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THE STATE OF CELL MEDIATED IMMUNITY AMONG HEPATITIS B SURFACE ANTGENI 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 HB_s Ag.

A significant decrease of total T-cells observed in HB_sAg 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 HB_sAg carriers to their lymphocytes reduced the number of detectable cells in HB_sAg 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 HB_sAg carriers detected by the leukocyte migration inhibition

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(LMI) test. This failure cannot be attributed to the presence of HB_sAg-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_sAg); Hepatitis B coreantigen (HB_cAg) and Hepatitis B e-antigen (HB_eAg), have been established as indicating ineffectivity in viral hepatitis B ((1, 6, 20, 28).

A number of infected individuals also developed clini cal evidence of disease and HB_sAg may remain present in the serum of some subjects for a long time (18). It has been suggested that the persistence of HB_sAg is related to a defect in CMI, whether liver disease is present or not, and impairment of the lymphocyte response to phyto-haemagglutinin (PHA) in this group is presented in evide· nce (8, 9, 13, 24, 25).In contrast, other workers report a normal response to PHA in healthy carriers of HB_sAg 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 lto et al. (14) reported that migration of leukocytes could be inhibited by serum containing

HB_sAg. Frei et al. (11) have also reported the effect of purified, partially purfied and non purified HB_sAg on lymphocytes in acute cases of hepatitis B using the leukocyte migration inhibition test.

The prevalence of HB_sAg 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_sAg carriers and applied the LMI test in the presence of PHA, purified protein derivative (PPD), crude HB_sAg positive serum and purified HB_sAg, in order to define CMI in HB_sAg carriers.

Materials and Methods:

Serum from one hundred voluntary blood donors was tested for HB_sAg by radio-immuno-assay using Ausria II (Abbott Co.).Carriers were defined as those who were HB_sAg 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_sAg positive serum from persistent carriers and purified HB_sAg(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-

ette 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 HB_sAg carriers is shown in Table 1. A significant decrease of total-T cells was observed in HB_sAg 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 HB_sAg carriers on their lymphocytes. We observed only a diminution of total-T cells in HB_sAg 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 HB_sAg carriers as a percentage. PHA caused inhibition in 59% of HB_sAg 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_sAg 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_sAg carriers and 25% of normal controls (P 0.005), whereas, with purified HB_sAg 35% of carriers and none of the controls gave a positive test result ($P < 0.005$)

Among anti-HB_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_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_sAg 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_sAg 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 inteaction 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 normal lymphocytes. (5, 21).

We have found a reduction of T-cells when the serum of HB_sAg carriers is reacted with their peripheral lymphocytes (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 peripheral blood of HB_sAg carriers.

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

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

The absence of correlation between L.M.I. and the presence of antibody indicates that inhibition of migration is unrelated to the presence of $HB_sAg/anti\text{-}HB_s$ complexes

Table 1:

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

Cells	Normal Controls	HB_sAg Carriers
Tt	73.9% ± 11.35*	49% ± 5.65
Ta	30.4% ± 9.43	28% ± 1.1
EAC	20.2% ± 4.45	16.45% ± 3.2

Tt : Total Cells

Ta : T active cells

EAC : Erythrocyte Antibody Complement

* : Siandard devation

Table 11: The effect of autologus serum from Normal Controls and HB_sAg carriers on their lymphocytes.

	Normal Controls			HB_sAg Carriers		
	Tt	Ta	EAC	It	Ta	EAC
Before serum	73.9 ± 11.35	30.4 ±* 9.43	20.2± 4.45	49 ± 5.65	22 ± 1.1	16.45± 3.3
After serum	68 ± 5.2	29.1 ± 4.2	18.2 ± 3.2	38 ± 2.4	27.1 ± 1.2	15.3 ± 2.1

Tt : Total T-cells

Ta : T-active cells

EAC : Erythrocyte Antibody Complement Cells(B-lymphocytes)

* : Standard devation

Table III: PHA and PPD induced Leukocyte inhibition among Normal Controls and HB_sAg carriers.

Ag	Normal Controls	HB_sAg Carriers
PHA	79 %	59.5 %
PPD	58 %	26.1 %

Ag : Antigen

PHA : Phytohemagolutinin

PPD : Purified Protein Drivitive

'Table IV: The effect of autologus serum and purified HB_sAg induced leukocyte inhibition among Normal Controls and HB_sAg carriers

Ag	Normal Controls	HB_sAg Carriers
20% autologus serum	25%	60.5%
Purified HB_sAg	0%	35 %

Table V: LMT with autologus serum in Anti HB_s Positive and Negative subjects among HB_sAg carriers.

Subjects	Anti HB_s Positive		Anti HB_s Negative	
	Cases	%	Cases	%
Positive LMT* with autologus serum	5	41.66	29	82.85
Negative LMT with autologus serum	7	58.34	6	17.14
Total	12	100	35	100

* LMT = Leukocyte migration fest.

in autologous serum which might have reacted with HB_sAg carrier lymphocytes.

The results we have obtained with the LMI test in HB_s Ag 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 HB_sAg. In contrast our observations are in agreement with Lee et al. (17,18) who found 26% positivity of LMI in the presence of purfied HB_sAg. The major difference between our study and previous reports lies in the use of an appropriate concentration of HB_sAg to increase the sensitivity of the in-vitro detection of CMI response to HB_sAg.

Desaules et al.(7) also employed the LMI test to demonstrate unresponsiveness to purified HB_sAg 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 HB_sAg specific CMI response and concurrent liver damage.

In our opinion the lack of liver damage in most HB_sAg 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.

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