THE STATE OF CELL MEDIATED IMMUNITY AMONG
HEPATITIS B SURFACE ANTENI CARRIERS IN IRAN.

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Summary

Cell-mediated immune (CMI) status and sub-populations of peripheral blood lymphocytes were investigated in one hundred voluntary blood donors who were carriers of HBsAg.

A significant decrease of total T-cells observed in HBsAg carriers as compared to normal controls. The percentage of active T-cells and B-lymphocytes did not differ significantly between the two groups.

Addition of autologous serum from HBsAg carriers to their lymphocytes reduced the number of detectable cells in HBsAg carriers. This reduction could be due to the presence of a rosette inhibitory factor in their serum. Our studies demonstrated a failure of CMI among HBsAg carriers detected by the leukocyte migration inhibition

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(LMI) test. This failure cannot be attributed to the presence of HB$_s$Ag–AB complexes in their serum. It is possible that specific failure of CMI allows the hepatitis B virus to remain harmless in carriers.

Hepatitis B surface-antigen (HB$_s$Ag); Hepatitis B core antigen (HB$_c$Ag) and Hepatitis B e-antigen (HB$_e$Ag), have been established as indicating ineffectivity in viral hepatitis B ((1, 6, 20, 28).

A number of infected individuals also developed clinical evidence of disease and HB$_s$Ag may remain present in the serum of some subjects for a long time (18). It has been suggested that the persistence of HB$_s$Ag is related to a defect in CMI, whether liver disease is present or not, and impairment of the lymphocyte response to phytohaemagglutinin (PHA) in this group is presented in evidence (8, 9, 13, 24, 25). In contrast, other workers report a normal response to PHA in healthy carriers of HB$_s$Ag and they conclude that the defective T-cell response is related to the liver disease rather than the immune system (31). Dudley et al (8) have suggested that liver damage occurring after hepatitis B infection, may be an effect of thymus-dependent lymphocytes (12).

A variety of in-vitro tests have been employed to demonstrate the status of cellular immunity (30, 35). Amongst these the inhibition of leukocyte migration by different specific and non-specific antigens appears to be a sensitive and convenient procedure (37). Leukocyte Inhibitory Factor (LIF) production in the presence of the virus itself has already been demonstrated (11). In a preliminary study Ito et al. (14) reported that migration of leukocytes could be inhibited by serum containing
HB\textsubscript{s}Ag Frei et al. (11) have also reported the effect of purified, partially purified and non purified HB\textsubscript{s}Ag on lymphocytes in acute cases of hepatitis B using the leukocyte migration inhibition test.

The prevalence of HB\textsubscript{s}Ag among some 200,000 voluntary blood donors in Iran was found to be 3.5% (10). We have evaluated the subpopulations of T and B lymphocytes in HB\textsubscript{s}Ag carriers and applied the LMI test in the presence of PHA, purified protein derivative (PPD), crude HB\textsubscript{s}Ag positive serum and purified HB\textsubscript{s}Ag, in order to define CMI in HB\textsubscript{s}Ag carriers.

Materials and Methods:

Serum from one hundred voluntary blood donors was tested for HB\textsubscript{s}Ag by radio-immuno-assay using Ausria II (Abbott Co.). Carriers were defined as those who were HB\textsubscript{s}Ag positive for at least three months before the study was initiated (26). Fifty healthy subjects with no history of liver disease and no detectable HB viral makers were selected as controls.

The following substances were used as antigens: PHA (Difco Labs.) in a concentration of 3ug/ml; PPD (Difco Labs) in a concentration of 100 units/ml; crude HB\textsubscript{s}Ag positive serum from persistent carriers and purified HB\textsubscript{s}Ag (10ug/ml protein) prepared by the method of Venek and Prince (34). Peripheral blood lymphocytes were obtained by Ficoll-Hypaque gradient separations.

Enumeration of T-active cells (Ta) was performed by the technique of Wybran et al. (34), and total-T cells (Tt) were measured by the technique of Jandal et al. and Lay et al. (15, 16). Erythrocyte-Antibody-Complement Ros-
atte forming cells (EAC-RFC) of B lymphocytes were determined as described by Mendes et al. (22).

LMI was performed by the technique of Soborg and Bendixen (30) with modifications described elsewhere (19). The migration pattern was projected, traced and the area of migration was measured by planimetry. Inhibition of migration was considered significant when the Migration Index (MI) in the presence of antigen was less than 75% of the control figure without antigen.

\[
MI = \frac{\text{Area of migration in the presence of antigen}}{\text{Area of migration in control chambers}} \times 100
\]

Results:

The distribution of lymphocyte subpopulations in normal controls and HBsAg carriers is shown in Table 1. A significant decrease of total-T cells was observed in HBsAg carriers as compared to normal controls (P < 0.005). The percentage of active T cells and EAC-RFC (B-lymphocytes) did not differ significantly in the two groups.

Table 2 shows the effect of autologous serum from normal controls and HBsAg carriers on their lymphocytes. We observed only a diminution of total-T cells in HBsAg carriers after contact with their serum which was not significant. The number of T-active and B lymphocytes remained relatively unchanged.

Table 3 demonstrated the number of subjects showing significant leukocyte inhibition in the presence of specific and non-specific antigens, comparing normal controls and HBsAg carriers as a percentage. PHA caused inhibition in 59% of HBsAg carriers as compared to 79% observed in normal controls (P < 0.01). Similarly, the leukocyte inhibition was 26% for PPD in the carriers and
58% in normal controls (P = 0.005), thus indicating a significantly decreased response by HB\textsubscript{s}Ag carrier lymphocytes in the presence of PHA and PPD.

The effect of adding autologous serum is indicated by the M.I. in Table 4 which shows significant inhibition in 60% of HB\textsubscript{s}Ag carriers and 25% of normal controls (P = 0.005), whereas, with purified HB\textsubscript{s}Ag 35% of carriers and none of the controls gave a positive test result (P < 0.005).

Among anti-HB\textsubscript{s} positive and antigen negative subjects (Table 5) and in the presence of autologous serum, the LMI shows that a greater number of the latter group demonstrated migration inhibition as compared to the former anti-HB\textsubscript{s} positive group (P = 0.005).

Discussion:

Our studies showed that total-T cells are both numerically and functionally reduced in the peripheral blood of HB\textsubscript{s}Ag carriers (Tables 1 & 3). In contrast, the number of EAC-rosette forming cells (B-lymphocytes) remains unaltered. The qualitative and quantitative diminution of T-lymphocytes in the presence of normal numbers of B-cells in HB\textsubscript{s}Ag carriers suggests the existence of a factor(s) present in the serum of this group, which selectively affects T-cells. (3,23; 27, 29, 33).

It appears that two distinct mechanisms are implicated in the generation and function of T-cells (4). One is probably related to an extrinsic serum factor, whereas the other mechanism appears to be intrinsic to the defective lymphocyte.

The extrinsic mechanism seems to operate via an interaction between T-lymphocytes and humoral substances which are found in blood such as virus itself or other inhibito-
rs present in the serum. In contrast, lymphocytes with
an intrinsic defect do not engender T-cell function, nor
does authochtonous serum inhibit T-cell function of nor-
mal lymphocytes. (5, 21).

We have found a reduction of T-cells when the serum
of HB_sAg carriers is reacted with their peripheral lym-
phocytes (Table 2). This reduction could be due to a
rosette inhibitory factor (RIF) which Chisari et al.
described in the serum of patients with HB_sAg(4).

There are several methods by which one can determine
CMI status (30,36). The leukocyte migration inhibition
(LMI) test using specific antigens or mitogens appears
to be a convenient in-vitro test (19).

Our studies demonstrate a failure of CMI among HB_sAg
carriers detected by LMI in the presence of PPD and PHA.
This finding contrasts with the inhibition we observed
in healthy HB_sAg negative blood donors. The failure of
this response in the presence of a normal range of active
T-cells may explain persistence of the virus in the peri-
pheral blood of HB_sAg carriers.

On the other hand, we have been able to demonstrate
a significant difference in leucocyte migration inhibi-
tion when autologous serum or purified HB_sAg are alter-
natively employed as antigen in HB_sAg carriers and normal
subjects.

These results confirm the existence of sensitive
cells in the peripheral blood of persistent HB_sAg carriers
which are directed against the virus; its associated par-
ticles or else other substances present in the serum.

The absence of correlation between L.M.I. and the pr-
essence of antibody indicates that inhibition of migration
is unrelated to the presence of HB_sAg/anti-HB_s complexes
Table 1:

Relative distribution of peripheral Blood lymphocytes (B, Tt and Ta) among Normal Controls and HBsAg carriers.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Normal Controls</th>
<th>HBsAg Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tt</td>
<td>73.9% ± 11.35*</td>
<td>49% ± 5.65</td>
</tr>
<tr>
<td>Ta</td>
<td>30.4% ± 9.43</td>
<td>28% ± 1.1</td>
</tr>
<tr>
<td>EAC</td>
<td>20.2% ± 4.45</td>
<td>16.45% ± 3.2</td>
</tr>
</tbody>
</table>

Tt : Total Cells
Ta : T active cells
EAC : Erythrocyte Antibody Complement
* : Standard deviation
Table 11: The effect of autologus serum from Normal Controls and HBsAg carriers on their lymphocytes.

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls</th>
<th>HBsAg Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tt</td>
<td>Ta</td>
</tr>
<tr>
<td>Before serum</td>
<td>73.9 ± 30.4 ±*</td>
<td>20.2 ± 49 ±</td>
</tr>
<tr>
<td></td>
<td>11.35</td>
<td>9.43</td>
</tr>
<tr>
<td>After serum</td>
<td>68 ± 29.1 ±</td>
<td>18.2 ± 38 ±</td>
</tr>
<tr>
<td></td>
<td>5.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Tt : Total T-cells  
Ta : T-active cells  
EAC : Erythrocyte Antibody Complement Cells (B-lymphocytes)  
* : Standard deviation
Table III: PHA and PPD induced Leukocyte inhibition among Normal Controls and HB$_s$Ag carriers.

<table>
<thead>
<tr>
<th>Ag</th>
<th>Normal Controls</th>
<th>HB$_s$Ag Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td>79 %</td>
<td>59.5 %</td>
</tr>
<tr>
<td>PPD</td>
<td>58 %</td>
<td>26.1 %</td>
</tr>
</tbody>
</table>

Ag : Antigen  
PHA : Phytohemaglutinin  
PPD : Purified Protein Drivative

Table IV: The effect of autologus serum and purified HB$_s$Ag induced leukocyte inhibition among Normal Controls and HB$_s$Ag carriers.

<table>
<thead>
<tr>
<th>Ag</th>
<th>Normal Controls</th>
<th>HB$_s$Ag Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% autologus serum</td>
<td>25%</td>
<td>60.5%</td>
</tr>
<tr>
<td>Purified HB$_s$Ag</td>
<td>0%</td>
<td>35 %</td>
</tr>
</tbody>
</table>
Table V: LMT with autologus serum in Anti HB$_s$ Positive and Negative subjects among HB$_s$Ag carriers.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Anti HB$_s$ Positive</th>
<th></th>
<th>Anti HB$_s$ Negative</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>%</td>
<td>Cases</td>
<td>%</td>
</tr>
<tr>
<td>Positive LMT* with autologus serum</td>
<td>5</td>
<td>41.66</td>
<td>29</td>
<td>82.85</td>
</tr>
<tr>
<td>Negative LMT with autologus serum</td>
<td>7</td>
<td>58.34</td>
<td>6</td>
<td>17.14</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100</td>
<td>35</td>
<td>100</td>
</tr>
</tbody>
</table>

* LMT = Leukocyte migration test.
in autologous serum which might have reacted with HBsAg carrier lymphocytes.

The results we have obtained with the LMI test in HBsAg carriers differ from those of Desaules et al (7) and Frei et al. (11) who did not find significant inhibition with PPD; PHA or purified HBsAg. In contrast our observations are in agreement with Lee et al. (17,18) who found 26% positivity of LMI in the presence of purified HBsAg. The major difference between our study and previous reports lies in the use of an appropriate concentration of HBsAg to increase the sensitivity of the in-vitro detection of CMI response to HBsAg.

Desaules et al. (7) also employed the LMI test to demonstrate unresponsiveness to purified HBsAg the a relatively normal response to PPD among antigen carriers. They concluded that these observations contribute to an explanation of the absence of lesions in 'health' carriers although they emphasize that the lesions of hepatitis are not exclusively produced by the virus itself. In our study, no association was observed between the existence of an HBsAg specific CMI response and concurrent liver damage.

In our opinion the lack of liver damage in most HBsAg carriers could be the consequence of the existence of some inhibitory factor (s) such as a rosette inhibitory factor or other substances in the serum of these subjects (25), which are responsible for the diminished response to PPD or PHA when tested by LMI. It is therefore possible that specific failure of CMI allows the hepatitis B virus to remain harmless in carriers.
References:


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