

Immunological Aspects of Persistent Hepatitis B in Children

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Abstract We studied the immunological status of 203 children having persistent Hepatitis B (positive HBsAg) ranging in age from 6 to 14 years in Semarang Municipality. All patients showed negative results of humoral immunity (IgM anti-HBc), excluding the possibility of acute hepatitis B (HB). Cellular immunity examination using CMI skin test showed positive result in 64.9% of persistent and 65.2% in non-persistent HB ($p>0.05$). T cell examination shows significant statistical difference ($p<0.01$) between persistent and non-persistent HB, and there was a significant difference ($p<0.01$) on CD4 cell examination; indicating a difference on immunoregulation function and response repression of anti-virus between the two groups. There was no significant differences on CD4/CD8 ratio between persistent and non-persistent HB. The specific function of cytotoxic T cell also shows no significant difference between the two groups. [Paediatr Indones 1998; 38: 224-232]

Introduction

It has been known that hepatitis B Virus (HBV) is not a cytopathic virus against liver cells. In healthy carrier, that although there are a lot of HBV particles in the liver cells, there is no pathological disorder in the liver tissues. The HBV infection is caused mainly by the immunological reaction of the carrier's against the liver cells infected by HBV.^{1,2}

The clinical manifestations of the infection depend very much on its immunological reaction, particularly the cellular immunological reaction against HBV. If there is no or

only minimal immunological reaction, the carrier will be healthy. Acute hepatitis B (HB) will occur if the immunological reaction causes liver cell necrosis followed by the disappearance of HBV from the liver cells. When the immunological reaction fails to destroy HBV from the liver cells, chronic hepatitis will develop. When the necrosis on the chronic B hepatitis is diffuse, it will become chronic aggressive, while if the necrosis is limited, there will be persistent chronic HBV infection.³⁻⁵ This study aimed to determine humoral and cellular responses of persistent chronic HBV infection.

Methods

Subjects of this study were children aged 6-14 years old randomly selected from 5,974 children with positive HBsAg in Semarang Municipality. The immunological factors examined were humoral immunity, i.e., IgM anti-HBc, and anti HBs; no anti-HBe, anti-Pre-S1, or Pre-S2 examinations were performed. For cellular immunity, total number of lymphocyte cells, CMI skin test, the number of T cells, T helper and T supressor, as well as the CD4/CD8 cell ratio were examined.

The immunological factors examined were skin test, T roset cell, CD4 cell, CD8, ratio of CD4/CD8 and specific function of T cytotoxic cells. The skin test was performed by using CMI multitest from Paris; it was called positive if by 2x24 hours the diameter of induration was more than 2 mm. T cells were examined in the form of T cell in roset form and was examined by using T roset method. CD4 cells (T helper cells) were examined by using ELISA and expressed in % unit; CD8 cells (T supressor cells) were examined by using ELISA and expressed in % unit. The cytotoxic cells were examined by MIF method. Statistical analyses were performed by using Student's t test or Chi-square test; the significance level was $P < 0,05$ for all tests.

Results

There were 203 children with positive HBsAg included in the study, of which 114 were persistent and 89 non-persistent HBV infections. IgM anti-HBc examination was performed in all 203 children, and it turned out that all children showed negative IgM anti-HBc. In this series of children with positive HBsAg, no symptoms were noted; in other words these children were asymptomatic HBsAg carriers. None of them was having acute HBV infection. The IgM anti-HBc examination was done to differentiate acute from chronic HB.⁶ Brunetto *et al.* (1993) stated that IgM anti-HBc was useful for diagnosis and follow-up of patients with chronic HB.⁷

The cellular immunity responses examined in this study included: (a) CMI skin test, (b) T lymphocyte cells, (c) CD4 cells, (d) CD8 cells, (e) CD4/CD8 ratio, and (f) specific function of cytotoxic T cells against pure HBsAg. The results are described below.

a. CMI Skin Test

Table 1 depicts the result of CMI skin test as related to the presence of persistent HBV infection. It shows that out of 203 children having skin test, 132 (65%) showed positive result. Positive CMI skin test was positive in 64.9% of children with persistent and in 65.2% of those with non-persistent HBV infection. There was no significant relationship between CMI skin test and the persistence status in the two groups ($p>0.05$).

Table 1. Relationship of skin test and persistent HBV infection

CMI skin Test	HBV Infection				Total	
	Persistent		Non-persistent		n	%
	n	%	n	%		
Negative	40	35.1	31	34.8	71	35
Positive	74	64.9	58	65.2	132	65
Total	114	56.2	89	43.8	203	100

χ^2 test: $p>0.05$

b. T Lymphocyte Cells

Figure 1 shows the difference of the average number of T lymphocyte cells in persistent and in non-persistent HBV infection. The figure depicts that the average number of T cells in persistent HB was less than in non-persistent ones, and the difference was statistically significant ($p<0.05$). This shows that if the T lymphocyte cells increased, then the cellular immunity response function would be more active, so that the increased T lymphocyte cells would destroy and eliminate HBsAg. This study shows that the number of T lymphocyte cells decreased, meaning that the cell number for cellular immunity also decreased, so that the prevalence of persistent HBV infection increased.

c. T Helper Cells or CD4 Cells

The difference between CD4 cells in persistent and non-persistent HBV infection can be seen in Figure 2. This figure shows that there was a statistically significant difference on the average number of CD4 cells between persistent and non-persistent HBV in children. Total number of CD4 cells in persistent HBV children was higher than in non-persistent ones, this indicated that an increase of CD4 cells was followed by an increase of the persistence. This is consistent with the function of CD4 cells as a regulator of immune response or even suppress the anti virus response.⁸

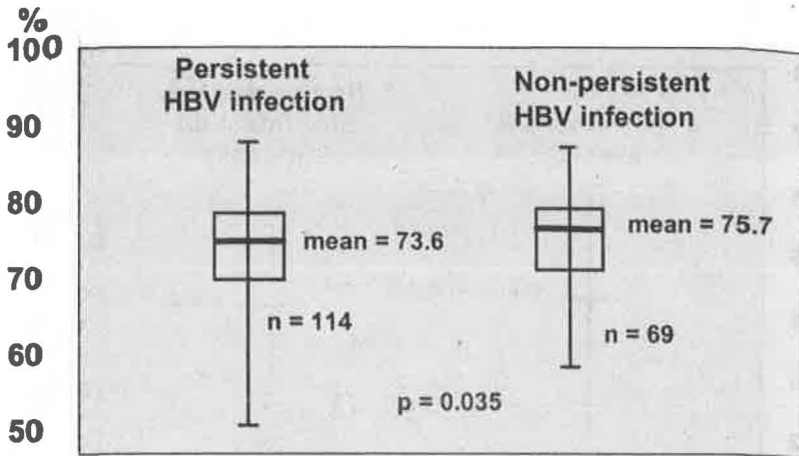


Figure 1. Comparison of T lymphocyte cells (in percentage of total lymphocyte count) in persistent and non-persistent HBV infection showing the significant difference between the 2 groups.

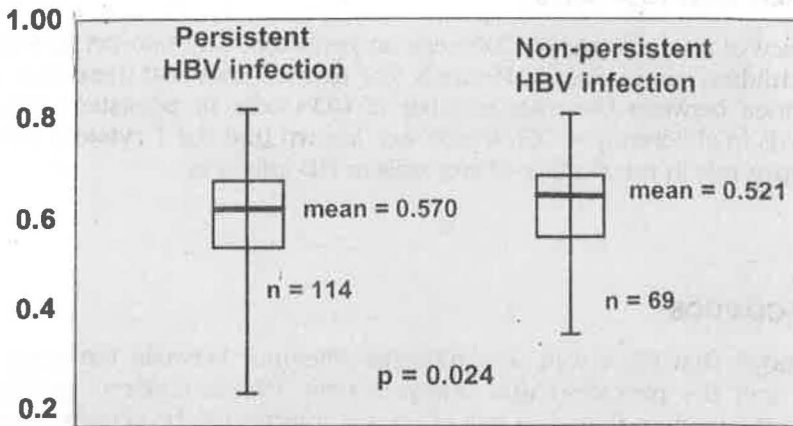


Figure 2. Number of CD4 cells in persistent and non-persistent HBV infection; the difference between the 2 groups was significant.

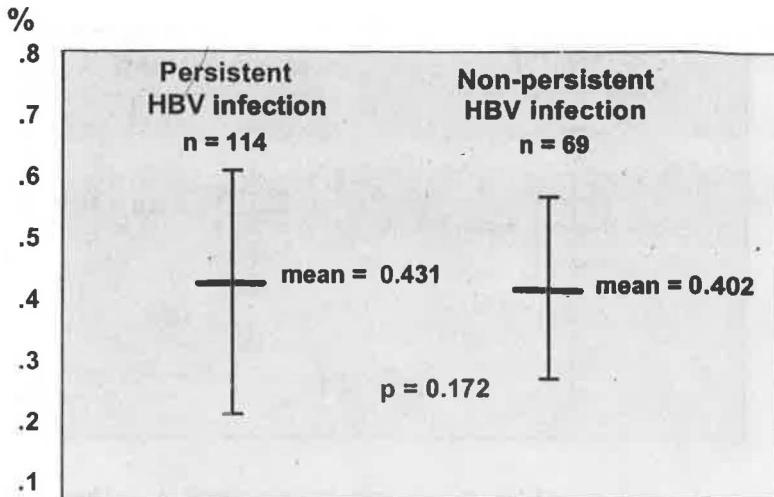


Figure 3. The total average of CD8 cells in persistent and non-persistent HBV infection in children. Vertical lines indicate range of values.

d. T Cytotoxic Cells (CD8 Cells)

The difference of total average of CD8 cells on persistent and non-persistent HBV infection on children can be seen in Figure 3. The figure shows that there was no significant difference between the total number of CD8 cells in persistent and in non-persistent HB in children ($p > 0.05$). It has been known that the T cytotoxic cells have a very important role in the damage of liver cells in HB infection.

e. Ratio of CD4/CD8

Figure 4 shows that there was no significant difference between the mean of CD4/CD8 ratio and the persistent and non-persistent HB in children ($p > 0.05$). If the CD4/CD8 ratio is more than 1, it will cause the infection to be persistent, and if it is less than 1, it will decrease the persistence. This is because the number of T cytotoxic cells will be increasing and the CD4/CD8 ratio becomes less than 1. In this case, the CD4/CD8 ratio has no relationship with the HBV infection persistency.

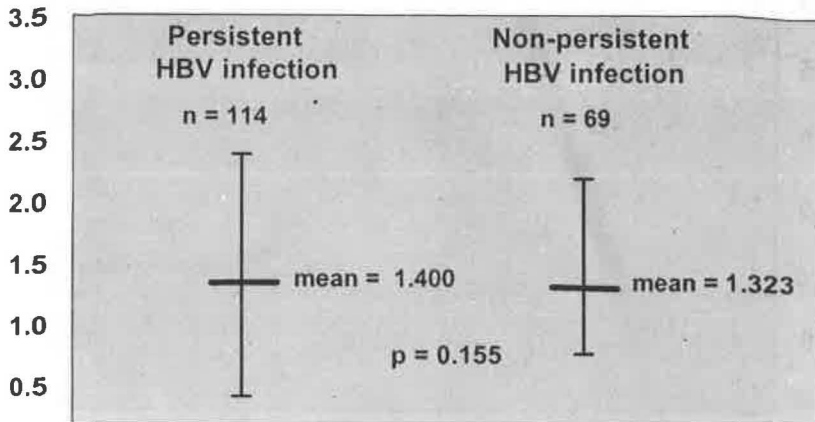


Figure 4. Comparison of CD4/CD8 ratio in persistent and non-persistent HBV infection.

f. Specific T cell function

Comparison between specific T cell function in persistent and non-persistent HBV infection is shown in Figure 5. It shows that there is no significant difference between the function of specific T cells in persistent and non-persistent HBV infection in children ($p > 0.05$). Migration inhibition factor (MIF) test is conducted by using pure HBsAg as the antigen. From this test, there is no difference observed between the group of children having persistent and non-persistent infection.

Discussion

There are two things required to cure HBV infection. The first is the cellular immune response which is effective to destroy the infected liver cells. The second is the humoral neutralizing antibody which can prevent the spread of the virus which flee into the healthy liver cells. When there is any disturbance in either one or both the two mechanisms above, the infection will become persistent.⁵

It has been known that there are several important antigen systems against the HBV infection, namely, HBsAg, HBcAg and HBeAg, and these three antigens will cause humoral immunity response of the body, namely Anti-HBs, Anti-HBc and Anti-

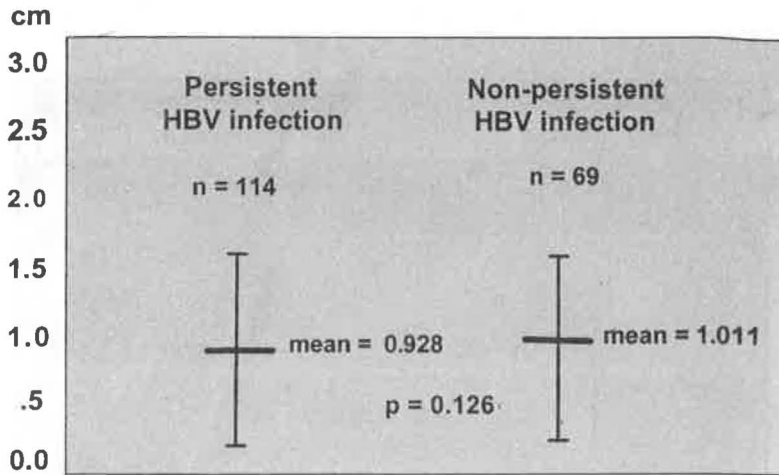


Figure 5. Comparison of specific T cell function in persistent and non-persistent HBV infection.

HBe, respectively. However, our data show that the persistent HBV infected children had no Anti-HBs formed yet and all the groups had negative Anti-HBc, so that they could not be differentiated statistically.

Anti pre-S1 and anti pre-S2 were not examined in this study, and latter it is known that HBsAg consists of S, pre-S1, and pre-S2 components, which will form anti S or anti HBs, anti pre-S1 and anti pre-S2. A study of Neurath *et al*⁹ shows that the immunodominant epitope of HBsAg is located in protein pre-S, and in pre-S2 to be precise. It has been proved that the binding site of HBsAg is located in pre-S2.⁹ In the recovery process of acute hepatitis B, before the anti-HBs appears, the first one to appear is anti pre-S2. Some authors show that both in acute and chronic hepatitis, pre-S2 is the indicator of HBV disappearance or recovery from HBV infection. Anti pre-S2 is not found in acute hepatitis B which tends to be chronic, and is not found in chronic hepatitis B.¹

Anti-pre S is the neutralizing antibody released during HBV infection. Anti-pre S has the role as the starter in the recovery process of HBV infection. This antibody is the first antibody in the recovery stage, and Anti-pre S titer is decreasing along with the disappearance of HBsAg.^{1,5}

The results of the cellular immunity examination shows that the CMI skin test was not different between persistent and non-persistent HBV infection. The negative result of skin test in persistent HBsAg shows that in persistent HBsAg of 35.1%, the cellular

immunity is generally low. This condition is caused by various situations, the growth of the immature immune cells, the status of nutrition, calory and protein or other vitamin. This is also stated by Stites that chronic infection will cause the decrease of cellular immunity.¹⁰ Lack of calory and protein also decreases the cellular immunity.¹¹

The conclusion is that the T lymphocyte cells have the role as the risk factor of persistent HBV infection in children. This is in accord with the report of Dinfeng et al that showed a decreased T cells in all hepatitis groups examined, particularly in fulminant hepatitis and active chronic hepatitis, which associated with less recovery and becomes persistent.¹²

Figures 1 and 2 show the total number of T cells and CD4 cells in persistent and non-persistent HBV which were statistically different ($p < 0.01$). This shows that total number of T cells in general in persistent hepatitis B is less than the non-persistent ones, so that the function to eliminate the virus is also decreased. the CD4 cells show that the immunoregulation function and immune response repression is still enough and is different between the persistent and non-persistent ones.

Our data show that the specific T cytotoxic cell function against HBsAg was not different in both persistent and non-persistent HB ($p > 0.05$). This is probably due to the antigen used for the MIF test is pure HBsAg, but does not use HBcAg, since in persistent hepatitis, the target antigen is HBcAg or HBeAg. If the MIF test is normal, this means that the T cell function is good and there is no liver cell damage. The damage of liver cells is caused by immunological response against the liver cells infected by HBV which cause the target antigen appears on the cell membrane.^{12,13} In acute hepatitis, the target antigen is HBsAg.¹⁴ In this case, the one having the important role is the T cytotoxic cell which is capable of identifying the HBV antigen created on the cell membrane.¹⁵ Dienstag finds that the liver cells damage is caused by the effort of the body to eliminate the HBV.¹³ This is influenced by several factors such as the existence of antiHBc in the circulation, immunoregulator in the serum, and the role of HLA class I. The T cytotoxic cell can identify the target antigen, if the target antigen is created and appears along with the HLA class I. On chronic hepatitis B, it is often that there is no HLA class I on the cell membrane, so that the immune cellular system can not touch the cells infected by HBV, which creates HBV antigen on the cell surface.¹⁴ It is understandable that the individuals on this research are children having persistent HBV infection whose MIF test is normal, since actually the cellular immune response against the HBV particle in vitro is probably still normal, while if it is in vivo, the result could be different. Besides, on chronic HBV infection, the target antigen created on the cell membrane is HBcAg or HBeAg, and not HBsAg.

In conclusion, the result of humoral immunity examination in this research of Ig Anti-HBc has been all negative. Cellular immunity examination in this research such as CMI skin test is not significant difference ($p > 0.05$), T cell examination show significant statistically difference, CD4 examination also significant different, CD8, CD4/CD8 ratio and the specific function of cytotoxic T cell show no significant difference.

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