Compositional Changes of PBL Population in Patients With Chronic Hepatitis B Virus Infection

Alireza Khabiri, Mohsen Abolhassani, Sudabeh Aslani, Hiedeh Darabi, Farideh Bagheri and Mohammad-Hossein Alimohammadian

Department of Immunology, Pasteur Institute of Iran, Tehran 13164, Iran

In this report we have analysed the peripheral blood lymphocyte of several patients with chronic hepatitis B virus infection with flow cytometry. Based on the presence and absence of the HBeAb, patients were divided into two groups. In both, all the patients were HBsAg positive with normal range of serum alanine aminotranferase (23.9 ± 17.8). We have found that the immunophenotypic profiles of patients were different from healthy donors with significant decrease in CD_3^+ T cells, specially CD_8^+ T cells and a siginificant increase in the CD_{19}^+ B cells. The differences were seen in other subset of T cells (CD_4^+) or NK cells (CD_{56}^+/CD_{16}^+) and HLA-DR markers were not significant. When the phenotypic profiles of both groups were compared with each other, such changes were more dominant in group II, with HBeAb positive than in group I, with HBeAb negative. Also, we have seen a correlation between the increase of CD_{19}^+ B cells and the decrease of CD_3^+ T cells. No such correlation was observed with other cells.

Key Words: Hepatitis B virus, chronic HBV infection, HBsAg, HBeAg, HBeAb, lymphocyte subset, flow cytometry.

Hepatitis B is one of the major diseases of mankind and is now preventable with safe and effective vaccines. Out of the 1 billion people who have been infected with the virus, more than 350 million are chronic carriers of the virus. The hepatitis B virus (HBV) is a noncytopathic, enveloped virus that causes acute and chronic liver disease and hepatocellular carcinoma [1]. These chronic carriers are at high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about 1 million people each year. Primary HBV infection with an infected host may be asymptomatic or results in varying degrees of acute liver injury [1]. Approximatelly 5% to 10% of infected adults will not resolve the primary infection and go to a persistent infection with different characteristics.

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Address for correspondence: Dr. Mohsen Abolhassani. Department of Immunology, Pasteur Institute of Iran, Tehran 13164, Iran. Phone/Fax: (98-21) 649-2596
E-mail: abolhassani@institute.pasteur.ac.ir

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Chronic carriers fall into at least three groups and may progress from one state to another. The first group is the immunotolerant phase, characterized by high levels of virus replication, viremia and antigenemia without any appreciable immunopathology. The second group is the immunoeliminative stage, which resembles that seen during the resolution of acute infections, consisting of active virus replication, immune reactivity and subsequent elevation in serum alanine aminotransferase (ALT) [2]. The chronic carrier may then serconvert with the loss of viral markers and the development of antibody to hepatitis B and antigen (HBeAg) latent phase [3]. In the third group, patients may experience a persistent, chronic infection, characterized by recurrent episodes of virus replication and active liver disease of varying duration. Expression of viral antigens on the surface of infected hepatocytes is believed to invoke a cytotoxic T-cell (CTL) response resulting in liver damage. The presence of the virus has been shown in several types of cells that are involved in the host immune response [4-7]. HBV DNA has been shown in T cells as well as B-cells and monocytes

of chronic HBV carriers [8]. These infected cells present in the microenviroment could modify T cell and T-B cell interaction to bring about *in vivo* suppression of the HBsAb response [8].

One approach to studying the mechanism for HBV immunopathology is to analyse the phenotypic composition of peripheral blood or liver-derived leukocyte populations from chronically infected patients [9,10]. Previous analyses of peripheral blood momonuclear cells (PBMC) from patients with chronic hepatitis B had led to controversial results [10]. Some investigators demonstrated an impared balance of the T-cell subsets related to decreases in the CD_4/CD_8 ratio [11,12], whereas others showed an increase or no significant changes [13,14]. Recently, it has been shown that one of the reasons for such divergent results is the fact that chronic hepatitis B represents a dynamic series of disease states, particularly when viewed from a perspective of virus-host interactions [2]. They have shown changes in populations of circulating lymphocytes not only in patients but also in healthy carriers who seroconverted with loss of HBeAg. These changes consist of a decrease in the percentages of CD_4^+ $\alpha\beta$ TCR^+ cells and an increase in both CD_4 and CD_8 cells bearing the $\gamma\delta$TCR [2].

In this study, we have analyzed the peripheral blood lymphocytes (PBL) of different patients with chronic hepatitis B in Iran and found differences not only in CD_4^+/CD_8^+ populations, but also in the B cell population.

Materials and Methods

Subjects. PBL of 25 patients (15 males and 10 females between 22 years old to 80 years old and the mean of 44.5 ± 17) with chronic HBV infection and 72 healthy individuals without HBV, hepatitis C virus (HCV) and HIV infection were used. All patients were admitted to different hospitals in Tehran, Iran, between 1997-1998. These patients were seropositive for HBsAg and seronegative for HCV and HIV, using Organon Teknika commercial kit. All patients had HBV infection for at least 6 months before selection, according to the hospital records, and were assigned into two groups based on HBeAb positive or negative (Table 1). These patients had normal range of ALT (23.9 ± 17.8).

Flow cytometry. Flow cytometry analysis was done on a Facscan with two colors of fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-labeled antibodies against CD_3^+/CD_4^+, CD_3^+/CD_8^+, CD_{3-}/CD_{19}^+, CD_3^+/CD_{19-}, $CD_{3-}/CD_{56-16}+$ and CD_3^+/HLA-DR+ (Becton Dickinson). Cells (1×10^4) were analyzed using LYSIS II software (Becton Dickinson).

Statistical analysis. Data were compared with normal subjects using analysisi of variance (ANOVA).

Results

Screening patients based on chronic HBV. All patients (n=25) used in this study were infected with HBV for at least 6 months and all were HBsAg positive (Table 1). Some patients in group I were positive for HBeAg (n=4) and some (n=4) were negative. No detectable HBeAg was seen in this group. We designated this group to the immunotolerant phase of HBV infection. In contrast, the Group II (n=17), all patients were positive for HBeAb and all were negative for HBeAg (except 2 patients that were HBeAg positive).

Distribution of lymphocytes subset. To determine whether any particular immunophenotypic profiles could be associated with disease outcomes, the patients PBL composition were compared with control healthy individuals. As shown in Figure 1, a statistically significant reduction was seen in the percentage of CD_3^+ cells ($p<0.005$) as well as CD_8^+ cells ($p<0.01$) in all patient as compared with 72 control subjects. In contrast, a significant increase in the percentage of CD_{19}^+ cells ($p<0.005$) was seen in PBL of patients. The differences were seen in the percentages of other subset of T cells (CD_4^+) or NK cells (CD_{56}^+/CD_{16}^+) and HLA-DR markers were not significant.

The phenotypic profiles were also compared between two groups (I and II) as well as within each

group of patients. Figure 2 shows the distribution of lymphocyte subsets between two groups of chronic HBV patients. A significant increase in percentage of CD_{19}^+ cells ($p<0.001$) was seen in group II having HBeAb+ when compared with group I (HBeAb-). Also, in group II a significant decrease of CD_3^+ cells was observed.

Figure 3 shows that there in a correlation between increase of CD_{19}^+ cells and decrease of CD_3^+ cells in group II patients. No such correlation was seen between CD_{19}^+ B cells and CD_4^+ or CD_8^+ cells (data not shown). No significant differences were seen between any of the two groups with respet to cells bearing the activation marker HLA-DR.

Discussion

In this report we analyzed the PBL of different patients with chronic hepatitis B infection with flow cytometry. Based on the presence and absence of HBeAb, these patients were divided into two groups. In both groups, all the patients were HBsAg positive with a normal range of ALT. In group I, all patients were HBeAb negative, but half of them were HBeAg negative, which represent the late incubation or early chronic HBV infection. The other half was HBeAg positive which represents late chronic HBV infection. Such a profile represents an inability to mount an immune response because of large amounts of circulating virus. All patients in group II were positive for HBeAb. In this group, two patients were also HBeAg positive representing early chronic infection or seroconversion. All other patients of group II were HBeAg negative representing the late seroconversion. This group had a functional immune response to HBV and was able to reduce the viral load following an initial flare up of liver disease, and supress further viral replication with the development of HBeAb.

We have found that the immunophenotypic profiles of patients were different from healthy donors with a significant decrease in CD_3^+ T cells, specially CD_8^+ T cells and a significant increase in the CD_{19}^+ B cells (Figure 1). When the phenotypic profiles of both groups were compared to with each other, such changes were more dominant in group II than group I (Figure 2). These changes may account for the presence or absence of HBeAg and the development of HBeAb.

It has been shown that the HBV nucleocapsid or core antigen (HBcAg) is extremely immunogenic during infection and after immunization. This antigen binds to specific membrane Ig antigen receptors on a high frequency of resting murine B cells sufficiently to induce B7.1 and B7.2 costimulatory molecules [15]. B cells can process and present HBcAg to naive Th cells more efficiently than macrophage or dendritic cells [15]. A unique feature of the HBV is the production of a secreted, nonparticulate form of the HBcAg designated hepatitis B precore Ag (HBeAg). The function of secretory HBeAg in the viral life cycle is unknown because it is not required either for infection or replication [16-18]. It has been proposed that the circulating HBeAg may have an immunoregulatory function in promoting viral persistance [19-21]. Recently, it has been shown that circulating HBeAg has the potential to preferentially deplete inflammatory HBeAg- and HBcAg- specific Th1 cells that are necessary for viral clearance, thereby, promoting hepatitis B virus persistence [22]. The mature HBeAg-specific Th1 cells preferentially depleted in the prephery after contact with secreted HBeAg by apoptosis [22]. HBeAg-specific Th2-like cells produce anti-inflammatory cytokines such as IL-4 and IL-10, that would be expected to inhibit the expansion of HBe/HBcAg-specific CTL and Th1 effector cells necessary for the clearance of this noncytolytic virus [22]. Recent serologic evidence suggests that a Th1/Th2 subset imbalance in favor of HBe/HBcAg-specific Th2 cells may play a role in promoting chronic HBV infection [23].

There are controversial reports regarding CD_4/CD_8 ratio in patients with chronic HBV infection. Some investigators [24] showed either a decrease or increase and yet others showed no changes at all in CD_4/CD_8 ratio [10]. It has been shown that during yhe seroconversion phase, the percentage of CD_3 lymphocytes, predominantly $\alpha\beta TCR^+$ CD_4 cells [24,25] decreases. However, if patients remained HBe-Ag-negative for 6 months after the hepatitis flare, a significant increase in $\gamma\delta$TCR-bearing cells of both CD_4

Table 1. Biochemical and virological characteristic of patients with chronic hepatitis B virus at the time of selection

Groups	Patients	Sex	ALT	HBsAg	HBeAg	HBeAb
I	1	F	29	+	-	-
I	2	M	39	+	-	-
I	3	F	38	+	-	-
I	4	M	13	+	-	-
I	5	M	34	+	+	-
I	6	M	54	+	+	-
I	7	M	15	+	+	-
I	8	F	37	+	+	-
II	9	F	15	+	+	+
II	10	M	17	+	+	+
II	11	F	13	+	-	+
II	12	M	13	+	-	+
II	13	M	8	+	-	+
II	14	M	18	+	-	+
II	15	M	13	+	-	+
II	16	F	55	+	-	+
II	17	F	5	+	-	+
II	18	M	48	+	-	+
II	19	M	17	+	-	+
II	20	F	11	+	-	+
II	21	M	35	+	-	+
II	22	M	38	+	-	+
II	23	F	15	+	-	+
II	24	M	11	+	-	+
II	25	F	33	+	-	+

Figure 1.

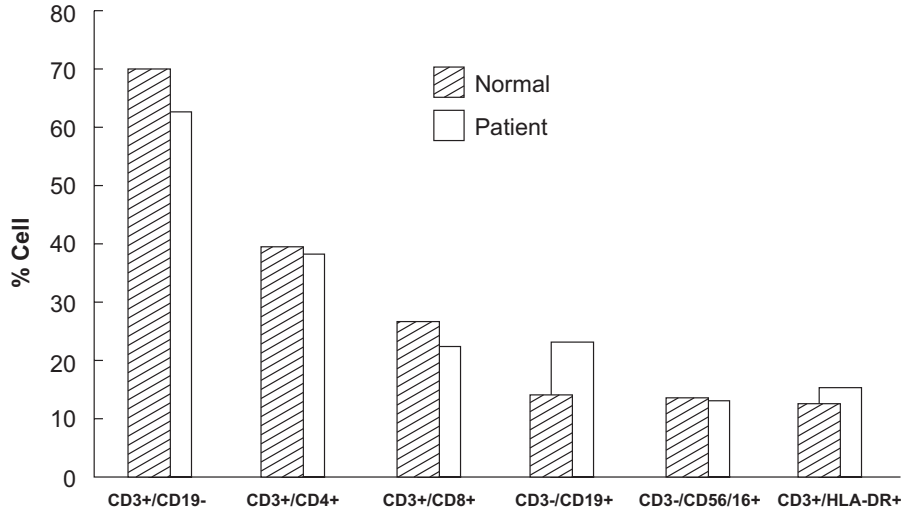


Figure 2.

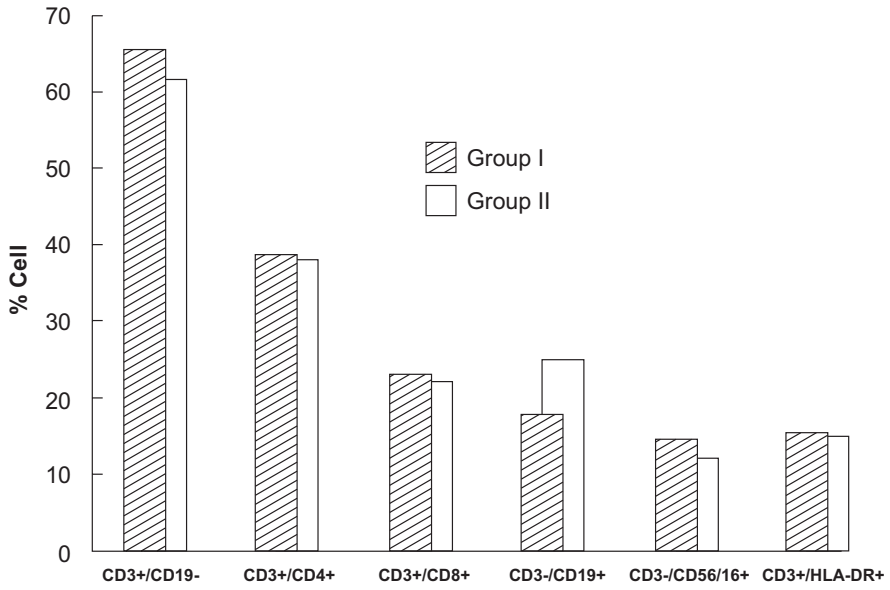
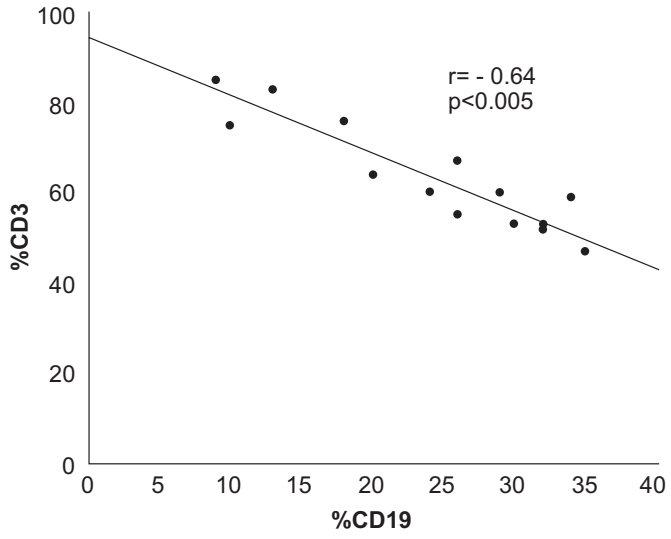


Figure 3.



and CD_8 subsets. T cells with γδ receptor that account for 1% to 5% of PBL in normal human blood, have been shown to be cytotoxic against a number of targets [26,27]. We have shown that because of the significant decrease of CD_8^+ T cells this ratio increases (1.56 in normal vs. 1.69 in patients). We have seen a correlation between the increase of CD_{19}^+ B cells, and the decrease of CD_3^+ T cells in group II patients with HBeAb positive. No such correlation was observed in group I with HBeAb negative or other cells. It seems, not only the ratio of CD_4/CD_8 in HBV infection is important but also the ratio of CD_{19}/CD_3 plays a role in chronic HBV infection.

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