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Adhesion of human haematopoietic ($CD34^+$) stem cells to human liver compartments is integrin and CD44 dependent and modulated by CXCR3 and CXCR4☆

Heather A. Crosby*, Patricia F. Lalor, Emma Ross, Philip N. Newsome, David H. Adams*

Centre for Liver Research and NIHR Biomedical Research Unit for Liver Disease, University of Birmingham and Queen Elizabeth Hospital Birmingham, Birmingham B15 2TT, UK

***Background/Aims*: Haematopoietic stem cells (HSC) have previously been shown in some studies to migrate to damaged and diseased liver where a small proportion will engraft. Such cells can promote liver repair in rodent models of liver injury and lead to improved liver function in uncontrolled clinical studies. In order to maximize the engraftment of cells for clinical applications it is necessary to understand the molecular mechanisms that regulate stem cell recruitment and retention. Our aim therefore was to determine which factors where involved in adhesion of circulating HSC to liver endothelium and sequestration around epithelial cells within the liver.**

***Methods*: We examined the ability of CD34+ populations from peripheral and mobilized blood and the CD34-expressing cell line KG1a to bind to human hepatic sinusoidal endothelial (HSEC) and biliary epithelial cells (BEC) *in vitro*.**

***Results*: We report that all $CD34^+$ populations express α4β1, β2 integrins and CD44. Liver tissue sections and primary liver cells expressed the corresponding ligands VCAM-1/ fibronectin, ICAM-1 and CD44. Pertussis toxin was shown to decrease binding of $CD34^+$ cells and the cells migrated to CXCR3 and CXCR4 ligands.**

***Conclusions*: $CD34^+$ populations use α4β1, β2 integrins and CD44 receptors to bind to the ligands VCAM-1/fibronectin, ICAM-1, and hyaluronic acid expressed on sinusoidal vessels in tissue sections and to primary human HSEC. Binding to BEC was mediated by the interaction of β1 and β2 integrins with VCAM-1 and ICAM-1 respectively. A role for chemokines is supported by our finding that pertussis toxin inhibits $CD34^+$ cell adhesion to BEC and HSEC and by the ability of $CD34^+$ cells to migrate to CXCR3 and CXCR4 ligands.**

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***Keywords*: Progenitor cells; Integrin; Chemokine; Adhesion; Homing**

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* Corresponding authors. Tel.: +44 121 4158784; fax: +44 121 4158701 (H.A. Crosby).

E-mail addresses: h.a.crosby@bham.ac.uk (H.A. Crosby), d.h.adams@bham.ac.uk (D.H. Adams).

Abbreviations: HSC, haematopoietic stem cells; HSEC, hepatic sinusoidal endothelial cell; BEC, biliary epithelial cells; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; SCF, stem cell factor; SDF-1, stromal derived factor-1; PBC, primary biliary cirrhosis; ALD, alcoholic liver disease; NL, normal liver (donor,control); PB, peripheral blood; MB, mobilized blood; TNF-α, tumor necrosis factor-α; VAP-1, vascular adhesion protein-1.

1. Introduction

Many studies have demonstrated that haematopoietic stem cells (HSC) are recruited to the injured liver from the circulation albeit in low frequencies [1,2]. Although the nature of the effects of the mobilised HSC within the liver remains controversial there is both experimental and clinical evidence that HSC can promote repair [3]. The mechanisms by which HSC contribute to liver repair include paracrine stimulation of endogenous hepatocytes [4], resolution of liver fibrosis [5] and also production of hepatocytes predominantly by a process of cell fusion [6–13]. This latter contribution to hepatocytes occurs at very low levels and the first two mecha-

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nisms are likely to be more important. Previously, our studies have reported that approximately 1×10^4 CD34 cells can be isolated per 30gm of human liver tissue and that these cells can differentiate into either epithelial or endothelial cells in culture [14]. Also $CD34^+$ cells can be detected in liver tissue from patients with a variety of liver diseases [15]. The factors that regulate HSC recruitment to the liver are poorly defined, but involve migration of cells from the blood via the liver endothelium into the liver.

Recent data shed light on some of the mechanisms which may promote stem cell incorporation into the injured liver. Kollet and colleagues [1] have demonstrated that SDF-1 and CXCR4 mediated signaling are important in migration of HSC to the murine liver. SDF-1 was expressed on BEC thus promoting retention of $CXCR4^+$ cells within the portal tracts [1,16,17]. Increased levels of HGF present after liver injury are also associated with recruitment of $CD34^+$ HSC to the damaged liver in response to SCF and SDF-1 [1,18]. Human studies confirm that SDF-1 activates and increases the expression of the integrins LFA-1 and VLA-4 on $CD34^+$ cells [19–21]. Since the ligands for LFA-1 and VLA-4 (ICAM-1 and VCAM-1 respectively) are present on HSEC during liver injury [22] it is likely that similar mechanisms promote HSC recruitment during liver injury or inflammation. Similarly, the hyaluronate receptor CD44 has been implicated in HSC homing to the bone marrow [21,23,24] and in leukocyte recruitment to the liver [25,26]. These studies led us to investigate potential roles for integrins, CD44 and chemokine-dependent signals in HSC homing to and retention within the liver.

In the present study we have defined the functional role of adhesion molecules expressed by mobilized and peripheral blood $CD34^+$ stem cells compared with the $CD34^+$ cell line KG1a. We have documented the expression of major adhesion and chemokine receptors on these cells and assessed their role in the binding of HSC to human liver endothelium and epithelium. We report that $CD34^+$ HSC migrate in response to chemokine ligands for CXCR3 and CXCR4 and use both CD44 and chemokine-dependent integrin-mediated pathways to bind to HSEC and BEC.

2. Materials and methods

2.1. Samples

All samples were obtained according to institutional guidelines with appropriate ethical approval and informed written consent.

Liver tissue was obtained from the adult liver transplant program, University Hospital Birmingham, UK. Hepatectomy specimens were obtained from patients with primary biliary cirrhosis (PBC) and alcoholic liver cirrhosis (ALD). Donor tissue surplus to surgical requirements was obtained from the paediatric transplant program and served as normal controls (NL). For immunohistochemistry and static adhesion assays, tissue was snap frozen and stored at −70 °C. For cell isolation, tissue was stored in Dulbecco's modified Eagle's medium (Invitrogen, UK) at 4 °C and used within 48 h post-collection.

Peripheral blood (PB) was obtained by therapeutic venesection of haemochromatosis patients, University Hospital Birmingham, UK. The mononuclear cell fraction was obtained from the gradient interface following density gradient centrifugation on lympholyte H (25 min, 800 g, VH Bio Ltd.) and was used for flow cytometry and $CD34^+$ cell isolation.

Mobilised blood (MB) was obtained from the National Blood Service, Birmingham using cells that were isolated for stem cell transplant but surplus to clinical requirements (thus over 24 h old) from human blood using standard leukopheresis techniques. The mobilised blood was then used for flow cytometry or $CD34^+$ cell isolation.

KG1a this $CD34^+$ cell line was cultured in RPMI containing 10% heat inactivated fetal calf serum (both Invitrogen, UK), penicillin, streptomycin, glutamine (100 U/ml, 100 μg/ml, 292 μg/ml; Invitrogen, UK) and was used as a positive control for flow cytometry and static adhesion assays to compare their behavior with PB and MB $CD34^+$ cells.

2.2. Primary cell isolation and culture

2.2.1. Human hepatic biliary epithelial cells (BEC) and sinusoidal cells

Human hepatic biliary epithelial and endothelial cells (BEC & HSEC) were isolated from 30 g human liver tissue as described previously [27,28]. HSEC were cultured on rat tail collagen 1 coated flasks in human endothelial media (Invitrogen, UK) containing 10% heat-inactivated human serum (H&D Supplies, Bucks, UK), penicillin, streptomycin and glutamine (100 U/ml, 100 μg/ml, 292 μg/ml), hepatocyte growth factor and vascular endothelial growth factor (both 10 ng/ml, Peprotech). BEC were cultured in Dulbecco's modified Eagle's medium, Ham's F12 media (1:1; Invitrogen), containing 10% heat-inactivated fetal calf serum, penicillin, streptomycin and glutamine (100 U/ml, 100 μg/ml, 292 μg/ml), epidermal growth factor (10 ng/ml, Sigma, UK), hydrocortisone (2 mg/ml), cholera toxin (10 ng/ml), triiodothyronine (2 nmol/L, all Sigma), insulin (0.124 U/ml, Novo Nordisk, W. Sussex, UK) and hepatocyte growth factor (10 ng/ml). Cells were cultured to confluence and used between passages 2 and 4 in all experiments. For static adhesion assays BEC and HSEC were cultured to confluence on glass coverslips in 24 well plates and stimulated with 10 ng/ml TNFα (Peprotech, UK) for 24 h. Cells were then fixed in cold 70% ethanol for 5 min, washed and stored in PBS prior to use in the static adhesion assay.

2.2.2. Preparation of CD34 cells from mobilised blood (MB) and peripheral blood (PB) for adhesion assays or immunohistochemistry

Mobilised blood cells were pelleted and re-suspended in ammonium chloride lysing reagent (BD Pharmlyse, BD Biosciences, Oxford, UK) for 10 min at room temperature to remove red blood cells. Leukocytes (MB and mononuclear cells from PB) were washed and then incubated with CD34 antibody (QBEND/10, 1:25, Novocastra, Newcastle-upon-Tyne, UK) for 30 min at 37 °C. Cells were rinsed to remove unbound antibody and then CD34 positive cells selected by immunomagnetic isolation using Dynal Cellection Pan mouse IgG kit (Dynal, Wirral, UK) according to the manufacturer's instructions. Viability of $CD34^+$ cells were checked using trypan blue exclusion and were only used if viable. $CD34^+$ selected cells still expressed CD45 (haemopoietic) and CD34 as determined by immunofluorescent staining (Supplemental Figure 1).

2.2.3. Flow cytometric analysis of KG1A, MB and PB cells

Cells from MB, mononuclear cells from PB and KG1a cells were resuspended in cold cytometry buffer consisting of PBS and 2% BSA (Sigma, UK). Approximately 5×10^6 cells were placed into tubes and resuspended in primary antibody solution (Table 1). Isotype-matched, fluorescently-labeled control antibodies were used to deter-

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