contin-

Table 3 Aspartate aminotransferase (AST) during ex vivo rat liver perfusion as mean ± SEM in percent of basal AST before start of perfusion

Group (treatment)	Time (min)			
	5	15	30	45
ISO	012 ± 07*	015 ± 07*	023 ± 07*	032 ± 07*
XENO	103 ± 13**	214 ± 32**	303 ± 40**	406 ± 54**
ASP	089 ± 07*, **	150 ± 30*, **	154 ± 16*, **	260 ± 19*, **
WEB	104 ± 16**	186 ± 25**	230 ± 26*, **	310 ± 27**
COMB	067 ± 10*, **	140 ± 23*, **	176 ± 12*, **	200 ± 30*, **
CVF	052 ± 21*, **	107 ± 26*, **	161 ± 35*, **	170 ± 30*, **
ABR	033 ± 08*, **	044 ± 10*, **	070 ± 07*, **	182 ± 77*, **

* $P < 0.05$ vs XENO perfusion group; ** $P < 0.05$ in vs ISO control group

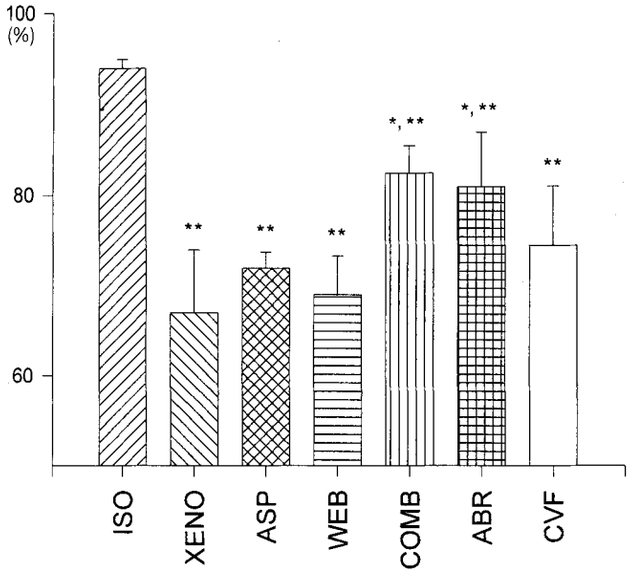

Fig. 1 Sinusoidal perfusion rate (number of perfused sinusoids per total number of sinusoids observed) as mean ± SEM in percent after 30 min of liver perfusion. Sinusoidal perfusion failed significantly in the XENO group; no difference was observed between the ASP and WEB groups. The sinusoidal perfusion rate improved significantly after apheresis of antibodies and in the COMB group (ASP + WEB). The difference between the CVF and XENO groups was not significant. * $P < 0.05$ vs XENO perfusion group; ** $P < 0.05$ vs ISO control group

uous quantification of such microhemodynamic parameters as sinusoidal/acinar perfusion rates, blood cell velocity, and leukocyte flux [21, 28].

This study was the first effort to quantify microhemodynamic changes during liver xenoperfusion in order to analyze the impact of the treatments used. In contrast to histological findings, which could not prove obvious differences in tissue damage at the end of liver perfusion between the groups treated, intravital microscopy was able to register the dynamic changes in microcirculation. Each of the treatment strategies used was shown to have some influence on the rejection mechanism. The best results were achieved after the removal of antibodies, underlining the importance of xenoreactive antibodies in hyperacute rejection [4, 24, 36]. The effect of acetylsalicylate and platelet-activating factor antagonist highlighted the important role of arachidonic acid products in the pathogenesis of xenogeneic rejection [32]. Inactivation of the complement system using CVF was only partly able to influence the function of the graft. Compared with isogeneic results, the blood supply in the liver was better in the CVF group, although there was less homogeneous sinusoidal perfusion. CVF did not appear to influence other parameters (perfusion pressure, bile production), in contrast with documented prolongation of survival after liver transplantation under CVF treatment [8]. It must be remembered, however, that the survival time of recipients, especially after xenogeneic liver transplantation, is not a sensitive measure of graft function. It is known that an anhepatic rat can survive for several hours [38]. The reason for the high perfusion pressure and low bile production in the CVF group is not known. Toxic side effects or products of complement activation, such as anaphylatoxins C3a and C5a, are thought to influence the microcirculation [3]. These products are developed during activation of the complement cascade by CVF [6] and obviously play an important role in the xenogeneic rejection process [30].

None of the treatments used was able to influence the trapping of leukocytes or platelets. Histological findings in hyperacute rejected grafts have provided evidence of the involvement of platelets in the formation of thrombi [5] and the adhesion of leukocytes to xenogeneic endothelial cells [9]. In vitro studies point to an involvement of leukocytes, especially natural killer cells, in the early rejection phase [12]. Their actions and importance in vivo during hyperacute xenogeneic rejection are not yet understood. We observed a massive

Table 4 Acinar perfusion index as an indicator of the perfusion status of the whole liver, given as mean ± SEM. Thirty acini were analyzed after a perfusion period of 45 min and the index calculated according to the formula: Index = [N (regular perfused) + 0.5xN (irregular perfused)]/N (total) with N = number of observed acini

	ISO	XENO	ASP	WEB	COMB	CVF	ABR
Index of acinar perfusion	0.91 ± 0.02*	0.48 ± 0.05**	0.53 ± 0.02	0.57 ± 0.02	0.62 ± 0.02*· **	0.68 ± 0.03*· **	0.74 ± 0.04*· **

* $P < 0.05$ vs XENO perfusion group; ** $P < 0.05$ vs ISO control group

Table 5 Number of sticking leukocytes in postsinusoidal venules as mean ± SEM in number per mm^2 endothelial cell surface

	ISO	XENO	ASP	WEB	COMB	CVF	ABR
n cells/mm^2	122 ± 27*	55 ± 13**	68 ± 17**	65 ± 27	71 ± 22	61 ± 14**	93 ± 15*

* $P < 0.05$ vs XENO perfusion group; ** $P < 0.05$ vs ISO control group

and rapid accumulation of leukocytes within the first 10 min of perfusion, particularly in periportal areas. The main fraction of accumulated leukocytes was considered to be granulocytes, as estimated from blood counts. It is not clear whether leukocytes were able to communicate via xenogeneic adhesion molecules and/or cytokines in a normal, physiological manner. The expression and distribution of vascular adhesion molecules in the liver is different in portal areas and sinusoids. It is known, for example, that in the human liver ELAM-1 and CD62 are expressed in portal, but not on sinusoidal, endothelial cells [35]. This could explain the observed trapping of leukocytes in portal vessels, which are the first point of contact for xenogeneic leukocytes. Complement products, especially factor iC3b, are known to induce rapid neutrophil-endothelial adhesion [17]. Mechanical, morphological, and rheological factors have also been found to play a role in sinusoidal leukocyte accumulation [14]. Our results prove the trapping of leukocytes and platelets and all consequences of their activation in the early phase of rejection during xenogeneic liver perfusion. Further investigations are necessary to identify the mechanisms of xenogeneic cell trapping, as leukocyte-endothelial cell interactions might initiate the delayed vascular rejection of xenografts.

In conclusion, intravital fluorescence microscopy is sensitive enough to quantify microhemodynamic changes and to analyze the efficiency of immunomodulation during xenoperfusion of rat livers with human blood. In addition to specific humoral factors (XNA and the complement system), nonspecific cellular interactions of leukocytes and platelets with xenogeneic endothelial cells are thought to play a role in microcirculatory disturbances during the early xenogeneic rejection phase. This will be analyzed further in future investigations.

References

1. Adachi H, Rosengard BR, Hutchins GM, Hall TS, Baumgartner WA, Borkon AM, Reitz BA (1987) Effects of cyclosporine, aspirin, and cobra venom factor on discordant cardiac xenograft survival in rats. Transplant Proc 19: 1145–1148
2. Bach FH, Robson SC, Winkler H, Ferran C, Stuhlmeier KM, Wrighton CJ, Hancock WW (1995) Barriers to xenotransplantation (comments). Nat Med 1: 869–873
3. Bjork J, Hugli TE, Smedegard G (1985) Microvascular effects of anaphylatoxins C3a and C5a. J Immunol 134: 1115–1119
4. Fischel RJ, Matas AJ, Perry E, Dalmasso A, Noreen H, Bolman RM III (1992) Plasma exchange, organ perfusion, and immunosuppression reduce "natural" antibody levels as measured by binding to xenogeneic endothelial cells and prolong discordant xenograft survival. Transplant Proc 24: 574–575
5. Forbes RD, Guttmann RD (1982) Evidence for complement-induced endothelial injury in vivo: a comparative ultrastructural tracer study in a controlled model of hyperacute rat cardiac allograft rejection. Am J Pathol 106: 378–387
6. Grier AH, Vogel CW (1989) The oligosaccharide chains of cobra venom factor are required for complement activation. Mol Immunol 26: 563–574
7. Hammer C (1987) Isohemagglutinins and preformed natural antibodies in xenogeneic organ transplantation. Transplant Proc 19: 4443–4447
8. Hengster P, Linke R, Feichtinger J, Hechenleitner P, Mark W, Eberl T, Klima G, Huemer H, Daha M, Margreiter R (1996) Mechanisms of hyperacute rejection of discordant liver xenograft. Xenotransplantation 3: 246–251
9. Henry ML, Bendle MD, Ferguson RM (1994) Leukocyte inhibiton and ex vivo whole blood xenoperfusion. Transplant Proc 26: 1311
10. Heuer HO, Casals Stenzel J, Muacevic G, Weber KH (1990) Pharmacologic activity of bepafant (WEB 2170), a new and selective hetrazepinoic antagonist of platelet activating factor. J Pharmacol Exp Ther 255: 962–968
11. Holzknecht ZE, Platt JL (1995) Identification of porcine endothelial cell membrane antigens recognized by human xenoreactive natural antibodies. J Immunol 154: 4565–4575
12. Inverardi L, Samaja M, Marelli F, Bender JR, Pardi R (1992) Cellular early immune recognition of xenogeneic vascular endothelium. Transplant Proc 24: 459–461
13. Kimikawa M, Agishi T, Teraoka S, Suga H, Hayasaka Y, Ota K (1992) Prolongation of cardiac xenograft survival by double filtration plasmapheresis and ex vivo xenoantibody adsorption. Transplant Proc 24: 447
14. Koo A, Breit G, Intaglietta M (1988) Leukocyte adherence in the hepatic microcirculation in ischemia reperfusion. In: Manabe H, Zweifach BW, Messmer K (eds) Microcirculation in circulatory disorders. Springer, Tokyo, pp 205–213
15. Leventhal JR, Dalmasso AP, Cromwell JW, Manivel CJ, Bolman RM III, Matas AJ (1993) Complement depletion prolongs discordant cardiac xenograft survival in rodents and non-human primates. Transplant Proc 25: 398–399
16. Makowka L, Chapman FA, Cramer DV, Qian SG, Sun H, Starzl TE (1990) Platelet-activating factor and hyperacute rejection. The effect of a platelet-activating factor antagonist, SRI 63-441, on rejection of xenografts and allografts in sensitized hosts. Transplantation 50: 359–365
17. Marks RM, Todd RF III, Ward PA (1989) Rapid induction of neutrophil-endothelial adhesion by endothelial complement fixation. Nature 339: 314–317
18. Marzi I, Walcher F, Menger M, Bühren V, Harbauer G, Trentz O (1991) Microcirculatory disturbances and leukocyte adherence in transplanted livers after cold storage in Euro-Collins, UW and HTK solutions. Transpl Int 4: 45–50

19. Marzi I, Takei Y, Knee J, Menger M, Gores GJ, Buhren V, Trentz O, Lemasters JJ, Thurman RG (1990) Assessment of reperfusion injury by intravital fluorescence microscopy following liver transplantation in the rat. Transplant Proc 22: 2004–2005
20. Meijer DK, Keulemans K, Mulder GJ (1981) Isolated perfused rat liver technique. Methods Enzymol 77: 81–94
21. Menger MD, Marzi I, Messmer K (1991) In vivo fluorescence microscopy for quantitative analysis of the hepatic microcirculation in hamsters and rats. Eur Surg Res 23: 158–169
22. Parker W, Saadi S, Lin SS, Holzknecht ZE, Bustos M, Platt JL (1996) Transplantation of discordant xenografts: a challenge revisited. Immunol Today 17: 373–378
23. Platt JL, Bach FH (1991) The barrier to xenotransplantation. Transplantation 52: 937–947
24. Platt JL, Fischel RJ, Matas AJ, Reif SA, Bolman RM, Bach FH (1991) Immunopathology of hyperacute xenograft rejection in a swine-to-primate model. Transplantation 52: 214–220
25. Pohlein C, Pascher A, Storck M, Young VK, Konig W, Abendroth D, Wick M, Thiery J, White DJ, Hammer C (1996) Transgenic human DAF-expressing porcine livers: their function during hemoperfusion with human blood. Transplant Proc 28: 770–771
26. Post S, Palma P, Rentsch M, Gonzalez AP, Menger MD (1993) Hepatic reperfusion injury following cold ischemia in the rat. In: Messmer K, Menger MD (eds) Liver microcirculation and hepatobiliary function. Karger, Basel, pp 152–166
27. Post S, Menger MD, Rentsch M, Gonzalez AP, Herfarth C, Messmer K (1992) The impact of arterialization on hepatic microcirculation and leukocyte accumulation after liver transplantation in the rat. Transplantation 54: 789–794
28. Post S, Menger MD, Rentsch M, Gonzalez A, Palma P, Messmer K (1992) New potentials of intravital fluorescence microscopy of the liver. Int J Microcirc Clin Exp 216
29. Riedel GL, Scholle JL, Shepherd AP, Ward WF (1983) Effects of hematocrit on oxygenation of the isolated perfused rat liver. Am J Physiol 245: G769–774
30. Rollins SA, Matis LA, Springhorn JP, Setter E, Wolff DW (1995) Monoclonal antibodies directed against human C5 and C8 block complement-mediated damage of xenogeneic cells and organs. Transplantation 60: 1284–1292
31. Saadi S, Platt JL (1995) Transient perturbation of endothelial integrity induced by natural antibodies and complement. J Exp Med 181: 21–31
32. Saumweber DM, Bergmann R, Gokel M, Hammer C (1994) Hyperacute rejection in an ex vivo model of renal xenografting. Role of the mediator response and its pharmacologic manipulation by the specific platelet-activating factor antagonist WEB 2086BS. Transplantation 57: 358–363
33. Scheringa M, Blanker R, Bemelman M, Schraa E, Bruin R de, Bouwman E, Ijzermans J, Marquet R (1995) Prolonged survival of guinea pig hearts in rats after combined antibody and complement depletion. Transplant Proc 27: 303–304
34. Seehofer D, Baatz H, Thiery J, Muller Hocker J, Muller Derlich J, Hammer C (1997) Fluorescence videomicroscopic assessment of xenogeneic microcirculation and impact of antibody removal by immunoadsorption. Transplantation 63: 460–465
35. Steinhoff G, Behrend M, Schrader B, Pichlmayr R (1993) Intercellular immune adhesion molecules in human liver transplants: overview on expression patterns of leukocyte receptor and ligand molecules. Hepatology 18: 440–453
36. Suga H, Hayasaka Y, Sato Y, Kimikawa M, Teraoka S, Agishi T, Ota K, Oguchi K (1992) Adsorption of natural antibodies with acetone-treated tissue powder. Transplant Proc 24: 704
37. Vollmer B, Menger MD, Lang G, Post S, Messmer K (1993) Hepatic microcirculation in hemorrhagic shock. In: Messmer K, Menger MD (eds) Liver microcirculation and hepatobiliary function. Karger, Basel, pp 85–105
38. Yamaguchi Y, Bollinger RR, DeFaria E, Landis B, Quarfordt S (1989) A simplified single stage total hepatectomy in the rat with maintenance of gastrointestinal absorptive function. Hepatology 9: 69–74