Cytokine 90 (2017) 130–134

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Human hepatic stellate cells and inflammation: A regulated cytokine network balance

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ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 21 October 2016
Accepted 11 November 2016

Keywords:
Hepatic stellate cells
Inflammation
Immunological cytokine profile
Immune response balance
Liver cell therapy

ABSTRACT

Aim: Uncertainty about the safety of cell therapy continues to be a major challenge to the medical community. Inflammation and the associated immune response represent a major safety concern hampering the development of long-term clinical therapy. *In vivo* interactions between the cell graft and the host immune system are mediated by functional environmental sensors and stressors that play significant roles in the immunobiology of the graft. Within this context, human liver stellate cells (HSC) demonstrated marked immunological plasticity that has main importance for future liver cell therapy application. *Methods:* By using qPCR technique, we established the cytokine gene expression profile of HSCs and investigated the effect of an inflammatory environment on the immunobiology of HSCs. *Results and discussion:* HSCs present a specific immunological profile as demonstrated by the expression and modulation of major immunological cytokines. Under constitutive conditions, the cytokine pattern expressed by HSCs was characterized by the high expression of IL-6. Inflammation critically modulated the expression of major immunological cytokines. As evidenced by the induction of the expression of several inflammatory genes, HSCs acquire a pro-inflammatory profile that ultimately might have critical implications for their immunological shape. *Conclusion:* These new observations have to be taken into account in any future liver cell therapy application based on the use of HSCs.

1. Introduction

Cell therapy holds promise for the treatment of liver diseases and has recently emerged as a promising alternative to liver transplantation or at least provides liver function support. Long-term engraftment in the liver may be achieved once the inflammatory and related immune responses are managed [1]. Liver fibrosis remains one of the most challenging consequences secondary to multiple pathological mechanisms including, but not limited to, drug and alcohol induced liver injury and viral hepatitis [2–6]. The curative treatment for chronic liver injury is still inexistent whilst liver transplantation despite some challenges and complications remains the only available effective therapy [7]. To facilitate liver regeneration, cellular therapies involving the use of resident liver stem or progenitor cells or non-liver stem cells are coming to fore. However, more preclinical data regarding mechanisms of action, long-term safety and efficacy are warranted before initiating large-scale clinical application [8,9]. Besides hepatocytes and liver progenitor cells which are considered potential cell candidates for liver transplantation [10,11], the liver is composed of several other cell types including stellate cells (HSCs). Gene expression profiling and secretome analysis have been found to differentiate HSCs from other liver derived cells [12]. In the healthy liver, HSCs are quiescent and play a major role in maintaining an optimal environment for the resident liver cells [13]. In response to liver injury, HSCs acquire an activated phenotype, a process stimulated by the presence of inflammatory cytokines [14]. Because HSCs play a critical role in fibrosis, they are currently regarded as therapeutic targets to prevent and treat the complications of chronic liver disease [15,16]. The liver is considered as an immune-privileged organ

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http://dx.doi.org/10.1016/j.cyto.2016.11.008
1043-4666/

with HSCs contributing to its tolerogenic property [17]. HSCs induce immune-suppressive responses during liver homeostasis, like induction of regulatory T cells (Treg), T cell apoptosis (via B7-H1, PDL-1) or inhibition of cytotoxic CD8 T cells. In contrast, during liver injury, HSCs are important sensors of altered tissue integrity and initiators of immune response [19,20]. Parallel to have determine their immunophenotype [18], our group have also provided the first epigenetic blueprint of HSCs by establishing their transcriptome, their miRNome, their genome-wide promoter methylome and the profile of their histone modification patterns [21,22]. The main goal of this new study was to establish the immunological profile of HSCs based on the expression of relevant immunological cytokines and to investigate the impact of an inflammatory priming on their immunobiology. As known, HSCs have been characterized as the main effector cells in liver fibrosis and are able to respond to many immunological triggers. As evidenced herein, HSCs under inflammatory settings such as observed in liver fibrosis, might acquire a pro-inflammatory profile by expressing more inflammatory genes. Locally, this positive feedback loop will maintain a sustained inflammatory process and subsequently induce liver tissue fibrosis [20]. Accordingly, the path toward successful cell transplantation in the liver inevitably requires a better understanding of the immunological shape of liver derived cells such as HSCs. Therefore, keeping HSCs in a non-inflammatory state seems to be a key event in any future cell transplantation attempt.

2. Materials and methods

2.1. Isolation, culture and priming of HSCs

All experiments performed in the current study were approved by local ethics committees of the St-Luc Hospital and faculty of Medicine of the Université Catholique de Louvain. A written and signed informed consent has been obtained for each human liver used in the current study. The cells have been isolated from non-fibrotic/cirrhotic patients, which do not have any other underlying pathology. HSCs were isolated from the non-parenchymal fraction with the use of Nycodenz gradient centrifugation (Myegaard, Oslo, Norway) from 6 different donors as described previously [21,22]. In brief, human pieces of liver are obtained through the hepatic tissue bank of Saint-Luc hospital. Non-parenchymal liver cells were separated from parenchymal cells by sequential perfusion of human liver pieces with pre-warmed EGTA-containing EBSS medium (Lonza, Verviers, Belgium) and a collagenase P digestion solution (Roche) followed by low-speed centrifugation steps. HSCs were obtained by plating the low-density cell population obtained after subjecting the dissociated and washed non-parenchymal cells to an 8% Nycodenz (Myegaard, Oslo, Norway) gradient centrifugation step. After isolation, HSCs were cultured in DMEM (Lonza, Verviers, Belgium) supplemented with 10% FBS (Lonza) at 37 °C in a humidified atmosphere with 5% CO_2. HSCs were plated at a density of 1.10^4 cells/cm^2 and passaged when reaching 80–90% confluence. Regarding the inflammatory priming of the cells, HSCs were incubated overnight in medium supplemented with an inflammatory cytokine cocktail consisting of 25 ng/mL IL-1β (Peprotech, Rocky Hill, NJ, USA), 10^3 U/mL IFN-γ, 50 ng/mL TNF-α and 3×10^3 U/mL IFN-α (all from Prospec Inc, Rehovot, Israel). After incubation, cells were used for cytokine analysis.

2.2. qPCR analysis

RNA was isolated from HSCs by using the TriPure Isolation Reagent (Roche Applied Science, Vilvoorde, Belgium). Complementary DNA (cDNA) was obtained by means of reverse transcription of 1 μg RNA with the use of qScript cDNA SuperMix (QUANTA Bioscience, Gaithersburg, MD, USA) for 5 min at 25 °C, 30 min at 42 °C and 5 min at 85 °C. To avoid potential DNA contamination to our RNA samples, DNase (Promega, Leiden, Netherlands) treatment was performed. Real-time PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Rotterdam, Netherlands). We used 25 ng of cDNA in a real-time PCR with SYBR Green PCR Master Mix (Applied Biosystems) and 0.32 mmol/L of gene-specific forward and reverse primers. Primer sequences specific for human cytokine messenger RNA (mRNA) were used as previously described by our group [23]. Values are representing mRNA levels after normalization with GAPDH gene.

2.3. Statistical analysis

Statistical significance of mRNA expression between control and treated cells was determined according to paired Wilcoxon test. P-Values < 0.05 (*), <0.01 (**) were considered significant. All analyses were performed using Graph-Pad Prism software 5.0 (GraphPad Software, www.graphpad.com).

3. Results and discussion

The immunological shape of HSCs is of major importance to balance the liver's immune response. To gain insight into this shape, we have determined the cytokine pattern of HSCs and critically evaluated the impact of an inflammatory environment on this profile. In this brief report (Fig. 1), we show that HSCs do not constitutively express major immunological cytokines. However, when exposed to an inflammatory cocktail made of a mixture of pro-inflammatory cytokines, the cytokine expression profile of HSCs was substantially modulated. Inflammation strongly modulated the expression of various genes considered as inflammatory and anti-inflammatory. Thus, IL-6, IL-1β, IL-8, CCL5, IL-12A, p28, EBI-3, p19, TNF-α, IL-Ra, IDO1 expression levels were significantly upregulated. In contrast, only the expression of TGF-β was down-regulated. The group of pro-inflammatory (IL-6, IL-8, CCL5, IL-1β, TNF-α, IL-12a, p28, EBI-3, p19), anti-inflammatory (IL1Ra, IDO1) and pro-fibrotic factors (TGF-β) modulated by HSCs can establish a positive feedback loop that ensures further activation of HSCs. Interestingly, IL-6, IL-8, IL-1β, TNF-α and IL-12a as well as TGF-β are key cytokines and profibrotic factors known to be released by the cells in vicinity of HSCs, i.e. hepatocytes, kupffer cells, and infiltrating T-cells and leukocytes, when challenged with an inflammatory stimuli following liver injury [24]. Cytokines are critical coordinators of the immune response. Interleukin 6 (IL-6) is a pro-inflammatory cytokine that stimulates the immune response. It is reported that IL-6 promotes the differentiation of naive T lymphocytes into helper cells able to promote B-cell activation and antibody secretion [25]. Among this pattern of cytokines, we noted that in basic state, IL-6 and IL-8 in a lesser extent, were the only cytokines highly expressed in HSCs. After inflammatory priming, IL-6 expression was further significantly increased. IL-1β is another potent inflammatory cytokine acting through the IL-1-receptor/Toll-like receptor family member. The biologic activity of IL-1 can be antagonized by its natural decoy IL-1-receptor antagonist (IL1-Ra). When IL-1Ra binds to the IL-1 receptor, the binding of IL-1β is blocked preventing downstream proinflammatory signaling [26]. Basically not expressed, IL-1 and IL1Ra were also modulated by inflammation in HSCs. Therefore, the release of IL1Ra could landmark the anti-inflammatory properties of HSCs important in inflammation resolution. Through activation of both innate and adaptive immune reactions, IL-1α and β play a cardinal role in inflammation response and can thus can affect almost all body tissues [27,28]. Similarly, IL-12 is an important proinflammatory