Cellular Immunology 311 (2017) 36–45

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Research paper

Hepatic expansion of virus-specific CD8$^+$BTLA$^+$ T cells with regulatory properties in chronic hepatitis B virus infection

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ARTICLE INFO

Article history:
Received 28 June 2016
Revised 3 October 2016
Accepted 6 October 2016
Available online 6 October 2016

Keywords:
B and T lymphocyte attenuator
Chronic virus infection
Hepatitis B virus
Regulatory CD8$^+$ T cells

ABSTRACT

Similar to programmed death-1 (PD-1), B and T lymphocyte attenuator (BTLA) is a co-inhibitory molecule of the CD28 family. PD-1 is involved in T cell exhaustion during chronic viral infection. However, the role of BTLA in virus-specific T cells is poorly defined. Here we investigated the expression and function of BTLA in T cells from patients with chronic hepatitis B virus (HBV) infection. The phenotype of peripheral and intrahepatic HBV-specific T cells from 43 patients with chronic HBV infection was assessed by flow cytometry. Functional evaluation was analyzed by T cell expansion and cytokine secretion after different treatments. In chronic HBV patients, a subset of inefficient interferon-γ producing antigen-specific CD8$^+$ T cells recruited to the liver expressed high BTLA levels. The BTLA$^+$ HBV-specific CD8$^+$ T cell suppressive function was antigen-specific, at least in the induction phase, because they were only activated by a pool of HBV peptides but not with a pool of unrelated peptides. Suppression of T cell responses was restored by a BTLA signaling blockade and neutralizing IL-10, indicating that BTLA signaling-mediated IL-10 secretion plays a key role in suppression. This study provides important evidence that there is a subset of liver infiltrated virus-specific CD8$^+$BTLA$^+$ regulatory T cells in patients with chronic HBV infection. This subset of cells plays a pivotal role in controlling hepatic effector CD8$^+$ T cell responses through BTLA signaling mediated regulatory factor IL-10 production.

1. Introduction

Hepatitis B virus (HBV) can persist in adults and, particularly, child hosts to establish a lifelong liver disease [1]. The establishment of persistent HBV infection is related to the severe dysfunction of HBV-specific CD8$^+$ T cells. The dysfunctional HBV-specific CD8$^+$ T cells prevent resolution of the infection, and favors the onset of chronic liver immunopathology [1,2]. However, it is still unclear why the effector's functions are impacted in the expanded HBV-specific CD8$^+$ T populations. Recent studies indicate that exhaustion of antigen-specific T cells, caused by propagation with an excess of co-inhibitory signals (such as PD-1, CTLA-4 and Tim-3) and a lack of CD4$^+$ T helper cells, contributes to HBV-specific CTL dysfunction [3–7]. Furthermore, different types of CD4$^+$ and CD8$^+$ regulatory T cells, which are potentially activated in an antigen-specific manner, suppress excess effector responses in an antigen-specific or -nonspecific manner [8–12]. The regulatory/effector T cell imbalance may contribute to a chronic low-grade inflammation which is critical to the survival of both the microbial agent and host.

Although overexpressing PD-1 has been demonstrated to link impairment of CD8$^+$ T cell functions in many types of chronic virus infection [4,5,13,14], the role of B and T lymphocytes attenuator (BTLA), which belongs to the same co-inhibitory family as PD-1, has not been well investigated in chronic viral infection. In this study, we found that patients with chronic HBV infection show a considerable enrichment of HBV-specific CD8$^+$ T cells in the liver (in contrast to the periphery) that express the co-inhibitory receptor, BTLA. Importantly, we demonstrated that the BTLA$^+$ HBV-specific CD8$^+$ T cells take regulatory roles in the liver, partly

Abbreviations: ALT, alanine aminotransferase; BTLA, B and T lymphocyte attenuator; CHB, chronic hepatitis B; CM, central memory; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EM, effector memory; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HBcAg, hepatitis B virus core antigen; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; IFN-, interferon-; IL-2, interleukin-2; LIL, liver infiltrated lymphocyte; PBMC, peripheral blood monouclear cell; PD-1, programmed death-1; TEMRA, CD45RA positive effector memory.

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http://dx.doi.org/10.1016/j.cellimm.2016.10.002
0008-8749/

depend on IL-10 secretion, and may have a major role in establishing chronic low-grade liver inflammation.

2. Patients and methods

2.1. Study population

Patients with HBV infection were studied according to the ethical guidelines of the 1975 Declaration of Helsinki and with *a priori* approval by the ethical committee of our hospitals. Patients with chronic HBV infection were diagnosed as previously reported[5]. Forty-three patients with chronic hepatitis B (Table 1) and 20 HLA-matched (HLA-A24$^+$) healthy donors (age range, 22–56 years; M/F, 13/7) were enrolled in this study. All enrolled patients were negative for anti-hepatitis C virus, δ virus, human immunodeficiency virus type 1 (HIV-1), HIV-2 antibodies, and other markers of viral or autoimmune hepatitis. No patients were being treated with Interferon (IFN)-α at the time of this study. Serum HBV-DNA and HBV genotypes were determined by real-time polymerase chain reaction (RT-PCR) (TaqMan; Qiagen, Indianapolis, IN, USA) and sequencing of the RT and S genes was performed using a Trugene HBV genotyping kit (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). All patients underwent percutaneous needle liver biopsy. The tissue samples were processed for pathological diagnosis and immunological analysis. Histology was graded using the histological activity index (HAI): periportal necrosis, intralobular necrosis, or portal inflammation (values range from 0 to 10, 0 to 4, or 0–4, respectively; thus their sum provides the "grading" or the "inflammatory index"), as well as fibrosis (ranging from 0 to 4; this leads to the "staging" or the "fibrosis index") and total score (grading + staging) [15] (Table 1).

2.2. Cell preparation

Peripheral blood mononuclear cells (PBMCs) and liver infiltrating lymphocytes (LILs) were isolated as previously described [16]. CD8$^+$ T cells were purified from PBMCs or LILs by immunomagnetic separation with mouse anti-CD8-conjugated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). BTLA$^+$ and BTLA$^-$ cells were purified from CD8$^+$ LILs or PBMCs by staining with biotin labeled anti-BTLA and magnetic microbeads conjugated with anti-biotin (Miltenyi Biotec). FACS analysis demonstrated more than 95% BTLA$^+$ CTLs in the positively purified population, and less than 10% BTLA$^+$ cells in the BTLA-depleted population. Immature dendritic cells (DCs) were derived from peripheral monocytes and purified by positive selection with CD14 monoclonal antibodies (mAb) coupled to magnetic beads (Miltenyi Biotec). Then, CD14$^+$

Table 1
Clinical parameters of patients with chronic HBV infection.

Pts	Age	Sex	ALT (U/mL)	HBV-DNA (U/mL)	Genotype	HAI		
						Total score	Grading	Staging
1	48	M	42	9.55E+05	B	3	3	0
2	56	M	60	3.02E+05	B	3	3	0
3	35	F	55	5.50E+04	C	4	3	1
4	24	F	43	5.25E+03	C	2	2	0
5	48	M	115	5.70E+03	B	6	5	1
6	40	M	82	4.61E+06	C	7	5	2
7	29	F	62	5.01E+06	B	3	3	0
8	28	F	305	1.82E+04	B	2	2	0
9	22	M	324	1.58E+03	B	2	2	0
10	48	M	82	1.29E+04	B	5	4	1
11	34	M	74	5.01E+03	C	7	5	2
12	65	F	44	2.95E+05	C	4	3	1
13	58	M	164	5.89E+04	B	8	7	1
14	28	M	50	1.66E+06	C	3	3	0
15	28	M	22	4.47E+04	C	3	3	0
15	34	M	89	1.58E+04	C	10	8	2
17	42	M	43	1.26E+04	B	9	7	2
18	39	M	66	3.39E+03	B	3	3	0
19	61	M	234	6.76E+04	B	4	3	1
20	44	M	110	1.07E+06	B	2	2	0
21	19	M	111	7.94E+04	B	2	2	0
22	64	M	99	5.00E+07	C	4	3	1
23	50	M	130	1.74E+06	C	8	6	2
24	37	M	134	1.62E+04	B	3	3	0
25	38	M	86	1.51E+04	C	3	3	0
26	59	F	35	7.41E+03	C	2	2	0
27	66	F	59	2.88E+07	B	5	4	1
28	23	M	145	1.32E+06	C	7	5	2
29	61	F	29	5.75E+04	B	4	3	1
30	45	M	33	3.89E+05	C	2	2	0
31	54	M	123	7.24E+04	B	3	2	1
32	37	M	126	3.55E+04	B	2	2	0
33	58	M	64	5.00E+07	B	2	2	0
34	44	M	105	1.45E+06	C	2	2	0
35	55	F	80	1.51E+06	B	5	4	1
36	50	M	87	1.82E+05	C	3	3	0
37	38	M	26	5.00E+07	C	3	3	0
38	35	M	66	1.70E+03	B	6	5	1
39	48	M	215	4.68E+03	B	4	3	1
40	37	M	107	8.51E+06	B	3	3	0
41	53	M	25	1.26E+07	C	2	2	0
42	35	F	97	9.55E+05	C	5	4	1
43	34	M	87	6.46E+03	B	8	7	1