Cytotherapy, 2015; 17: 174–185

International Society for Cellular Therapy
ISCT®

Influence of inflammation on the immunological profile of adult-derived human liver mesenchymal stromal cells and stellate cells

GORDANA RAICEVIC[1,*], MEHDI NAJAR[1,*], MUSTAPHA NAJIMI[2], ADIL EL TAGHDOUINI[3], LEO A. VAN GRUNSVEN[3], ETIENNE SOKAL[2] & MICHEL TOUNGOUZ[1,4]

[1]*Laboratory of Clinical Cell Therapy, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium,* [2]*Laboratory of Pediatric Hepatology and Cell Therapy, Institute of Experimental & Clinical Research, Université Catholique de Louvain, Brussels, Belgium,* [3]*Liver Cell Biology Laboratory, Vrije Universiteit Brussel, Brussels, Belgium, and* [4]*Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium*

Abstract

Background aims. Stem cell therapy for liver diseases has recently emerged as a promising alternative to liver transplantation. Eligible cells should have an appropriate immunophenotype. The aim of the present study was to define the immunological profile of two human liver-derived mesenchymal stromal cell populations, namely, stem cells (ADHLSC) and hepatic stellate cells (HSC). *Methods.* The study was conducted under normal and inflammatory conditions with the use of human bone marrow mesenchymal stromal cells (BM-MSC) as reference. *Results.* Like BM-MSC and ADHLSC, HSC were negative for hematopoietic (CD45) and endothelial (CD34) markers but positive for stromal markers. All cell types were constitutively positive for HLA class I and negative for human leukocyte antigen (HLA) class II and co-stimulatory molecules (CD80, CD86, CD134 and CD252). Inflammation induced the expression of CD40 in all cell types, but the highest values were observed on HSCs; high CD252 expression was only observed on HSC as compared with ADHLSC and BM-MSC. The expression of various adhesion molecules (CD54, CD58, CD106 and CD166) was dissimilar in these three cell types and was differentially influenced by inflammation as well. ADHLSC and HSC constitutively expressed the immunosuppressive molecule HLA-G, whereas CD274 expression was induced by inflammation, as in the case of BM-MSC. Moreover, all cell types expressed the two major natural killer ligands CD112 and CD115. *Conclusions.* Toll-like receptors (TLR) 1, 3, 4 and 6 messenger RNA was expressed by both cell types, whereas TLR 2, 5, 7, 9 and 10 were only expressed by ADHLSC. Inflammation increased the expression of TLR 2 and 3 by ADHLSC and HSC. Finally, both liver-derived cell types were immunosuppressive because they inhibited the proliferation of mitogen-activated T cells.

Key Words: *adult-derived human liver stem cells, cell adhesion molecules, inflammation, stellate cells, Toll-like receptors*

Introduction

Acute and chronic liver diseases may cause a loss of the self-repair capacity of this organ. These clinical conditions are often life-threatening. The only available treatment in these circumstances is liver transplantation. However, because of organ shortage, novel therapeutic options are urgently needed to treat patients who lack a donor. Liver cell transplantation has been suggested as a promising alternative to whole-liver transplantation [1–4]. Mature hepatocytes but also liver progenitor cells and mesenchymal stromal cells (MSC) are considered as potential cell candidates [5,6].

Besides hepatocytes and liver progenitor cells, the liver is constituted of other cell types such as liver-specific endothelial cells, Kupffer cells (liver-resident macrophages) and hepatic stellate cells (HSC). HSC, non-parenchymal liver cells, are in a quiescent state and are characterized by the presence of lipid droplets, long cytoplasmic processes and a balanced production of matrix proteins and matrix remodeling enzymes to maintain an optimal environment for the resident liver cells [7,8]. When the liver is damaged, HSC become activated, a process stimulated by the presence of inflammatory cytokines and resulting in a myofibroblast-like phenotype [9,10]. Adult-derived

*These authors contributed equally to this work.

Correspondence: **Gordana Raicevic,** LCCT, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium. E-mail: gordana.raicevic@ulb.ac.be, gordana30@yahoo.com

(Received 25 April 2014; accepted 3 October 2014)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2014.10.001

Table I. Conjugated monoclonal antibodies used for flow cytometry analysis.

Markers	Antibodies	Origin
Endothelial		
CD34	CD34-PC5	Becton Dickinson, NJ, USA
Stromal		
CD90	CD90-PE	Miltenyi Biotec, Leiden, Netherlands
CD105	CD105-FITC	Ancell Corp, MN, USA
Hematopoietic		
CD45	CD45-PC7	Becton Dickinson, NJ, USA
Immunological		
HLA-ABC	HLA-ABC-PE-Cy5	eBioscience, San Diego, CA, USA
HLA-DR	HLA-DR-PerCP	BD Biosciences, Erembodegem, Belgium
HLA-G	HLA-G-PE	ExBio, Vestec, CZ
CD40	CD40-PE	Miltenyi Biotec, Leiden, The Netherlands
CD80	CD80-FITC	eBioscience, San Diego, CA, USA
CD86	CD86-APC	Miltenyi Biotec, Leiden, The Netherlands
CD134	CD134-FITC	BioLegend Europe, San Diego, CA, USA
CD252	CD252-PE	BioLegend Europe, San Diego, CA, USA
Adhesion proteins		
CD29	CD29-PE-Cy5	BD Biosciences, Erembodegem, Belgium
CD49e	CD49e-PE	BD Biosciences, Erembodegem, Belgium
CD54	CD54-PE	BD Biosciences, Erembodegem, Belgium
CD58	CD58-FITC	BD Biosciences, Erembodegem, Belgium
CD106	CD106-PE-Cy5	BD Biosciences, Erembodegem, Belgium
CD166	CD166-PE	BD Biosciences, Erembodegem, Belgium
CD44	CD44-FITC	Miltenyi Biotec, Leiden, The Netherlands
CD146	CD146-PC5	Beckman Coulter, Analis, Namur, Belgium
Immunoregulatory		
CD39	CD39-FITC	BioLegend Europe, San Diego, CA, USA
CD200	CD200-APC	BioLegend Europe, San Diego, CA, USA
CD73	CD73-PE	BD Biosciences, Erembodegem, Belgium
CD274	CD274-PE-Cy7	BD Biosciences, Erembodegem, Belgium
HO-1	HO-1-PE	Enzo Life Sciences, Antwerp, Belgium
NK ligands		
CD112	CD112-PE	BioLegend Europe, San Diego, CA, USA
CD155	CD155-PE	BioLegend Europe, San Diego, CA, USA
ULBP-3	ULBP-3-PE	R&D Systems Europe, Abington, United Kingdom

human liver stem cells (ADHLSC), another liver cell population, are obtained after primary culture of the liver parenchymal fraction. These cells are of fibroblastic morphology and have a hepato-mesenchymal phenotype and have the potential to differentiate into hepatocyte-like cells [11].

ADHLSC are considered as MSC-like cells. Previous studies have suggested that MSC may have an anti-fibrotic effect [12,13]. MSC are described as fibroblast-like adherent cells able to differentiate into lineages of mesodermal origin [14,15]. MSC do not have any specific marker; therefore their immunophenotypic characterization is based on the expression of certain molecules (CD73, CD105 and CD90), together with the absence of others (CD45, CD19 and CD14) [16]. An important feature of MSC is their low immunogenicity related to a low level of human leukocyte antigen (HLA) class I expression and the absence of HLA class II and co-stimulatory molecules (CD40, CD80 and CD86) [17,18]. A number of *in vitro* studies have shown that MSC are also immunoregulatory because they alter the differentiation, maturation, activation and cytokine secretion profile of different immune cells including dendritic cells [19,20], B cells [21], natural killer (NK) cells [22] and T cells [23–25]. Their immunosuppressive function is dependent on both cell-to-cell contact and secretion of different cytokines, chemokines and growth factors such as human growth factor, prostaglandin E2, Heme oxygenase (HO)-1, Indoleamine-pyrrole 2,3-dioxygenase (IDO), Leukemia inhibitory factor (LIF), galectins, HLA-G and others [26–32]. Because of these unique features, MSC have become attractive candidates as therapeutic agents in transplantation to improve graft outcome and reduce the need for chronic immunosuppression. In this context, it is worth noting that the immunoregulatory properties of MSC are strongly affected in certain conditions such as inflammation or infection, thereby compromising graft tolerance [33,34]. Indeed, the activation of Toll-like receptors (TLR) expressed by MSC modifies their immune properties. TLR constitute a family of germline-encoded pattern-recognition receptors evolved to detect danger signals. Among the