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Peripheral CD27⁻CD21⁻ B-cells represent an exhausted lymphocyte population in hepatitis C cirrhosis☆

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Abstract Hepatitis C cirrhosis is associated with a profound disappearance of memory B-cells. We sought to determine if this loss is associated with the expansion of the CD27⁻CD21⁻ tissue-like memory B-cells with features of B-cell exhaustion. To this end, we quantified the frequency of CD27⁻CD21⁻ B-cells in healthy, non-cirrhotic HCV-infected, and cirrhotic patients. We examined the expression of putative inhibitory receptors, the proliferative and immunoglobulin-secreting capacity of CD27/CD21-defined B-cell subsets upon B-cell receptor and/or CD40 stimulation. We found that CD27⁻CD21⁻ B-cells are significantly increased in frequency relative to healthy donors in HCV-infected patients. CD27⁻CD21⁻ B-cells were hypoproliferative relative to naïve and resting memory B-cells upon agonistic stimulation, but retained similar capacity for antibody secretion. Conclusion: CD27⁻CD21⁻ tissue-like memory B-cells with exhausted proliferation circulate at increased frequency in cirrhotic and non-cirrhotic HCV-infected patients. This B-cell subset does not appear anergic, exhibiting immunoglobulin-secreting capacity on CD40 agonism indistinguishable from other CD27/CD21-defined B-cell subsets.
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Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; TLR, toll-like receptor; BCR, B-cell receptor; LAIR-1, leukocyte-associated immunoglobulin-like receptor 1; FcRL4, Fc-receptor like protein 4.

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1. Introduction

An estimated 170 million individuals worldwide including 3 million persons in the United States are infected by the hepatitis C virus [1]. Over 70% of the persistently infected individuals develop chronic hepatic inflammation (hepatitis), which progresses to cirrhosis in approximately 20–30% of infected individuals usually over the course of 2–3 decades [2]. Hepatitis C infection is characterized by profound hyperglobulinemia consisting of non-virus-specific antibodies [3,4] produced by oligoclonally-activated B-cells [5,6]. Somewhat unexpectedly, chronic B-cell activation in chronic hepatitis C does not result in expansion of the memory B-cell pool in cohorts of mostly non-cirrhotic individuals [7–9]. Possible reasons cited for the lack of peripheral memory B-cell expansion include increased plasma cell differentiation [8], increased activation-induced B-cell apoptosis [8], and intrahepatic compartmentalization [10]. Among these explanations, activation-induced apoptosis has been contradicted by more recent data suggesting that B-cells in HCV-infected individuals are relatively resistant to apoptosis [11,12]. Rather than being expanded, we previously demonstrated that the circulating memory B-cell population disappears in cirrhotic but not non-cirrhotic HCV-infected patients [13]. The reduction in memory B-cells strongly correlated with multiple parameters of liver dysfunction and portal hypertension, also occurred in individuals with cirrhosis from other causes, and associated with a reduction in B-cell antigen-presenting cell function.

An alternative hypothesis to explain the disappearance of peripheral $CD27^+$ memory B-cells is the conversion of activated memory B-cells into $CD27^-CD21^-$ "tissue-like memory" B-cells that manifest evidence of B-cell anergy. A virus-specific anergic $CD27^-CD21^-$ B-cell population has been described in HIV disease that may be identified by the expression of FcRL4 [14,15]. In common variable immunodeficiency, a tissue-homing peripheral $CD21^{lo}$ B-cell population with impaired proliferation but exaggerated IgM secretory capacity with phenotypic similarities to the $CD27^-CD21^{lo}$ B-cell population in HIV has also been described [16]. Limited investigation in HCV disease has not identified an expansion of a similar $CD27^-CD21^-FcRL4^+$ B-cell population [9,17] but did suggest that a hyporesponsive $CD27^-CD21^{lo}$ B-cell population does exist in HCV patients with cryoglobulinemia [17]. FcRL4 putatively mediates its inhibitory effect on B-cell activation via its cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM). Several other ITIM-containing receptors including CD22, CD72, CD300a, CD305 (LAIR-1), FcγRIIB, and CD85j are expressed on B-cells [18–22,24], but the association of these ITIM-bearing receptor expression and B-cell activation in HCV disease remains largely unexamined.

The purpose of this study was to determine if HCV-related cirrhosis is associated with expansion of the $CD27^-CD21^-$ B-cell population and to determine if this population indeed represents an anergic B-cell population. We found that $CD27^-CD21^-$ B-cells have an increased frequency relative to healthy donors both in cirrhotic and non-cirrhotic HCV-infected patients. We confirm that $CD27^-CD21^-$ B-cells proliferate to a significantly lesser degree than naïve and resting memory B-cells after agonistic stimulation but retain similar capacity for antibody secretion. The expression of ITIM-containing CD305, CD22 and CD72 was lower in $CD27^-CD21^-$ than naïve $CD27^-CD21^+$ B-cells. Overall these data suggest that proliferative exhaustion of $CD27^-CD21^-$ B-cells does not infer functional anergy.

2. Methods

2.1. Patients

Subjects and controls were recruited from the Gastroenterology Clinics at the Philadelphia Veterans Affairs Medical Center following informed consent on an institutional review board-approved protocol. All patients were assessed for baseline demographics, hepatitis viral serologies, alcohol use history, and radiological findings. HIV-infected patients were excluded. Healthy donors (HD) had no evidence of liver disease or malignancy. Study subjects with HCV infection confirmed twice by commercial PCR assays were classified in this study as having: 1) early fibrosis (non-CIR HCV) based upon a liver biopsy within 3 years of the bleed date showing ≤Metavir F2 fibrosis and/or Fibrotest ≤ F1–2 testing within 6 months; 2) cirrhosis (HCV CIR) based upon clinical decompensation (ascites, jaundice, encephalopathy, thrombocytopenia), radiological finding (splenomegaly, nodular liver, varices, ascites), liver biopsy within 5 years, and/or Fibrotest F4; or 3), hepatocellular carcinoma (HCV HCC) based on standard American Association for the Study of Liver Disease diagnostic guidelines [23]. Non-HCV infected cirrhotic patients (non-HCV CIR) were recruited as an additional control group.

2.2. Cells isolation

Peripheral blood mononuclear cells were isolated using Ficoll-Histopaque (Sigma, St. Louis, MO) density centrifugation and either cryopreserved in liquid nitrogen or used immediately. Functional assays were performed with freshly isolated PBMC. Surface phenotyping was performed on thawed cryopreserved PBMC. For some experiments, B-cells were isolated using an autoMACS platform with B-cell isolation kit II (Miltenyi Biotech).

2.3. Flow cytometry

Surface phenotyping of cryopreserved peripheral blood mononuclear cells was performed using antibodies against CD3 (PerCP, SK7), CD14 (PerCP, M□P9), CD19 (APC-H7, SJ25C1), CD20 (2H7, PE-Cy7), CD21 (APC and V450, B-ly4), CD22 (APC, S-HCL-1), CD72 (FITC, J4-117) (24), CD305 (FITC, DX26), CD27 (PE and V450, M-T271), CD38 (FITC, HIT2), CD56 (PerCP, NCAM16.2), CD95 (APC, DX2), IgD (Alexa Fluor 700, IA6-2), IgG (V450, G18-145), IgM (FITC, G20-127), that were obtained from Becton Dickinson (Franklin Lakes, NJ). FcRL4 (PE-Cy7, 413D12) was obtained from BioLegend (San Diego, CA). Live/Dead Aqua was obtained from Invitrogen (San Diego, CA). All data were acquired on FACSCanto (BD) and analyzed using FlowJo (Tree Star Inc., Ashland OR) using cutoffs based on isotype antibody staining.