Intrasplenic Preconditioning: A Model for the Study of Xenostimuli Accommodation

Roman Telpner, M.D.,* Ron Ben-Abraham, M.D.,* Yifat Klein, Ph.D.,* Richard Nakache, M.D.,† Wisam Khoury, M.D.,† and Avi A. Weinbroum, MD*,‡,1

*Department of Anesthesia and Critical Care Medicine; †Transplantation Unit; and ‡Post-Anesthesia Care Unit and Animal Research Laboratory, Tel Aviv Sourasky Medical Center affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Submitted for publication May 11, 2009

Background. Discordant xenotransplantation, the grafting of organs from one phylogenic species to another, results in hyper-acute rejection (HAR). HAR is associated with the deposition of recipient preformed xenoreactive natural antibodies and complement on the endothelium of the donor organ, leading to activation and apoptosis of the endothelium, an event associated with xenograft rejection. Endothelial resistance to HAR, termed “accommodation,” an active protection of graft endothelium, may be achieved by previous stimulation of endothelial cells by discordant xenantibodies.

Materials and Methods. Forty-eight male Wistar rats were used to evaluate HAR induction in an isolated, dually perfused in-situ rat liver transfused with human blood. This ex-vivo model served to mimic rat-to-human liver xenotransplantation. Preconditioning of the liver endothelium was induced by rat intrasplenic injection of human blood (n = 8) or effluent of previously xenotransfused rat liver (n = 8), i.e., high versus low xenantibody solution, each undertaken 1d before liver xenotransfusion. Two other groups were not preconditioned. Preconditioned and non-preconditioned rats were perfused directly with human blood, and eight rats were used as controls. The effluent that exited these first-line livers was used to perfuse the second-line livers.

Results. Portal and hepatic artery perfusion pressures, resistances, rates of oxygen extraction, lactic acid and pH, and wet-to-dry weight ratio values were significantly increased in livers xenotransfused with blood indicating HAR, compared with unchanged values in livers perfused with Krebs solution. Portal pressure and resistance were best protected from HAR by the blood preconditioning in the blood perfused group, while the hepatic artery perfusion system was better protected by the perfusate precondition-blood perfused group. The physiologic effects of HAR were attenuated in most second-line livers.

Conclusions. Attenuation of HAR in rats’ livers is achieved by preconditioning with xenantibodies and/or by “filtering out” xenantibodies present in the circulation, and is suggestive of accommodation. This novel method may be useful in future studies aimed at refining methods for accommodating xenotransplantation.

Key Words: xenotransplantation; hyper-acute rejection; acute vascular xenograft rejection; accommodation.

INTRODUCTION

The scarcity of human organs available for transplantation has directed attention to xenografts. Discordant xenotransplantation refers to the grafting of organs from members of one species to another, which are not closely related phylogenetically. Hyper-acute rejection (HAR) is a likely outcome of discordant xenotransplantation. It occurs within minutes, and is associated with the deposition of the recipient’s antigraft antibodies and complement on the endothelium of the donor organ, leading to the initiation of pathologic changes within the endothelium, and eventually to graft rejection. Endothelial cell pathophysiology includes the loss of vascular integrity, resulting in edema and hemorrhage, and the development of a strongly procoagulant phenotype, resulting in thrombosis [1–5].
Two strategies, the prevention of complement activation [6–8] and the depletion of xenoreactive antibodies [9–13], effectively prevent HAR in animal models. However, vascular rejection usually resumes once serum complement levels return to baseline when there are detectable deposits of C3 [7, 8], or when IgM antibodies reappear in the circulation [14–16].

The immunosuppressive therapy used in clinical allograft transplantation is not capable of countering the xenograft HAR, although survival of an incompatible transplant in the presence of a humoral response has been reported [17]. Moreover, there are several documented instances in which antibody depletion prior to xenografting resulted in longer-term graft survival, even after the return of the xenoreactive antibodies [18–20]. This phenomenon, termed “accommodation,” has led researchers to reason that induction of accommodation might improve the clinical outcomes of xenotransplantation. Accommodation depends on active changes within the xenograft that render the endothelium resistant to host reactions [20]. The nature of the stimulus, which triggers accommodation of xenotransplants, is poorly defined. It may involve prior exposure of the xenograft to low concentrations of anti-graft IgG antibodies [21], or heme [22], as the initiating factor. In support of this hypothesis, in–vitro accommodation has been obtained by the incubation of immortalized porcine endothelial cells with low concentrations of human IgG prior to HAR induction [23, 24]. A comparable model for studying whole organ accommodation to HAR has not been described to date.

We introduced a model of dually perfused isolated liver in an earlier publication [25]. This model allows selective recording of physiologic and metabolic changes in the portal and arterial hepatic circuits. These changes accompany various injurious phenomena, such as those induced by HAR, and indicate the grade of damage to liver endothelium.

In the present study, we utilized this model to study the effect of preconditioning (by intrasplenic administration of xenoreactive antibodies), or graft exposure to low-dose xenon antibodies via filtering, on antibody-mediated HAR in the vascular bed of semi-xenotransplanted livers. We hypothesized that these treatments may induce accommodation of the “transplanted” liver endothelium and thereby attenuate the manifestations of HAR, as deduced from previous findings in isolated endothelial cells [23, 24].

**MATERIALS AND METHODS**

All the male Wistar rats (n = 48, 310–400 g) used in this study received humane care in compliance with the “Principles of Laboratory Animal Care” of the Tel Aviv Sourasky Medical Center, and accorded with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Institute of Health publication, 1985).

Isolated, Dually Perfused Liver Preparation

Following the protocol of our previously described dually perfused isolated rat liver model [25], all the animals were anesthetized with an intraperitoneal injection of phenobarbital sodium 50 mg/kg. They underwent a tracheotomy and were ventilated with 95% air-5% CO₂ using a piston-type rodent ventilator set to deliver 1 mL/100 g body weight tidal volume at a rate of 40 breaths/min. A thoracolumbar incision was then performed, and combined perfusion and pressure monitoring cannulae (14-gauge) were placed in the portal vein and in the suprahepatic inferior vena cava. Isolation of the liver caused the animals to die from hemorrhage while under general anesthesia. The aorta was occluded above the superior mesenteric artery and cannulated (21-gauge catheter) above the celiac artery. Double metal clips were placed on all arterial branches except for the common hepatic artery towards which the cannula was advanced. The liver was separated from the surrounding tissue. The carcass was then placed in an environmental chamber designed to control temperature and minimize evaporative water loss.

All experimental livers were initially perfused with hemoglobin-free, modified Krebs-Henseleit (Krebs) solution (in mM: 118 NaCl, 4.7 KCl, 27 NaHCO₃, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 0.05 EDTA, 11.0-D-glucose) at 4 mL/min/g liver weight, readjusted to normal pH in the exiting Krebs during the stabilization period. The total hepatic flow was divided so that two-thirds were driven into the portal vein and one-third into the hepatic artery. The perfusate had an osmolality of 298–305 mOsm. The effluent was allowed to exit freely from the hepatic veins via the vena cava, with drainage pressure maintained at 0 cmH₂O, and was stored for further perfusion of livers (see below). The incoming perfusate had a constant temperature of 37 °C and was equilibrated with 95% O₂–5% CO₂ to achieve an influent pO₂ > 300 mmHg, a pCO₂ of 34–38 mmHg and a pH of 7.34–7.46. The liver at this stage of isolation and stabilization was perfused in a single-pass mode.

A thermometer and a bubble trap were set up immediately in front of the isolated liver. A second thermistor was placed under the right hepatic lobe to constantly detect the liver’s temperature (maintained between 37.0 °C and 37.5 °C). Pressure transducers and sample ports for the collection of aliquots for use in assessing liver status were positioned in front of and behind the hepatic circuits.

**Specific Experimental Protocol**

**Perfusion Protocol**

All parameters were continuously assessed during the 30-min stabilization of each isolated liver. In the event of failed cannulation or deviation of the stabilization hepatic indices from the known ranges [25], the experiment on that liver was terminated and the data were discarded.

The 48 rats were equally divided into four groups. Each protocol consisted of two lines of livers: the first line (L1) was perfused with Krebs or unwashed O–negative human packed red cells (see below), whereas the second line (L2) was always perfused with the L1 liver-exiting effluent. Following the stabilization phase, L1 and L2 livers were allocated into one of the following protocols:

- **Group 1:** L1 livers were perfused with Krebs containing 5% bovine serum albumin, and the L2 livers were then perfused with the L1-exiting perfusate. Group 1 livers served as a negative control group.
- **Group 2:** L1 livers were perfused with human blood, and L2 livers were then perfused with the respective L1-exiting perfusate.
- **Group 3:** L1 livers were isolated from rats that had been pretreated (preconditioned) with an intrasplenic injection of 1 mL of the human blood. The rest of the experimental protocol followed that described for group 2.
- **Group 4:** L1 livers were isolated from rats that had been pretreated (preconditioned) with an intrasplenic 1 mL of effluent...
that had exited an L1-like liver perfused with the human blood in an earlier experiment. The rest of the experimental protocol followed that described for group 2.

Perfusion of each liver was executed separately, in a close circuit mode, for 60 min.

Preconditioned livers (groups 3, 4, see above) were isolated from rats that had undergone a minilaparotomy 16 h before the experiment and had received an intrasplenic injection of 1 mL blood (group 3) or L1 effluent, as specified above (group 4).

Following previous studies, which demonstrated a modulation effect on endothelial cells incubated in vitro with low concentrations of human IgG, we used the intrasplenic injection as the most direct access to the liver venous inflow in order to challenge the liver endothelium with human immunoglobulin that had not been processed by the recipient immune system whatsoever or to any consequential extent. Indeed, intrasplenic injection has frequently been used as an experimental technique for hepatocyte transplantation [26]. Finally, xenotransplantation studies have used 40–50 mL of human blood, an amount considered adequate to cause xenograft native antibody molecules to be deposited on all endothelial cells. The 1 mL of human blood used in our study is the equivalent amount appropriate for one rat liver.

Data Collection and Assessment of Variables

Hepatic arterial and portal perfusion pressures were recorded continuously via a Statham Medical P132284 pressure transducer (Men- nen Medical, Inc., Clarence, NY) on a physiologic polygraph (7D polygraph; Grass Instruments Co., Quincy, MA) connected to a hemodynamic monitor (CS/3, Datex-Ohmeda, Helsinki, Finland). The resistance values were calculated later. Changes over time were calculated at the following experimental time points: 15 and 30 min during stabilization of each liver and at min 1, 5, 10, 15, 30, and 60 during their perfusion.

Aliquots of perfusates were collected before the perfusate entered the L1 liver and upon exiting from the L1 and L2 livers. Samples were put on ice and processed in duplicates within 24 h for the following:

- Metabolic state was assessed by the rate of oxygen extraction, base deficit, and lactic acid-released levels. Oxygen content (mL) was calculated as 
  \[ 1.36 \times \text{hemoglobin (g\%)} \times \text{oxygen saturation (\%)} + 0.003 \times \text{PO}_{2} \text{ (mmHg)} \]. These parameters were directly measured using an AVL OMNI 8 (AVL, Graz, Austria) analyzer (adapted for asanguineous solution when appropriate).
- Hemoglobin (g\%) was assessed by the AVL OMNI 8 analyzer.
- The wet-to-dry weight ratio (WDR) of each organ was measured upon completion of the experiment: a part of each liver was weighed and put to dry in a 70 °C oven for 7 d, after which it was weighed again to extrapolate the resultant WDR.

Statistical Analysis

The data retrieved from the variables are summarized as means ± SD. At each time point, a post hoc analysis was done using analysis of variance with comparisons between group means carried out by the Student-Newman-Keuls test (α = 0.05). Repeated measurements of analysis of variance were performed (α = 0.05) to compare trends in each group.

RESULTS

Perfusion Pressures

Portal perfusion pressure (PP) significantly (P < 0.05) increased in all livers xenotransfused with blood, indicating HAR, compared with the unchanged values in the livers perfused with the Krebs solution (Fig. 1A). Among the L1 livers, the highest PP was observed in those isolated from rats pretreated (preconditioned) with perfusate (group 4), followed by those perfused with human blood without preconditioning (group 2) (Fig. 1A, left column). These values were significantly (P < 0.01) higher compared with the pressure of L1 livers recorded in blood-perfused livers isolated from rats preconditioned with blood (group 3) or from the control L1 livers.

The average maximal PP recorded in the L2 livers was lower by approximately 40 mmHg than that in the L1 livers (Fig. 1A, right column). Although it was less than in the L1 livers, all experimental groups had a significantly (P < 0.01) higher PP compared with controls, while the PP in the experimental groups did not significantly differ among each other.

The hepatic artery perfusion pressure (HP) increased maximally in the L1 livers of groups 2 (non-preconditioned) and 3 (blood preconditioned) (Fig. 2A, left column). The lowest HP was recorded in the group 4 L1 livers (perfusate preconditioned), and these values were significantly (P < 0.05) lower than the other two experimental groups.

The average maximal HP registered in the L2 livers was ~100 mmHg lower than that registered in the L1 livers (Fig. 2A, right column). The L2 2 (non-preconditioned) livers had the highest significant (P < 0.05) values compared with all other groups, while those of group 4 exhibited the lowest pressure, similar to that of the controls.

Resistance Data

The effects of the events occurring within each of the circuits of the specific groups and lines of livers resulted in calculated resistances similar to those described in the perfusion pressure data (Figs. 1B and 2B). Resistances in the L1 livers were 2-fold higher than those in the L2 livers.

Metabolic and Electrolytes Data

Wide differences were noted in the rate of oxygen extraction among the various groups, from the lowest extraction rate, which was recorded in the control group (1) and attributable to the absence of hemoglobin in the perfusion solution, to the experimental groups in which the extraction rate was high (Table 1). Oxygen extraction was significantly (P < 0.05) higher in the L1 livers compared to the L2 ones. Among the experimental groups, the highest values of extraction were observed in group 2, which was perfused with human blood, and the lowest in group 4, which had been preconditioned with intra-splenic effluent.
Overall, base deficit was greater in L2 livers compared to L1 livers. The highest values in the L2 livers were observed in the ones in group 4 (perfusate preconditioned). Lactic acidosis was evident only in the xenotransfused groups; however, there was no distinctive group pattern (Table 1).

Hemoglobin and Wet Weight-to-Dry Weight Ratio (WDR)

Hemoglobin loss did not differ significantly among the experimental groups (the controls were Krebs-albumin-perfused). Overall, the WDR was significantly \( P < 0.05 \) higher in the L1 livers compared with their L2 counterparts (Table 1). Thrombi were noted only in the hepatic capillaries of the L2 group 2 livers (data not shown).

**DISCUSSION**

The present study offers an *ex-vivo* xenotransplantation-like model of portal- versus arterial-induced HAR and the variable creation of accommodation. HAR was evoked in this isolated, dually perfused model, as reflected by the exacerbation of vascular and metabolic...
parameters among the experimental livers and versus the control ones. Such reactivity was attenuated when the perfusate that exited the first-line exposed and damaged livers (L1) entered the second-line “protected” and thus accommodated livers (L2). In addition, preconditioning with either human blood or L1-derived perfusate also variably modified the physiologic manifestations of HAR, thus attesting to its differential attenuation in human-to-animal discordant conditions.

With the increasing problem of donor organ shortage, successful discordant xenografting is of significant importance worldwide. However, major hurdles, including immunologic ones, must be overcome before such an option becomes a reality. HAR is a prominent
obstacle to xenotransplantation. HAR is a complement-mediated response in recipients with preexisting antibodies to the donor. It occurs within minutes from exposure and requires prompt removal of the transplant in order to prevent severe systemic inflammatory response syndrome (SIRS) [1–5]. HAR may be avoided by a number of interventions, each resulting in a process termed accommodation, i.e., the survival of the transplanted organ despite the presence of anti-endothelial cell antibodies and complement, which otherwise would have lead to graft rejection [20–22]. Accommodation has been described in human and baboon renal and heart allotransplantation across the ABO barrier [27–33], in patients with anti-graft HLA antibodies following plasmapheresis [33, 34], in concordant cardiac xenotransplantation in hamster-to-rat, mouse-to-rat, and rat-to-mouse combinations [21, 35–37], and in pig endothelial cells in vitro [23, 24, 38]. Accommodation has not been demonstrated in discordant models of xenotransplantation.

HAR is often characterized by the loss of vascular integrity with increased permeability, leading to interstitial hemorrhage and edema. In addition, capillary vasospasm is associated with erythrocyte and platelet entrapment as well as to adherence of activated platelets to the endothelium [1–5]. One probable consequence of these conditions is the formation of thrombi, which is a major event in xenograft rejection [1–5]. The obstruction to perfusion is reflected by an elevation of PP and vascular resistance, an increase in oxygen requirements, and an abnormally elevated WDR. In our current study, these were severely but variably altered in the L1 livers of groups 2–4 that first encountered damaging factors contained in human blood. The L1 livers that were preconditioned with perfusate (group 4) exhibited the maximal increase in PP following the perfusion with human blood. The same livers had the highest WDR values, indicating severe edema related both to vascular damage and occlusion. These are the probable consequences of xenotransfused blood in livers that had been preconditioned with low concentrations of xenoreactive antibodies. In comparison, there was only a moderate increase in PP and WDR in the blood-pretreated and blood-transfused L1 livers (group 3), possibly since the endothelium of these livers had been preconditioned with perfusate (group 4) was associated with a rather more substantial attenuation in pressures than those obtained after pretreatment with human blood.

### TABLE 1

<table>
<thead>
<tr>
<th>Group (No.)</th>
<th>Line (L) of liver</th>
<th>Oxygen use (mL)</th>
<th>Hemoglobin loss (g %)</th>
<th>Base deficit</th>
<th>Lactic acid (mM)</th>
<th>Wet-to-dry weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>0.09 ± 0.03</td>
<td>–</td>
<td>–3.40 ± 0.18</td>
<td>0.47 ± 0.10</td>
<td>3.42 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>0.03 ± 0.02</td>
<td>–</td>
<td>–3.37 ± 0.72</td>
<td>5.45 ± 0.35</td>
<td>3.47 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>L1</td>
<td>22.42 ± 5.54</td>
<td>0.6 ± 0.11</td>
<td>–9.88 ± 3.25</td>
<td>77.48 ± 3.80</td>
<td>3.53 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>1.56 ± 0.64</td>
<td>1.31 ± 0.56</td>
<td>–23.07 ± 1.85</td>
<td>70.90 ± 23.90</td>
<td>3.38 ± 0.20</td>
</tr>
<tr>
<td>3</td>
<td>L1</td>
<td>11.57 ± 7.67</td>
<td>0.65 ± 0.64</td>
<td>–15.80 ± 11.88</td>
<td>30.25 ± 7.42</td>
<td>3.78 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>2.20 ± 0.28</td>
<td>0.55 ± 0.64</td>
<td>–41.65 ± 13.36</td>
<td>89.45 ± 5.16</td>
<td>3.54 ± 0.51</td>
</tr>
<tr>
<td>4</td>
<td>L1</td>
<td>4.94 ± 0.27</td>
<td>0.70 ± 0.44</td>
<td>–18.40 ± 5.30</td>
<td>15.70 ± 3.50</td>
<td>3.93 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>1.72 ± 0.17</td>
<td>0.63 ± 0.32</td>
<td>–49.10 ± 1.55</td>
<td>17.50 ± 7.10</td>
<td>3.50 ± 0.23</td>
</tr>
</tbody>
</table>

Group 1 (negative control): L1 livers were perfused with Krebs containing 5% bovine serum albumin; group 2: L1 livers were perfused with human blood; group 3: L1 livers were isolated from rats that had been pretreated (preconditioned) with an intrasplenic injection of 1 ml of the human blood; group 4: L1 livers were isolated from rats that had been pretreated with an intrasplenic 1 ml of effluent that had exited an L1-like liver perfused with the human blood in an earlier experiment. In all groups, L2 livers were perfused with the respective L1-exiting perfusate. Oxygen usage was significantly higher in the experimental groups compared to control. Among those groups, oxygen extraction was significantly higher in L1 livers compared to L2 ones, the highest values of extraction were observed in group 2, which was perfused with human blood, and the lowest in group 4, which was preconditioned with intra-splenic L1-effluent. Hb loss did not vary significantly among groups. Base deficit was greater in L2 livers than in L1 livers, and was the highest in the L2 livers of group 4. Lactic acidosis was evident only in the experimental groups, but did not exhibit a coherent pattern. WDR was significantly higher in L1 livers compared with L2.

P < 0.05 versus the corresponding L1 livers.
P < 0.01 versus the corresponding L1 livers.
P < 0.001 versus the corresponding L1 livers.
P < 0.05 versus group 1 L1 livers.
P < 0.01 versus group 1 L1 livers.

[References: 1–5, 21, 35–37, 23, 24, 38]
blood (group 3) or no preconditioning (group 2). Capillary leak increased during that pressure reduction, as demonstrated by interstitial edema and the ballooning of hepatocytes, with subsequent increased wet-to-dry weight ratio.

In the L2 (“protected”) livers, blood preconditioning resulted in lower arterial pressure and resistance compared with no preconditioning. At the same time, there was a transient increase in capillary leak in the former, as was also demonstrated by the higher portal pressure/resistance and the increased WDR. Interestingly, and similarly to what was reported in in-vitro studies [17], there was a negligible capillary leak after preconditioning with low concentration xenoantibodies (the perfusate, group 4).

A prerequisite for accommodation to occur involves the previous depletion of anti-graft antibodies and their slow return once the graft is in place, or merely gradual introduction of anti-graft antibody [27, 31, 32, 39]. Based on these data, it could be postulated that gradual exposure of the graft to anti-graft antibodies results in a sort of endothelial cell “education” process, which leads to vasculature resistance and graft survival. One hypothesis that we had proposed with regard to our model is that binding of anti-graft antibodies to xenograft endothelial cells could trigger the expression of protective genes, as is supported by others who investigated other organ systems [17, 23, 35, 40–42]. Such events would lead to a double stage “protection” feasibly generated in the present model, possibly an early rather a late one, which would result not only from the filtering effect of L1 livers but also from different concentrations of xenoantibodies (high versus low). These phenomena depicted isolated circulatory responses following different reactions in the two separate circulation areas of the liver, an organ where important events of HAR would take place.

Our second mechanistic hypothesis was that attenuation of the physiologic effects of HAR would be achieved by the perfusion of L2 livers with the L1-exited perfusates. We reasoned that the L1 liver would act as a filter in adsorbing xenoantibodies present in the circulation, independently of whether or not the L1 liver originated from a pretreated (preconditioned) rat. This proved to be correct, and we now contend that this mechanism of attenuating HAR is supported by the clinical techniques used routinely for filtering undesirable proteins from the blood upon its infusion. Thus, the relatively prolonged function of a guinea pig heart in the rat after it had undergone the removal of anti-xenoantibodies by a whole-body rinse–out [43] is equivalent to the L1’s filtering the same anti-xenoantibodies for the L2 liver. We also contend that the combination of both above-mentioned mechanisms helped in attenuating HAR in the L2 group 3 livers by approximately 25%–35% in this model.

The herein described metabolic changes indicate that the process of neutralization of the xenogenic stimulus is energy dependent: the level of $O_2$ extraction paralleled the level of HAR. Indeed, changes in $pO_2$ and lactate values that signal energy consumption were the highest in the L1 group 2 livers (the non-preconditioned livers later perfused with human blood, which contains high concentrations of xenoantibodies). This was in contrast to the L1 group 4 livers (isolated from rats pretreated with the L1 perfusate, i.e., low–concentration xenoantibodies), which required the least amount of energy among the experimental groups, because they were least damaged by HAR. Combining the findings of increased arterial and portal pressures together with the excessive level of lactic acid in the perfusate and oxygen requirement, occurring mainly in the non-preconditioned group (2), point to the severe destructive process that the blood cells and hepatocytes underwent during transfusion, apparently due to the antigenic activity as well as being the result of the massive generation of thrombi and the subsequent damage to the cells. These signs of tissue damage, together with the increase in base deficit, were attenuated by the process of “filtering” the xenoantibodies and/or the preconditioning the livers.

CONCLUSIONS

In this study, we used a dually perfused rat isolated liver model of ex-vivo discordant xenotransplantation to demonstrate active diverse protection of the rat liver endothelium from HAR, i.e., accommodation. Accommodation was achieved to varying degrees via preconditioning of livers by xenoantibodies, even at low concentrations. While preconditioning with liver perfusate better protected the arterial circuit against rejection, blood pretreatment was more effective in the portal system. The combination of such an immunological phenomenon, together with “filtering” of the xenostimulus, improved accommodation. This novel and heretofore unreported method may serve as a means for future studies aimed at further understanding of HAR and for refining the methods of enhancing accommodation in the presence of xenotransplantation.

REFERENCES


