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Autoimmune hepatitis type 1 and primary biliary cirrhosis have distinct bone marrow cytokine production☆

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Abstract

We have recently reported differences in the hematopoiesis between autoimmune hepatitis type 1 (AIH-1) and primary biliary cirrhosis (PBC). In view of the notion that cytokines are regulators of hematopoiesis, we investigated in our tertiary center the cytokine production in the bone marrow (BM) of the same consecutive cohort of patients (13 AIH-1, 13 PBC, 10 healthy and 7 patients with cirrhosis due to chronic hepatitis B). Interferon-γ (IFN-γ), interleukin-4 (IL-4), inteleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) were determined in the supernatants of long-term BM cultures by ELISAs. IL-4, TNF-α and TGF-β were found significantly increased in the BM of PBC patients compared to AIH-1 and both control groups. AIH-1 patients had significantly higher BM IL-10 compared to PBC patients and higher IL-10, IL-4 and TNF-α compared to controls. BM IFN-γ was significantly higher in PBC and AIH-1 patients compared to controls. In AIH-1 patients, IL-10 was positively correlated with CD34+, CD34+/CD38− and CD34+/CD38+ cell proportions. In conclusion, the BM cytokine microenvironment of PBC and AIH-1 patients differs significantly compared to that of healthy individuals and cirrhotic patients of non-autoimmune etiology. Differences were also found between patients with PBC and AH-1. The implication of BM in the pathogenesis of autoimmune liver diseases is possible and needs further investigation.

Keywords: Autoimmune liver diseases; Autoimmune hepatitis; Primary biliary cirrhosis; Hematopoiesis; Cytokines; Autoimmunity; Hepatitis

1. Introduction

Autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC) are autoimmune diseases of the liver, characterized by hepatocyte and biliary epithelial cell damage, respectively, leading to cirrhosis and liver failure [1–3]. The exact mechanisms responsible for the induction of cellular and humoral autoimmune responses and subsequent liver damage remain poorly understood in both diseases.

Experimental evidence suggests that BM stem cells are able to migrate to the liver and differentiate into hepatocytes and biliary epithelial cells [4–6] indicating their potential role in the pathogenesis of autoimmune liver diseases. This notion is also supported by the detection of the stem cell marker CD34 in 0.8–2.35% of isolated hepatic mononuclear cells [7].

We have recently shown an increased proportion of hematopoietic progenitor cells (CD34+) within the BM mononuclear cell (BMMC) fraction and normal hematopoiesis

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supported by BM stromal cells in patients with AIH type-1 (AIH-1) [8]. On the contrary, PBC patients were characterized by a significant quantitative and functional defect in hematopoiesis [8]. In view of the notion that several cytokines can potentially affect the survival, proliferation and optimal growth of BM progenitor cells [9], we investigated the cytokine production in long term cultures of BMMCs from the same cohort of patients included in the previous study [8] in order to assess first, whether the BM cytokine microenvironment is different between patients with AIH-1, PBC, and controls, and second, whether the abovementioned differences in hematopoiesis between AIH-1 and PBC patients published by our group previously [8], could be cytokine-driven.

For this reason, we determined in the supernatants of long term BM cultures from patients with AIH-1 and PBC as well as from controls the production of interferon-γ (IFN-γ), a hallmark cytokine of T helper 1 (Th1) immune response, interleukin-4 (IL-4) and interleukin-10 (IL-10), representatives of T helper 2 (Th2) immune response, tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine and transforming growth factor beta (TGF-β), a multipotent immunoregulatory cytokine [10,11].

2. Materials and methods

2.1. Patients

BM was collected from 13 consecutive AIH-1 and 13 PBC patients, as described previously [8]. As control groups, 7 age and sex matched patients with histologically proven cirrhosis due to chronic hepatitis B virus infection (cirrhotic controls, CC) and 10 healthy controls (HC) were also investigated. Demographic, clinical, serological and histological characteristics of patients and controls have been described previously [8] and are summarized in Table 1. Liver biopsy had been performed 3–12 months prior to the BM aspiration. All subjects consented to participate in the study. The human research review committee of Larissa University Hospital approved the study protocol.

2.2. Purification of BM

Immediately after aspiration BM samples were diluted 1:1 in Iscove's modified Dulbecco's medium (GIBCO-BRL, USA) with preservative-free heparin and antibiotics [12,13]. BMMCs were isolated on Lymphoprep (Nycomed, Oslo, Norway). CD34+, CD34+/CD38+ and CD34+/CD38− cells were isolated by indirect magnetic labeling, quantified by an indirect immunofluorescence technique and analyzed on an EPICS ELITE flow cytometer (Beckman-Counter, Lutton, UK) as described previously [8]. Long-term BM cultures (LTCs) from BMMCs from patients and controls were performed according to standard methods [14,15]. Briefly, unfractionated BMMCs (10^7 cells/well), CD34+ (5×10^4 cells/well), CD34+/CD38+ (500–5000 cells/well) and CD34+/CD38− (100–500 cells/well) from patients and controls were added to normal stromal layers (same stroma for patients and controls), and co-cultures were maintained by weekly 50% medium changes (Myelocult H5100, Stem Cell Technologies). After 5 weeks, non-adherent and adherent cells were plated in methylcellulose cultures, as has been described [14]. Colony forming cells (CFCs) were scored after an additional culture period of 12–14 days.

Table 1
Demographic, clinical, serological and histological characteristics of patients

	AH-1 patients ($n = 13$)	PBC patients ($n = 13$)	Cirrhotic controls ($n = 7$)
Sex (male/female)	3/10	1/12	2/5
Median age/age range (years)	62/27–79	60.5/17–74	56/38–70
Disease duration (months)	56 ± 44.8	29.5 ± 20.9	82.8 ± 53.4
Immunosuppression (yes/no)[a]	7/6	1/12	0/7
UDCA (yes/no)	5/8	12/1	0/7
Biochemical activity (yes/no)	2/11[b]	7/6[c]	4/3[b]
AST (U/L)	35.2 ± 15.7	31.5 ± 14.8	49.3 ± 31
ALT (U/L)	31.7 ± 13.2	34.1 ± 23	58.2 ± 40.4
ALP (U/L)	98.8 ± 31.3	149.9 ± 80.8	65.3 ± 19.8
Cirrhosis (yes/no)[d]	7/6	3/10[e]	7/0

Abbreviations are the same as in the text.
[a] Continuous administration of immunosuppression at least 6 months before and during the study period. Four AIH-1 patients were not receiving any treatment, two were under ursodeoxycholic-acid (UDCA), one was on prednizolone, four on combination therapy with prednizolone and azathioprine (one was also taking UDCA) and two were on prednizolone, mycophenolate mofetil and UDCA.
[b] AST (aspartate aminotransferase) or ALT (alanine aminotransferase) >1.5 upper normal limit at the time of BM aspiration.
[c] ALP (alkaline phosphatase) >1.5 upper normal limit at the time of BM aspiration.
[d] All cirrhotic patients with AIH-1 and PBC as well as cirrhotic controls had Child A cirrhosis without evidence of hypersplenism.
[e] From ten non-cirrhotic patients with PBC, four had stage I, four stage II and two stage III according to the classification of Ludwig.

2.3. Cytokine assays

Cell-free supernatants were harvested on confluence (weeks 3–4) and stored at −80 °C. IL-4, IL-10, IFN-γ, TNF-α and TGF-β were determined in the supernatants using commercially available enzyme-linked immunosorbent assays (ELISAs; R&D systems Minneapolis, USA, MN), according to the manufacturer's instructions. The sensitivity of the assays was <10 pg/ml for IL-4, <0.5 pg/ml for IL-10, <8 pg/ml for IFN-γ, <4.4 pg/ml for TNF-α and <7 pg/ml for TGF-β.

2.4. Statistical analysis

Results are expressed as mean ± standard deviation (SD) or median (range) as appropriate. Data were analyzed by unpaired *t*-test, Mann–Whitney *U*-test (MWU), analysis of variance (ANOVA) and the Fisher post-hoc least significant difference (Fisher PLSD) as the post-hoc test corrected for multiple comparisons, as well as the non-parametric test Kruskal–Wallis, where applicable. Pearson's coefficient of correlation (r) and Spearman's coefficient of correlation (r_s) were also used where appropriate. A two sided p value <0.05 was considered as statistically significant.