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Hepatocyte Transplantation-Granulocytes Recognize Surface of Isolated Autologous Hepatocytes as Non-Self and Destroy Them

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HEPATOCYTE (HC) transplantation (Tx) could be an alternative to whole liver Tx. Also, for hepatic gene therapy, Tx of HC is critical. Familial hypercholesterolemia, ornithine transcarbamylase deficiency, and hemophilia B are among the first diseases to be treated with HCTx. Successful Tx of HC is hampered by lack of proper cellular (stromal) and humoral environment and nutrient supply at the site of implantation and by rapid destruction of most of the implanted cells. Only few transplanted HC survive compared to the large numbers of implanted cells. The mechanism of fast destruction of both autologous and allogeneic HC is not site-dependent. It takes place intravascularly as well as in all tissues.[1,2] There may only be differences in the tempo of destruction. The molecular process of HC recognition and cytolysis remains unclear. It is likely that isolated HC, with their uncovered surface intercellular adhesion molecules (cadherins[3]), are recognized after intravenous infusion or implantation into tissues as "nonself," preferentially by granulocytes and macrophages and subsequently destructed.

The question arises, what is the mechanism of rapid destruction of hepatocytes transplanted intravenously, intraportally, intraperitoneally, subcutaneously and into spleen and liver? In this study we investigated the recipient cellular processes responsible for destruction of Tx syngeneic HC immediately after Tx into various tissues.

MATERIALS AND METHODS

In Vivo

Group 1. Lewis (LEW) rat HC were isolated according to Seglen's method, labelled with ^{51}Cr and infused i.v. Six hours after infusion, organ distribution of radioactivity was measured. Data were compared with distribution of ^{51}Cr-labelled lymphocytes. Distribution studies were repeated in leukopenic (agranulocytic) rats after 7 days of Endoxan (20 mg/kg) administration, and 7.5Gy irradiation.

Group 2. FITC-labelled HC were injected intravenously, intraportally, intraperitoneally and subcutaneously. Their distribution was evaluated after 6- and 24-hours in tissue sections by staining with anti-FITC moab (M878 DAKO). Some experiments were also performed in agranulocytic rats. To block HC surface receptors, in some other experiments HCTx were preincubated with HC-surface specific, anti-class I and anti-ICAM1 moabs and Tx subcutaneously. The specimens were evaluated after 24-hours on frozen sections. The phenotypes of infiltrating host cells were identified with moabs.

Group 3. To prove that HC transplanted in small liver fragments (HC in their spatial relationship with stroma) may survive after intratissue implantation, 2 × 2 × 2 mm large blocks were transplanted subcutaneously, intraperitoneally, into liver and spleen. Twenty-four hours later frozen sections were evaluated after staining with moabs.

In Vitro

Cell-mediated cytotoxicity (CMC) of buffy coat granulocytes, splenocytes, mesenteric lymph node lymphocytes, and adherent and nonadherent PBM to syngeneic HC was measured in a standart 6-hour ^{51}Cr test. Blocking of CMC with moabs against class I and II antigens, adhesion molecules and HC-surface specific antigens was performed. CMC of PBM in the presence of decomplemented rat serum (ADCC) was carried out.

RESULTS

Group 1. As in the previous investigation[1] only around 10% of HCTx-bound radioactivity was recovered after 6 hours in lymphoid tissues, whereas the rate for Tx lymphocytes was around 30%. Again, low levels of radioactivity were found in spleen, mesenteric lymph node and bone marrow, but high levels were found in kidneys and serum. For comparison, radioactivity of Tx lymphocytes was high in spleen, lymph nodes, liver and bone marrow, but low in serum and kidneys. In agranulocytic rats after Endoxan therapy a high level of radioactivity was recovered in lungs (Fig 1), and after 7-Gy irradiation in lungs and spleen (Fig 2).

Group 2. FITC-labelled HC behaved differently depending on the route of transplantation. Six hours after IV of Tx only, HC debris could be detected in the lungs. Twenty-four hours following intraportal, Tx clusters of HC with preserved structure but surrounded by granulocytes and mono-

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0041-1345/97/$17.00
PII S0041-1345(96)00455-1

Table 1. Cell-Mediated Cytotoxicity of Leukocytes to Syngeneic Hepatocytes (^{51}Cr-Labeled HC, 6-h Test, E:T 100:1, Percent of Cytotoxicity)

Effector Cells	Cytotoxicity (%)
Buffy coat	11.7 ± 8.0
Granulocytes	22.3 ± 15.1
Peripheral blood mononuclears	1.0 ± 1.0
Splenocytes	4.6 ± 5.0
Mesenteric lymph node lymphocytes	7.0 ± 6.0

cytes were seen in portal tributaries. Two and 6 hours after intraperitoneal HCTx the peritoneal fluid aspirate showed HC in clusters with granulocytes and macrophages. The percentage of granulocytes adherent to HC was 55.0 ± 8.7 and 42.2 ± 9.3 (mean SD), and the number of granulocytes adherent per 1 HC was 1.1 ± 0.5 and 1.0 ± 0.5, respectively. The percentage of granulocytes in peritoneal fluid rose from 24.1 ± 4.8 to 53.4 ± 5.5 and 56.5 ± 22.1 at 2- and 6-hours after HCTx. After 24 hours, no HC were found in the peritoneal fluid. In the subcutaneous tissue the HCTx were found infiltrated by granulocytes. They released FITC and lost their normal structure. The percentage of infiltrating granulocytes rose from 1 to 2 to a mean value of 69 and 76 after 6- and 24-hours, respectively. Among the infiltrating cells were some OX6-positive and ED1-positive cells.

Agranulocytic rats with subcutaneously HCTx revealed only minor granulocytic infiltrates. The structure of HCTx was largely preserved. Preincubation of HC with anti-ICAM1 and anti-HC-specific moabs before subcutaneous Tx protected some HC against destruction. Island of relatively normal HC could be seen after 24 hours.

Group 3. Fragments of liver (HC with stroma) Tx subcutaneously, into omentum and into liver tissue revealed after 24-hours foci of relatively normal HC in the necrotic hepatic tissue densly infiltrated at the periphery by granulocytes. Fragments of liver Tx into spleen were totally destroyed.

Group 4. A high level of cell-mediated cytotoxicity by buffy coat and isolated granulocytes was observed (Table 1). Splenocytes, mesenteric lymph node lymphocytes, and blood mononuclears revealed only marginal cytotoxicity (Table 1). Blocking of target HC with moabs against MHC class I, CD 11a/18, CD54 did not inhibit granulocyte cytotoxicity. In contrast, blocking with moabs against HC-specific antigens Hepa 10 and 34 decreased the level of cytotoxicity.

DISCUSSION

Our studies revealed that isolated syngeneic HCTx, IV or into tissues, undergo a rapid destruction. A number of factors may be responsible for this phenomenon. Isolation of HC from liver fragments with enzymatic methods may damage the cellular membrane and make it fragile. After Tx, these cells are carried with blood stream to lymphoid organs where the damaged membrane will be recognized. After topical Tx, the subcellular structures released from damaged HC may be chemoattractive for macrophages and granulocytes. Granulocytes, once they become in situ activated, may nonspecifically destroy normal HCTx. Interestingly, when lymphoid cells were isolated from spleen or lymph nodes and transplanted IV or topically, they did not undergo destruction and local attack by granulocytes. These observations led to formulation of a concept that the surface molecules of isolated HC (cadherins) are recognized either in blood by circulating granulocytes or in the tissue by dendritic cells and macrophages and subsequently granulocytes as "nonself" and attacked. This phenomenon would be similar to that seen in a wound where the molecular structures of fragmented cells are recognized by migrating macrophages and subsequently exposed to infiltrating immune cells. We showed that placing a fragment of autologous tissue in the lumen of a vein produces a rapid accumulation of granulocytes on the implant and its lysis.[4] Leukopenic rats after Endoxan therapy and irradiation destroyed IV HCTx in a slow tempo. HC accumulated in lungs and spleen. These data strongly support our concept that destruction of HCTx is a recipient cell-mediated process.

We noticed that the speed of elimination of HCTx depends on the route of Tx. FITC-labelled HCTx IV could not be recovered after 24 hours in lungs and those Tx intraperitoneally were not detected in the peritoneal fluid, whereas HCTx intraportally were found in portal areas or sinusoids. Interestingly, they formed clusters with many adherent granulocytes and large mononuclear cells. Most remarkable were histological pictures of HCTx subcutaneously where clusters of HCTx were infiltrated by granulocytes. This could be seen already 2 hours after Tx and reached its peak at 6 hours. After 24 hours debris of HC could be seen. Agranulocytic rats did not show granulocytic infiltrates but macrophages accumulating first after 24 hours at the margin of the relatively well preserved clusters of HC. Preincubation of HC with anti-CD54 (ICAM1) and anti-HC-specific moabs partly blocked infiltration by granulocytes.

HCTx require proper chemical environment for implantation and subsequent growth.[5,6] The putative stromal cells may be necessary for it. We transplanted minute fragments of liver into various tissues and found foci of preserved HC in the subcutaneous, intraomental and intrahepatic implants. Interestingly, the Tx fragments were surrounded in a typical fashion by granulocytes and only few of them penetrated the liver parenchyma. Two factors seem to be responsible for survival of some HC in Tx small fragments of liver. One would be inability of granulocytes to penetrate the block of adherent HC and get an access to individual HC, the other maintaining HCs in their functional spatial relationship with own stroma.

The in in vitro cell-mediated cytotoxicity (CMC) tests evidently showed high granulocyte cytotoxicity toward syngeneic HC. The cytotoxicity exerted by other cells like PBM, splenocytes, and mesenteric lymph node lymphocytes remained at a very low level. The granulocyte cytotoxicity to

HC was observed by other authors. The release of proteolytic enzymes and not reactive oxygen species was found responsible for HC membrane damage.[7] The blood mononuclear cells and liver infiltrating lymphocytes may also be cytotoxic for autologous HC infected with virus but not for normal HC.[8,9] Peritoneal macrophages destroy intraperitoneally Tx HC within 24 hours.[10,11] HC express MHC class I, CD 18, and CD54 (ICAM1) antigens. These molecules did not play any role in contact with granulocytes as blocking procedures with moabs directed against them did not inhibit the in vitro cytotoxicity. Two out of 34 moabs against various HC antigens raised in our laboratory, Hepa 10 and 34, partly blocked cytotoxicity. Taken together, isolated HC transplanted intravenously or into tissues undergo rapid destruction by autologous granulocytes. According to our study, granulocytes recognize exposed surface molecules (cadherins) on HC previously hidden and inaccessible to them in the liver tissue, and initiate the cytotoxic process. Blocking experiments with moabs support this concept. Destruction of HC may also be partly due to fragility of HC membrane after enzymatic isolation.

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