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Blockade of cell adhesion molecules enhances cell engraftment in a murine model of liver cell transplantation

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ABSTRACT

Aim: OLT is the best alternative for patients with end-stage liver diseases. However, as the need for organs surpasses donor availability, alternatives to OLT are required. LCT could be a useful option *versus* OLT in several patients even though its low cell-engraftment hampers its efficiency. Endothelial cell barrier is the main obstacle for the implantation of cells into the parenchyma. Our study has focused on the modification of the endothelial barrier with monoclonal antibodies against adhesion molecules in order to increase cell engraftment in a mouse model of liver cell transplantation.
Methods: Anti-mouse CD54 and anti-mouse CD61 antibodies were administered intrasplenically to healthy mice within 60 min prior to stem cell transplantation. Animals were sacrificed either short term at 2 h or middle term seven days after transplantation. Immunohistochemical techniques to detect alkaline phosphatase activity were used to identify the transplanted cells within the liver parenchyma.
Results: Anti-CD54 and anti-CD61 administration increases vascular patency and cell engraftment. This represents a 32% and 45% increase, respectively, of engrafted cells compared to the control ($p < 0.05$).
Conclusion: Modification of the vascular wall with monoclonal antibodies against endothelial adhesion molecules before cell transplantation enhances cell engraftment into the mouse liver.

1. Introduction

The mortality rate in patients with end-stage liver diseases has elevated in recent years. LT in the treatment of these patients has improved their outcome [1]. However, the increase of patients demanding a liver for transplantation has far exceeded the number of available donor organs. The mismatch between people on the waiting list for a liver transplantation and the availability of suitable organs has led to an undesirable increase in the liver transplantation waiting list and an increase in patient deaths [2].

Nonetheless, there are different alternatives to LT addressing this problem. An especially promising one is the application of regenerative medicine techniques, above all CT in the liver because of the liver's remarkable capacity to regenerate [3]. CT consists of liver parenchyma repopulation with donor cells to restore liver function [4]. Although the safety and potential of the CT to treat several liver diseases has been demonstrated [5] at present it has only shown its efficiency as a "bridge" supporting the liver function until a suitable organ becomes available for LT [6]. No long-lasting whole liver function restoration has been reported using CT exclusively [7]. The principal hurdle to the clinical use of CT is its low engraftment rate [8]: Transplanted cells cannot pass the endothelial cell barrier and die soon after perfusion. It has been reported that 70–80% of cells die within the first 24–48 h due to hypoxia, anoikis, and cell destruction by the host immune system [9]. Only cells that reach the liver plate can survive and regenerate the liver mass at this location [10]. The scientific community has focused its attention on the importance of the endothelial barrier in an effort to identify new approaches that can modify endothelial patency and promote cell engraftment. Some of these approaches are cytotoxic drug administration [11], hepatic irradiation [12] or ischemia–reperfusion models [13]. However, none of these approaches are without possible harmful side effects, thus making it difficult to apply them in the clinical scenario.

In 2010, we reported an *in vitro* test based on the culture of endothelial cells to evaluate molecules that are capable of disrupting endothelial

Abbreviations: OLT, orthotropic liver transplantation; LCT, liver cell transplantation; LT, liver transplantation; CT, cell transplantation; MoAb, monoclonal antibodies; ICAM-1, intercellular adhesion molecule I; HUVECs, human umbilical vein endothelial cells; ESCs, embryonic stem cells.

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intercellular cell-to-cell adhesion [14]. With this test, we demonstrated that the addition of MoAb against intercellular adhesion molecules to a confluent endothelial cell culture leads to an increase of patency *in vitro* [15].

2. Objective

This study has aimed to investigate the effect of MoAb against two different endothelial adhesion molecules: anti-CD54 (also known as ICAM-1) and anti-CD61 (also known as β3-integrin protein) that takes part in the glycoprotein II_aIII_b on vascular patency both *in vitro* and *in vivo* to enhance transplanted cell extravasation and engraftment.

3. Materials and methods

3.1. Drugs and chemicals

Monoclonal antibodies anti-human CD61, anti-human CD54, anti-mouse CD54 and anti-mouse CD61 were provided by BioLegend Inc. (San Diego, CA). Anti-human CD4 was provided by Chemicon International Inc. (Tamecula, CA).

3.2. Cell culture

HUVECs were isolated as previously described [16] and cultured on 0.1% gelatin-coated Petri dishes in Medium 199 with 10 mM Hepes, 100 μl heparin, 100 μg/ml endothelial cell growth factor (all from Sigma Chemical Co., CA) and 20% fetal calf serum (Invitrogen Co., CA). HUVECs phenotype was determined by fluorescence microscopy using FITC-labeled *Ulex europaeus* lectin (Sigma Chemical Co., CA). The purity of cultured endothelial cells was constantly ≥98%.

For confluence-loss assays, HUVECs were cultured until full confluency. Then, the medium was replaced with complete medium containing 2 μg/ml anti-human CD54 or 2 μg/ml anti-human CD61. As negative control, we used a complete medium with 2 μg/ml anti-human CD4 MoAb. To evaluate the cell-free areas, cultures were first fixed and permeabilized with ethanol 50% and then stained with Alexa-488 Phalloidin (Invitrogen Co., CA) as we have previously reported [14]. All the experiments were performed at least three times.

R1 murine ESCs were used for transplantation assays because of their strong alkaline phosphatase activity [17]. Until their transplantation, ESCs were kept in culture as described by Torres [18]. R1 mouse embryonic stem cells were incubated for 3 to 5 min with trypsin at 37 °C until they were detached from the culture flask. Cells with viability >80% based on trypan blue exclusion assay were transplanted within 2 h from detachment.

3.3. Animals

Cell transplant assays were performed using 12–16 week-old (30–35 g) male HsdWin: NMRI mice (Harlan). All experimental protocols were approved by Hospital Universitario 12 de Octubre Laboratory Animal Care and Use Committee and complied with European Union guidelines. Animals were kept with unrestricted access to food and water in the Research Institute at "12 de Octubre" Hospital.

3.4. Hepatic preconditioning and cell transplantation

To evaluate the effect of anti-mouse monoclonal antibodies *in vivo*, a subcostal laparotomy incision was used to locate the lower pole of the spleen. Afterwards, 100 μl of the MoAb saline solution (20 μg/kg body weight) was administered by intrasplenic injection. The mice were sacrificed 2 h after the MoAb injection.

Cell transplantation: For liver endothelial wall preconditioning, mice were divided into 3 groups ($n = 3$ for each group) and received a single intrasplenic injection of 100 μl monoclonal antibody solution (anti-mouse-CD54, anti-mouse-CD61 or anti-human-CD4 as negative control) in sodium saline (20 μg/kg body weight) under anesthesia with inhaled Forane (Abbott). Animals were reanimated and kept in their cages until the transplantation. Two to four hours after preconditioning 4×10^5 cells were suspended in 0.2 ml of sodium saline and were slowly injected through the portal vein. All transplantation assays were performed three times.

3.5. Histology

Livers were fixed with an intrasplenic perfusion of paraformaldehyde 4% in sodium chloride and then maintained 30 min at room temperature embedded in the same solution. Sections of 40 μm from different liver lobules were prepared for histology using a VT1200S vibratome (Leica Biosystems, Inc., IL).

Liver vessel sections were stained with FITC-*Griffonia simplicifolia* lectin (Sigma Chemical Co., CA) for 30 min at room temperature. Images of the entire thickness of the tissue were taken with an Olympus IX71 fluorescence inverted microscope coupled to a Nikon DXM 1200 digital camera. Images were stacked and processed using ImageJ (U. S. National Institutes of Health, Bethesda, MA, USA).

For transplanted cell engraftment, the tissue sections were stained with NBT/BCIP (Roche), for 30 min at room temperature using the manufacturer's protocol.

3.6. Image analysis

Images from multiple liver lobes were analyzed and quantified with ImageTools (University of Texas Health Science Center at San Antonio,

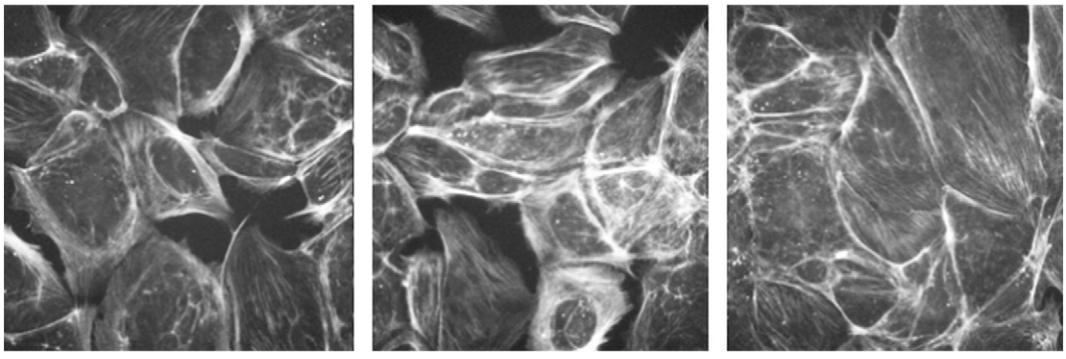

Fig. 1. Molecular antibodies treatment of a confluent endothelial cell culture induces acellular gap emergence due to cytoskeletal reorganization. HUVEC cell culture incubated 120 min with (A) anti-CD54 MoAb, and (B) anti-CD61. The cell stress fibers are blurred and located beneath the plasma membrane. Cells are partially detached from the substrate. The control cell culture (C) shows actin filaments that are well-defined and parallel to fibers in adjacent cells. Images of HUVEC stained with Alexa-488 Phalloidin to visualize the actin cytoskeleton. (Magnification 200 ×).