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MHC antigen presentation on the surface of hepatocytes: modulation during and after hypoxic stress

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Abstract Presentation and recognition of MHC antigen on the surface of cells is the basic process of initiating rejection and distinguishing "self and not self". This is considered to begin when a transplanted organ is reperfused with the blood of the recipient. In this study, the modulation of MHC I antigen presentation during preservation was investigated in a cell culture model using primary hepatocyte cultures of male Wistar rats. By incubation with different preservation solutions used in clinical transplantation, expression of the MHC antigen was observed during cold hypoxia. Primary hepatocyte cultures were isolated from male Wistar rats by a modified Seglen technique and seeded to glass slides, thus obtaining monolayer cultures. After 1 day of resting the cultures were incubated under different conditions using Krebs Henseleit solution (KH), Euro-Collins solution (EC), HTK solution of Bretschneider (HTK) and University of Wisconsin solution (UW) as incubation media. The conditions of incubation were warm normoxia (37 °C, pO_2 100 mm Hg) and cold hypoxia (4 °C, $pO_2 < 0.1$ mm Hg), which is the main condition of organ preservation. Incubation time was 6 h. Before starting the incubation reference cultures were fixed and every 60 min several cultures were withdrawn from the experiment and fixed also. MHC expression was studied by staining the cultures with monoclonal antibodies against rat MHC class I and class II. As expected, MHC class II was not present on the surface of hepatocytes, while MHC class I was demonstrated on the controls as well as on the cultures that were incubated using KH and EC independent of temperature and hypoxia or normoxia. At the end of the incubation they were still positive but not as strong as in the beginning. During incubation using HTK and UW, MHC I was not detectable at all phases of the experiment. In conclusion, hypoxic stress did not completely eradicate MHC I expression in rat hepatocytes, while the composition of the preservation medium may result in a hepatocyte surface negative for MHC I. Hydroxyethylstarch may be the substance in UW that covers the MHC antigen, while in HTK, mannitol or the histidine complex may play the same role. MHC negativity of the cells of a preserved transplant is another reason for the benefit of UW or HTK.

Key words Preservation
Reperfusion · Simulation
Hepatocyte culture
MHC expression

Introduction

Primary liver cell cultures have been used to evaluate hypoxic liver cell injury caused by graft preservation [9]. Previous examinations have shown that endothelial cells have a potent ability to express both MHC class I and class II antigens. Opinions differ concerning the intensity of their expression by hepatocytes. The aim of our research was to evaluate the impact of different preservation solutions on the expression of transplantation antigens and to evaluate whether there were any changes in the expression of these antigens during the preservation period. In addition, we evaluated the influences of temperature, preservation solution and hypoxic and reoxygenation stress on the intensity of the expression of these antigens during this preservation period. The major question of our study was if hypoxic stress and/or direct reoxygenation injury can modulate the expression of MHC antigens in primary hepatocyte cultures.

Materials and methods

Male Wistar rats (120–200 g) receiving a standard pellet diet and water ad libitum were used for the isolation of cells. The isolation of the hepatocytes was performed according to the technique described by Seglen et al. [6] and modified by Sies [7], using a two-step perfusion technique.

Anaesthesia was achieved with an intraperitoneal injection of pentobarbital and the abdomen was opened using a median laparotomy. The rat was then heparinized systemically. Portal vein cannulation was achieved. Perfusion was started using calciumfree buffer at a perfusion rate of 6 ml/min. To avoid increasing the pressure in the circulation, the inferior vena cava was incised and the blood flow was then increased to 20 ml/min. The system was then prepared for the recirculating perfusion with the calcium- and collagenase-containing buffer, by ligating the inferior vena cava and inserting a cannula into the right atrium. After perfusion of approximately 4 min, the liver was well congested by the collagenase. It then was removed and striked out in the calcium-containing buffer.

Hepatocytes were concentrated by washing and sedimentation. The pure cell suspensions were seeded on histology slides, which were then placed in chambers. The cells were cultured in 15 l medium with additional supplements. Hepatocytes were characterized by their morphology using haematoxylin-eosin staining.

Experimental procedure

For the incubation of the cell cultures, gas-impermeable chambers were developed in which the following incubation conditions were easily sustained: warm normoxia (37 °C; $pO_2 > 70$ mm Hg), warm hypoxia (37 °C; $pO_2 < 0.1$ mm Hg), cold normoxia (4 °C; $pO_2 > 70$ mm Hg) and cold hypoxia (4 °C; $pO_2 < 0.1$ mm Hg). For each different condition four preservation solutions were used as incubation media to evaluate their influences: University of Wisconsin (UW), HTK solution of Bretschneider (HTK), Euro-Collins (EC) and Krebs Henseleit solution (KH).

They were equilibrated before incubation with anoxic or normoxic gases. After 3 h, 6 h and after reoxygenation, the cultures were fixed by the addition of cold methanol (– 40 °C). Immediately after the fixation, a direct immunofluorescent staining was performed (antibodies by Dianova/Pharmingen: RTIA and RTIB; FITC conjugated). The intensity of the staining was evaluated under immunofluorescent microscopy at 488 nm. The intensity of the staining was graded from zero to four. Foci of increased intensity were graded by adding 1, 2 or 3 points to the scale, depending on the number of focal staining points. Classification was performed by two experienced cell culture researchers (C. E., B. M.) by independent estimation.

To evaluate the expression in the normal liver of the rat, two native cryostat sections were stained in the same manner. The results of this staining were comparable to previous studies. All cultures were stained and tested at the same time after preservation. Each experiment was repeated 3–5 times for all incubation conditions in a parallel manner (comparison of the conditions in the cell of the same liver) and, subsequently, with the livers of ten different animals.

Results

Native cryostat sections

Kupffer cells showed a very strong expression (grade 4) of MHC class I, endothelial cells showed an almost comparable strong staining (grade 3), while hepatocytes showed a very weak staining (grade 1). Staining for class II antigens expressed by Kupffer cells was grade 3, endothelial cells staining was grade 2 and hepatocytes showed no staining.

Isolated cell cultures

Hepatocytes showed a very weak staining (grade 1.4) for class I and and almost non-visible staining (grade 0.5) for class II antigens. Focal positive staining of single cells was detected in these cultures.

Hepatocytes hypoxia and reoxygenation (Figs. 1 and 2)

Under cold hypoxia there was only a very small, insignificant increase in the MHC class II expression, and no changes were seen in the expression of the class I antigens. After reoxygenation, no changes were found in class I expression, but a significant increase in the expression of MHC class II was found: UW (grade 2.4), HTK (grade 3.1), EC (grade 4.5) and KH (grade 2.1). Under warm hypoxia similar observations were made as those found under cold hypoxia. However, there was an even higher increase in MHC class II antigen after reoxygenation of the cell cultures: UW (grade 4.6), HTK (grade 4.5), EC (grade 4.2) and KH (grade 4.1).

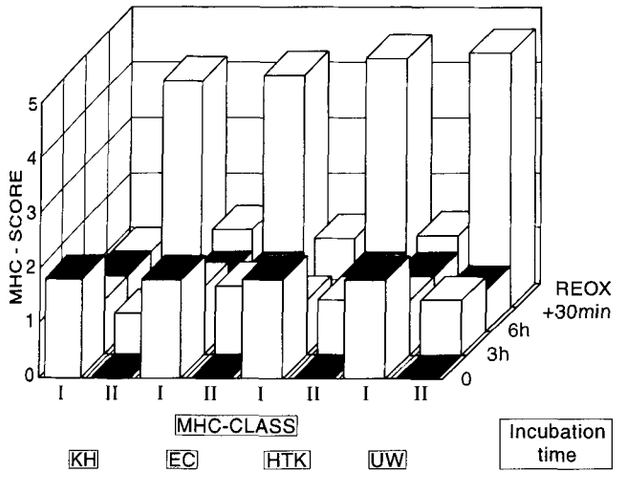

Fig. 1 Expression of MHC I and II on hepatocytes undergoing warm hypoxic incubation in different preservation solutions

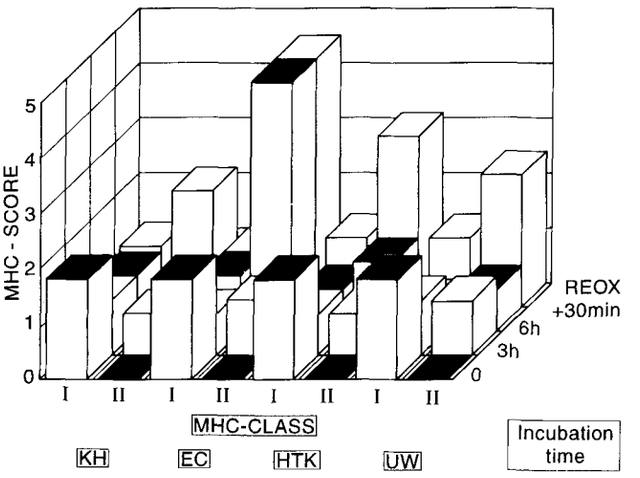

Fig. 2 Expression of MHC I and II on hepatocytes undergoing cold hypoxic incubation in different preservation solutions

Hepatocytes and normoxia

After 3 h in cold normoxia only a small increase in class II expression was seen. After 6 h, the class II expression remained the same, but in addition to that an increase in MHC class I appeared. These same findings were found in warm normoxia.

Discussion

The data presented here showed that under stressful conditions such as hypoxia and reoxygenation, which have proven to be a good simulation of the conditions of the transplantation processes of preservation and revascularisation, MHC surface antigens on the hepatocytes can be upregulated significantly. If conditions were not ideal (warm hypoxia or second choice preservation medium) even MHC II was strongly present. It must be pointed out that warm hypoxia is not only an unwanted condition during organ preservation but also corresponds to conditions of shock.

According to general immunological knowledge, MHC II is only expressed by antigen-presenting cells such as reticular cells and not by parenchymal cells. On the other hand, there is some evidence that even hepatocytes can express MHC II in certain diseases such as primary biliary cirrhosis or graft rejection [1, 3–5]. Thus, the interpretation of upregulated MHC I and II on the surface of liver cells after organ preservation and reoxygenation as well as in shock is not yet clear. It is most likely a stress reaction of the cells [2, 8] but whether this upregulation is immunogenic, attracting infiltrating cells, or immunosuppressive, inducing tolerance, cannot be decided from these data. Further experiments are in progress.

References

1. Ballardini G, Bianchi FB, Doniach D (1984) Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. Lancet 1:1009
2. Burke EC, Martinez OM, Preise CE (1991) MHC-expression on human hepatocytes before and after isolation. Transplant Proc 23:1428–1429
3. Calmus Y, Arvieux C, Gane PC (1992) Cholestasis induces major histocompatibility complex class I expression in hepatocytes. Gastroenterology 102:1371–1377
4. Gridelli B, Colledan M, Grendele M (1989) Induction of class I and class II major histocompatibility complex antigens (MHC) in rejecting human liver grafts. Transplant Proc 21:2215–2216
5. Ines GK, Nagafuchi Y, Fuller BJ (1988) Increased expression of major histocompatibility antigens in the liver as a result of cholestasis. Transplantation 45:749–752
6. Seglen PO (1976) Preparation of isolated rat liver cells. Cell Biol 13:29–30

7. Sies H, Ackerboom TPM, Tager JM (1977) Mitochondrial and cytosolic NADPH systems and isocitrate dehydrogenase indicator metabolites during ureogenesis from ammonia in isolated rat hepatocytes. Eur J Biochem 72:301–307

8. Trautwein C, Gerken G, Lohr H (1993) Lack of surface expression for the B-cell autoepitope of cytochrome P450 Il D6 evidenced by flow cytometrie. Z Gastroenterol 31:225–230

9. Viebahn R, de Groot H, Lauchart W, Becker HD (1991) Primäre Hepatocytenkulturen als Modell zur experimentellen Untersuchung der Leberkonservierung. Langenbecks Arch Chir 376:268–272