Cellular immune response in acute hepatitis B leading to chronic carrier state

M.G. Morsi[1], S.A. Zaki[1], M.K. Kamal[2], S.A. Sadaka[1] and Y.M. El Shimy[3]

استجابة المناعة الخلوية في التهاب الكبد البائي الحاد المؤدية إلى حالة الحمل المزمن للفيروس

منى جمال مرسي وسعاد علي زكي وكريم محمد كمال وسلامة محمد صدقة ويسري محمد الشيمي

خلاصة : كشفت الدراسات المناعية على مرضى اليرقان عن تغيرات ملموسة في الخلايا التائية المساعفة والخلايا التائية الكابتة بين المصابين بالتهاب الكبد البائي المزمن بالمقارنة بحالات الالتهاب الحاد والمجموعة الشاهدة . ولقد أبدت الحالات المزمنة انخفاضا كبيراً يعتد به في قيمَ الإنترليوكين -2R . أما المستويات المنخفضة بها للإنترفيرون γ فلم تكن ذات فرق يعتد به إحصائيا بالمقارنة بالمجموعة الشاهدة . وسجلت الحالات الحادة أعلى مستويات للإنترليوكين -2R والإنترفيرون γ وبإجراء مقايسة تكوين أرومات اللمفاويات استجابةً للميتوجينات والمستضدات المختلفة ، تبين وجود مجموعتين : مجموعة مستجيبة (الحالات الحادة) وأخرى غير مستجيبة (الحالات المزمنة) . أما المجموعة المستجيبة فقد أبدت استجابات مناعية خلوية نوعية ولا نوعية أكثر . ولم تختلف المجموعتان فيما يتعلق بالاستجابة التكاثرية للمستضد السطحي لفيروس التهاب الكبد البائي ، ولكن ظهرت استجابة قوية لدى المستجيبين تجاه المستضد اللبّي لفيروس التهاب الكبد البائي .

ABSTRACT Immunological studies among jaundiced patients revealed significant changes in T-helper and T-suppressor cells among chronic HBV cases from the acute and control groups. The chronic cases revealed a highly significant decrease in interleukin-2R expression but their low interferon-γ values were statistically nonsignificant from the control group. The acute cases recorded the highest interleukin-2R and interferon-γ values. Lymphocyte blastogenesis assay in response to different mitogens and antigens produced two groups: responders (acute cases) and nonresponders (chronic cases). The responders revealed more intact specific and nonspecific cellular immune responses. Neither group differed with regard to their proliferative response to HBsAg, but vigorous response to HBcAg was a significant feature of the responders.

La réponse immunitaire cellulaire menant à l'état de porteur chronique dans les cas d'hépatite aiguë B

RESUME Les études immunologiques effectuées chez des malades atteints d'un ictère ont révélé des changements importants au niveau des lymphocytes T suppresseurs et des cellules T auxiliaires chez les cas chroniques d'hépatite B par comparaison avec les groupes de cas aigus et de témoins. Chez les cas chroniques, il y avait une diminution très importante de l'expression du récepteur de l'interleukine 2 mais leurs faibles valeurs d'interféron-γ étaient statistiquement non significatives par rapport au groupe de témois. Les cas aigus enregistraient les valeurs les plus fortes en ce qui concerne le récepteur de l'interleukin 2 et l'interféron-γ. Le test de prolifération lymphocytaire en réponse aux différents mitogènes et antigènes a produit deux groupes: les patients répondants (cas aigus) et les patients non-répondants (cas chroniques). Les patients répondants avaient des reponses immunitaires cellulaires spécifiques et non spécifiques plus intactes. Les deux groupes étaient semblables en ce qui concerne leur réponse proliférative aux antigènes de surface de l'hépatite B (HBsAg) mais une réponse vigoureuse aux antigènes centraux de l'hépatite B (HBcAg) était une caractéristique importante chez les patients répondants.

[1]Microbiology Department, Faculty of Medicine, University of Alexandria; [2]NAMRU-3 Cairo Immunology Division; [3]Alexandria Fever Hospital, Directorate of Health, Alexandria, Egypt.

Introduction

The last two decades have witnessed an explosion in knowledge of viral hepatitis, a major public health problem throughout the world affecting several hundred million people. Viral hepatitis is a cause of considerable morbidity and mortality in the human population both from acute infection and the chronic sequelae which include, with at least two types of infection, chronic active hepatitis, cirrhosis and primary liver cancer. Hepatocellular carcinoma is one of the ten most common cancers worldwide [*1*].

Infection with hepatitis B virus (HBV) is a problem of immense dimension with over 200 million people being infected worldwide [2]. The virus is believed to be non-cytopathic. The major features of immunological interest in HBV infection are: that a proportion of those exposed to the virus become chronically infected, and that once chronic infection is established, most will gradually eliminate the nucleocapsid antigen from the liver, while the envelope antigens continue to be expressed. During the elimination of nucleocapsid antigens many patients develop permanent liver damage [*3*].

There are multiple factors and regulatory mechanisms operating to modulate the immune responses during HBV infection. In this respect, the current study was designed to understand the way in which the cellular immune response (presented as T-cells) complemented by the non antigen-specific amplification systems (T-cell cytokines) is involved in the efficient elimination of HBV.

Knowledge gained should not only lead to the design of more effective immunotherapy, but should also allow the construction of the most effective and immunogenic vaccines.

Aim

This study was designed:

1. to delineate the incidence rate of HBV infection among jaundiced cases admitted to Alexandria Fever Hospital compared to other hepatotropic viruses, and to assess the sociodemographic risk factors associated with the presence of HBV markers, and thus to evaluate the scope of the problem of HBV infections in Alexandria, Egypt;
2. to measure the cell-mediated immune competence of cases with acute HBV with a view to early detection of chronic carriers;
3. to investigate defects in the cellular immune elements of chronic HBV cases with a view to future design of an effective immunotherapy.

Subjects

The study was carried out on 302 jaundiced male and female patients admitted to Alexandria Fever Hospital during 1992. A group of healthy relatives were included for control purposes.

Cases were selected after full clinical assessment and history taking. Their ages ranged from 20 to 40 years with a mean of 33.8 years ± 1.5 years.

Extremes of age were not included in order to exclude possible immunological immaturity and waning.

Liver function tests and serological markers of hepatitis were carried out. The results of these tests led to the classification of the study patients into the following groups which were subjected to thorough immunological assessment.

1. Acute hepatitis B virus cases (Group I) comprising patients displaying the following markers:
   - HBs and anti-HBc IgM positive
   - anti-HBc IgM positive only
   - HBs, anti-HBc IgM and anti-delta IgM positive (coinfection of HBV and HDV).
2. Chronic hepatitis B virus cases (Group II) comprising patients who were:
   - HBs positive or
   - HBs and anti-delta IgM positive (HBs-antigen carrier with HDV superinfection

A control group comprising 50 healthy subjects, selected from the close relatives of the patients and of the same socioeconomic status was included in the study. They were chosen to match the age and sex of the study population to serve as negative controls for the immunological tests. These subjects were selected after exclusion of HBV by serological markers (HBs and anti-HBc IgM).

Methods

Every patient was subjected to the following:

- full clinical assessment and history taking;
- liver function tests using commercial kits for serum bilirubin (total and direct), SGOT, SGPT and alkaline phosphatase.
- assessment of serological markers of HBV infection using commercial kits for HBV surface antigen (HBsAg) (third generation ELISA): anti-HBV IgM ELISA formats were used.

Immunological studies were performed for both HBV patients and controls and included [*4,5*]:

- phenotypic analysis of total T-lymphocytes (CD3) and its subpopulations (CD4, CD8) as well as anti-Tac receptors (interleukin IL-2R) using specifically labelled monoclonal antibodies purchased from Behring in an indirect immunofluorescent technique;
- blast transformation assays using a panel of mitogens PHA (phytohaemagglutin), Con A (concanavalin A), PWM (pokeweed mitogen), staphage lysate and antigens (HBs and HBc);
- monitoring of the levels of IL-2 and (interferon) IFN-γ in cell culture supernatant using a bioassay (Con A blast) and an ELISA assay respectively.

For the immunological assays the procedure described by Boym (1988) for mononuclear separation was followed with minor modifications [*6*]. For quantification of IL-2R (Tac antigen)-bearing lymphocytes the procedure of Azogui et al (1983) for induction of IL-2R expression was followed [*7*]. The blastogenesis assay adopted was a modification of Aisenberg (1965) [*8*].

For interleukin-2 bioassay the method applied by Makonkawkeyoon and Kasinrerk (1989) was followed with minor modifications [*9*].

Results and discussion

The incidence rate of acute HBV infection among jaundiced cases included in this study was 39%, while the rate of chronic cases was 11%. Summed together the rate of HBV infection among the studied population was 49.7% (Table 1). Similar high incidence of HBV infection was reported

Table 1 **Classification of the hepatitis study population according to the serology data**

Group	No.	%
1 HAV IgM+ve	8	2.65
2 HBsAg+ve	21	6.95
3 HBsAg + anti-HBc IgM+ve	92	30.46
4 Anti HBc IgM+ve	10	3.31
5 HBsAg + anti-HBc IgM + anti-HDV IgM+ve (co-infection)	16	5.30
6 HBs+ anti HDV IgM+ve (superinfection)	11	3.64
7 Negative all (NANB)	144	47.68
8 Group (3) + Group (4)	102	33.77
9 Group (3) + Group (4) + Group (5)	118	39.07
10 Group (2) + Group (6)	32	10.60

NANB: non-A, non-B

by Omran [*10*] (1991) and Bassily et al (1986) [*11*]. The results obtained in these studies and the current one revealed that almost 50% of virus hepatitis cases admitted to fever hospitals were due to HBV, thus indicative of the magnitude of the problem.

Sociodemographic risk factors

Sex and occupation were the sociodemographic factors showing a very highly significant association ($P < 0.001$) with the presence of HBV markers (Table 2). Among the acute HBV cases, 74.6% were males and 46% were farmers and unskilled and semiskilled urban labourers, whereas the rates among the chronic HBV cases were 84% and 53% respectively; the male predominance among patients with HBV infections was in agreement with earlier reports [*11,12,13*]. This was probably due to occupational and other risk factors leading to the exposure of males in developing areas in Egypt.

Since low socioeconomic status has previously been found to be associated with an increased risk for acquisition of HBV infection, the high unemployment rate among our acute HBV cases was not unexpected.

Biochemical indices of HBV infection

Comparing the biochemical markers of hepatic injury in HBV infection, SGPT and/or TBIL proved to be significant indices for the early assessment of the degree of hepatic insult, being higher in the acute than in the chronic HBV studied patients (Table 3).

Immunological findings: phenotypic analysis of T-cells and subsets

In the current study, the frequencies of T-helper and T-suppressor subsets in reference to the total T cell population were monitored using specifically labelled monoclonal antibodies in an indirect immunofluorescent technique. The analysis revealed a nonsignificant decrease ($P > 0.05$) in total T-lymphocytes (CD3+) among the acute, and chronic HBV cases compared to the controls. Also CD4+ and CD8+ populations were nonsignificantly different among the acute cases as compared to the control subjects. However, a highly significant ($P < 0.001$) decrease in the CD4+ in chronic cases compared to the acute cases and controls was observed. The frequencies of CD8+ cells in such patients were subsequently elevated. The decreased T-helper subset and the increased T-suppressor subset observed in the chronic cases resulted in a diminished T-helper to T-suppressor ratio of 1:1 compared with 2:1 observed in the acute and control groups (Table 4).

In accordance with our study, Abdel-Ghaffar (1990) and Ahmed (1990) in similar studies reported significant increase in

Table 2 **Evaluation of the sociodemographic risk factors associated with the presence of HBV markers**

Risk factors	Groups studied						χ^2	Degrees of freedom	*P*
	Control (n = 50)		Acute (n = 118)		Chronic (n = 32)				
	No.	%	No.	%	No.	%			
Sex									
Male	25	50	88	74.6	27	84.4	18.4	3	<0.001
Female	25	50	30	25.4	5	15.6			
Residence									
Urban residence	33	66	78	74.6	21	65.6	9.5	3	<0.05
Rural residence	17	34	40	25.4	10	34.4			
Occupation									
Low risk[a]	25	50	54	46	14	44	34.3	6	<0.001
Intermediate risk[b]	10	20	54	46	17	53			
High risk[c]	15	30	10	8	1	3			
Previous history of jaundice									
No	50	100	111	94	24	75	10.9	2	<0.01
Yes	–		7	6	8	25			
Family history of jaundice									
No	–		108	91.5	27	84.4	6.1	2	<0.05
Yes	–		10	8.5	5	15.6			
History of schistosomiasis									
No	50	100	87	73.7	19	59.4	8.4	2	<0.05
Yes	–		31	26.3	13	40.6			
Clinical severity									
Mild	–		37	31.4	13	40.6			
Moderate	–		38	32.2	9	28.1	3.3	4	>0.05
Severe jaundice	–		43	36.4	10	31.3			
Smoking									
No	23	46	67	56.8	13	40.6	2.9	2	>0.05
Yes	27	54	51	43.2	19	59.4			
Alcohol intake									
No	50	100	112	94.9	31	96.9	0.2	2	>0.05
Yes	–		6	5.1	1	3.1			
History of surgical operations									
No	50	100	104	88.1	28	87.5	0.9	2	>0.05
Yes	–		14	11.9	4	12.5			
Needle injections									
No	40	80	81	68.6	19	59.4			
Yes	10	20	28	23.7	9	28.1	5.2	4	>0.05
Addict	–		9	7.7	4	12.5			

[a] *Housewives and unemployed personnel*
[b] *Farmers and unskilled and semiskilled urban labourers*
[c] *Medical and paramedical personnel*

Table 3 **Score of liver function tests among the study populations**

Liver function tests	Studied cases	Mean ± s	Range	F ratio	P
SGOT	Acute	157.1 ± 76.7	(35–429)	2.7	>0.05
	Chronic	130.2 ± 57.1	(50–280)		
SGPT	Acute	212.1 ± 122.7	(50–800)	7.1	<0.05
	Chronic	149.9 ± 77.8	(50–400)		
TBIL	Acute	7.9 ± 4.8	(0.2–18)	3.7	<0.05
	Chronic	5.4 ± 3.2	(1.2–14)		
DBIL	Acute	6.9 ± 4.2	(0.2–17)	2.9	>0.05
	Chronic	4.8 ± 3	(0.8–13)		
AP	Acute	16.4 ± 6.3	(5–40)	2.2	>0.05
	Chronic	15 ± 4.3	(8–25)		

s – standard deviation; P < 0.05 – significant
AP: alkaline phosphatase; DBIL: direct bilirubin; TBIL: total bilirubin
Chronic cases n = *32; acute cases* n = *118*

Table 4 **Phenotypic profile of peripheral blood T cells and IL-2 receptor positive cells among study populations**

Positive cells (%)	Groups studied			F ratio	P
	Control (*n* = 50)	Acute (*n* = 88)	Chronic (*n* = 26)		
CD3					
mean ± SE	61.1 ± 8.6	58.6 ± 6.2	54.7 ± 10.7	2.5	>0.05
range	41–82	25–85	34–75		
CD4					
mean ± SE	41.1 ± 5.8	39.1 ± 4.1	30 ± 5.9	11.5	<0.001
range	29–66	12–60	12–50		
CD8					
mean ± SE	19.9 ± 2.8	20.5 ± 2.2	25.8 ± 3.2	11.6	<0.001
range	10–27	8.5–35	10–45		
CD4:CD8	2.1 ± 2.1	1.9 ± 1.9	1.16 ± 1.8		<0.001
IL-2R					
mean ± SE	50.1 ± 7.1	52.9 ± 5.6	24.2 ± 4.7	66.7	<0.001
range	30–75	25–75	12–42		

SE = *standard error*

Table 5 **Analysis of T-cell receptor expression levels, IL2 and IFN-γ values among the study populations**

Groups	CD3+ (%)	CD4+ (%)	CD8+ (%)	IL-2R+ (%)	IL-2 (μ/ml)	IFN-γ (μ/ml)
Acute–control						
D	–2.5	–2	0.66	2.9	92.7	32.6
P	>0.05	>0.05	>0.05	>0.05	<0.05*	<0.001*
Chronic–control						
D	–6.3	–11.1	5.9	–25.9	–445.6	–5.7
P	>0.05	<0.001*	<0.001*	<0.001*	<0.001*	>0.05
Acute–chronic						
D	3.8	9.1	–5.3	28.8	538.3	38.3
P	>0.05	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

D = *difference of means;* P = *probability*
Acute n = *88; chronic* n = *26; control* n = *50*
* *Significant values*

the frequency of T-suppressor subsets in their studied cases [*14,15*]. Evidence that specific T-suppressor cells present in the circulation of patients with HBsAg positive chronic hepatitis prevent both anti-HBs antibody production *in vitro* [*16*] and T-LIF release by T lymphocytes sensitized by HBsAg [*17*] were reported. Whether these T suppressor cells appeared early in the course of the infection and contributed to the development of the HBV chronic carrier state or were secondary to the HBsAg overload present in chronically infected subjects was unknown.

Cytokines findings, IL-2 and IL-2R

When our patients' lymphocytes were stimulated *in vitro* with PHA, the IL-2 levels in culture supernatants and the frequency of IL-2R-bearing cells revealed low values among the chronic group as compared to the control and acute cases and the decrease was highly significant ($P < 0.001$). In contrast the frequency of IL-2R-bearing cells displayed a nonsignificant increase ($P < 0.05$) among the acute cases as compared to the controls. However, induced IL2 levels in the former population were significantly ($P < 0.05$) increased (Table 5). Our data were in accordance with Ozeki et al (1990) who reported highly significant ($P < 0.001$) low IL-2 values among their studied chronic HBV patients [18]. Also, Abdel-Ghaffar (1990) reported a significant decrease of both IL-2 levels and IL-2R among chronic HBV cases associated with schistosomiasis [*14*].

Saxena et al (1985) also reported similar observations [19]. They attributed the decrease to a central defect in the response to IL-1 resulting in an insufficient expression of IL-2 receptors. This was evident by the still consistently impaired proliferative response when exogenous IL-2 was included in the in-vitro cultures. Furthermore and although IL-2 production was decreased, exogenous IL-2 or IL-1 was unable to correct the low proliferative response, evidenced by Anastasskos et al (1987) [*20*]. In the present study, we failed to demonstrate

Table 6 **Levels of IL-2 and IFN-γ secreted by PHA-stimulated PBMNC from the study subjects**

Cytokine (μ/ml)	Groups studied			F ratio	P[a]
	Control (n = 50)	Acute (n = 88)	Chronic (n = 26)		
IL-2					
mean ± SE	555 ± 78.5	647.9 ± 69.1	109.6 ± 21.5	97.6	<0.001
range	150–1000	400–1000	7.5–800		
IFN-γ					
mean ± SE	14.6 ± 2.1	47.3 ± 5	8.9 ± 1.7	94.9	<0.001
range	10–22	10–80	2.5–80		

[a] *Denoting the differences between the three study populations*

a correlation between IL-2 levels in the culture supernatants and the frequency of IL-2 receptor (Tac antigen)-bearing cells of the same cultured cells among all HBV cases studied, acute and chronic. Abdel-Ghaffar (1990) reported similar observations [*14*]. This might be due to the fact that IL-2 receptors are of two types, high affinity and low affinity receptors. Both react and are monitored with the Tac monoclonal antibody following different experimental manipulations for each receptor type. An adequate number of high affinity receptors are mandatory for the mitogenic action of IL-2 [*21*].

Interferon-γ findings

The data on IFN-γ production by PHA stimulated peripheral blood mononuclear cells from the studied patients revealed a highly significant ($P < 0.001$) increase among the acute HBV group as compared to the healthy subjects and chronic cases (Table 6). However, IFN-γ production among the chronic cases revealed a highly significant decrease ($P < 0.001$) in comparison to the acute cases, yet statistically non-significant ($P > 0.05$) when compared to the controls (Table 6). The results published by Kakumu et al (1989) [*22*], Inowa et al (1989) [*23*], Fuji et al (1987) [*24*], Ikeda et al (1986) [*25*] and Abb et al (1985) [*26*] were in accordance with the present data. The findings of defective IFN production in patients with chronic HBV infection reported in our study and by other investigators had led to the hypothesis that this might be a primary defect which could have been instrumental in the early stages of infection in permitting continued viral infection. These have led to the speculation that a defect in the ability to produce sufficient IFN during acute viral hepatitis may lead to chronic infection. Our data enabled us to suggest that IFN levels were statistically highly correlated ($P < 0.001$) to IL-2 levels. Both cytokines increased in acute HBV cases and control subjects, and both decreased in the chronic cases (data not presented).

The release of IL-2 by Th1 cells seemed to be the main stimulus for the sequential synthesis of IFN-γ. When exogenous IL-2 was added to peripheral blood lymphocytes (PBL) in culture, there was a significant increase in the amount of IFN-γ [*23*]. When both cytokines were correlated to Th subsets they were statistically insignificant.

Table 7 **Multiple comparison (Scheffe's method) of means of blastogenic responses to various antigens tested among the study populations**

Group	Blastogenic response of PBMNCs to								
	SPL			HBc			HBs		
	E-cpm	D-cpm	SI	E-cpm	D-cpm	SI	E-cpm	D-cpm	SI
Acute–control									
D	8 628	–7 832	14	2 685	2 880	4.4	40	–161	0
P	>0.05	>0.05	>0.05	<0.001*	<0.001*	<0.00	>0.05	>0.05	>0.05
Chronic–control									
D	46 599	–46 551	–41	161	233	0.1	250	–144	–0.1
P	<0.05*	<0.05*	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Acute–chronic									
D	38 572	38 720	55	2 874	2 647	4.3	211	17	0.1
P	>0.05	<0.05*	>0.05	<0.001*	<0.001*	<0.00*	>0.05	>0.05	>0.05

D = *difference of means;* P = *probability*
SPL: Staphage lysate
E-cpm: mean counts per minute, D-cpm: delta counts per minute; SI: stimulation index
Acute n = *88; chronic* n = *26; control* n = *50*

This was explained by the fact that measurement of Th subset in the present study did not specifically identify Th1, the main inducer of IFN-γ and IL-2.

So, not only the phenotypic analysis of Th subsets was a must but also their functional differences should be studied to confirm the hypothesis proposed that defective Th1 was the main element underlying viral persistence in chronic HBV infections.

Functional analysis of T cells

The proliferative response of peripheral blood lymphocytes to nonspecific (mitogens) and specific stimuli (HBV envelope and core antigens; HBs and HBc antigens) was analysed in the studied subjects. This was aimed at better understanding of the cellular mechanisms responsible for the clearance of HBV, development of hepatocellular injury and evolution towards chronicity of HBV infection. The most evident and relevant finding was the observation that HBV-infected subjects who developed self-limited acute hepatitis showed a much stronger peripheral blood mononuclear cell (PBMNC) response to HBcAg than did the chronic cases. The differences between the mean cpm, Delta-cpm and SI peaks in the two population groups were highly significant ($P < 0.001$) (Table 7). Patients with acute HBV showed high levels of lymphocyte sensitization to HBcAg whereas patients with chronic HBV infection displayed non-significant levels of response to this antigen.

A comparison of the magnitude of lymphocyte response to HBV core antigen and envelope antigen in patients with acute hepatitis B, revealed a dramatically stronger ($P < 0.001$) HBcAg-driven lymphocyte responsiveness (Table 7). HBV core antigen appeared to be much more stimulatory than HBV envelope antigen with respect to activation of PBMNCs during acute HBV infection. This might result from a higher intrinsic immunogenicity of HBcAg, as recently demonstrated in the murine system

Table 8 **Multiple comparison (Scheffe's method) of means of blastogenic responses to various mitogens tested among the study populations**

Group	Blastogenic response of PBMNCs to								
	PHA			Con A			PWM		
	E-cpm	D-cpm	SI	E-cpm	D-cpm	SI	E-cpm	D-cpm	SI
Acute–control									
D	39 924	–40 810	–54	17 456	–17 181	64	19 388	–19 193	–19
P	<0.001*	<0.001*	>0.05	>0.05	>0.05	>0.05	<0.01*	<0.01*	>0.05
Chronic–control									
D	66 738	–66 325	–131	–61 797	–61 348	–12	–41 461	–41 068	–52
P	<0.001*	<0.001*	<0.01	<0.001*	<0.001*	>0.0	<0.001*	<0.001*	<0.05*
Acute–chronic									
D	26 814	25 515	77	44 341	44 203	184	22 703	21 874	33
P	<0.01*	<0.01*	>0.05	<0.001*	<0.001*	>0.05	<0.01*	<0.01*	>0.05

PHA: phytohaemagglutinin; Con A: concanavalin A; PWM: pokeweed mitogen
E-cpm: mean counts per minute; D-cpm: delta counts per minute; SI: stimulation index
Acute n = 86; chronic n = 26; control n = 50
D = *difference of means;* P = *probability*

[27]. It might even reflect the final balance between suppressive and stimulatory influences operative during HBV infection [28]. Reporting similar results, Ferrari et al (1990) assumed that the result obtained with exogenous HBcAg mostly reflected the behaviour of a CD4+, class II restricted T cell population preactivated *in vivo* by HBcAg recognition [8]. The possibility which followed was that of temporal association between core-specific T cell response and envelope-specific B cell response via a direct cell–cell interaction of HBcAg specific T cells with envelope specific B cells through the mechanism described by Milich et al [27]. In addition to this mechanism, HBV envelope-specific T and B cell responses might be amplified by the release of antigen-nonspecific lymphokines by activated core specific T cells. This phenomenon might be particularly relevant within the microenvironment of specific compartments (such as liver, lymph nodes) where HBV-specific T and B cells were likely to be more concentrated [8]. If these hypotheses were correct, it would follow that the T cell response to HBcAg could contribute *in vivo* to HBV clearance by amplifying and accelerating the development of the neutralizing anti-envelope immune response. Of course, the absence of a detectable T cell response to HBV envelope antigen in acute hepatitis did not mean that this response was not needed for HBV clearance because all patients with uncomplicated acute infection eventually developed anti-envelope antibody responses (which were protective and virus neutralizing), whereas patients with chronic HBV infection did not produce detectable levels of anti-HBs antibodies [8]. Therefore the temporal association between T cell response to HBcAg and clearance of HBsAg suggested the possibility that the T cell response to nucleocapsid antigen represented an additional factor cooperating with the anti-envelope immune response to bring about viral clearance.

Apparently in line with this possibility, PBMNCs from patients with chronic HBV infection who did not clear the virus appeared to be completely unresponsive to HBcAg with very highly significant ($P < 0.001$) lower values than that expressed by cells from patients who developed a self-limited acute hepatitis. However, with respect to this finding we cannot exclude the possibility that the poor cellular immune response detectable in the peripheral blood during chronic HBV infection was a consequence of the selective presence of HBcAg-specific T cells within the liver, as suggested by previous studies [29]. In this case, the different peripheral PBMNCs response to HBcAg between patients with acute and chronic HBV infection would reflect different distributions of HBcAg-responsive T cells rather than a specific defect of the immune system responsible for the inability to eradicate HBV infection. This would also explain the apparently intriguing observation that patients with chronic HBV infections showed levels of serum anti-HBc antibodies (total) higher than patients with acute infection as reported by other workers, even though the peripheral blood T cell response to HBc Ag was much stronger in the latter population of patients. This apparent discrepancy, however, could also be explained assuming that the higher level of anti-HBc antibodies detected in chronic patients were the result of a chronic exposure of B cells to HBcAg, which could also be a T cell-independent B cell immunogen [27].

When each group studied was compared to the other regarding the lymphoproliferative responses to the various mitogens test ed (Tables 7 and 8) the acute HBV group response was nonsignificantly decreased when compared to the control group using the SI. In contrast, the chronic HBV population displayed significantly decreased ($P < 0.05$) values in response to PHA and PWM. Although their response to Con A and SPL was significantly decreased by cpm, yet their SI values were not affected ($P > 0.05$) (Tables 7 and 8). The nonsignificant SI values among the studied cases were attributed to high base line DNA synthesis in PBMNCs of HBV infected patients as a result of HBV active replication. Similar results were reported in many studies [*15,30*].

Conclusion

Although variable degrees of immunodepression were manifested among HBV subjects in the current study, a valid conclusion of overall depressed cell-mediated immunity in the chronic infection group was obtained. This was, in part, evidenced first by diminished numbers of peripheral blood T helper/inducer lymphocytes as detected by indirect immunofluorescent technique. Second, generalized hyporesponsiveness was apparent when mitogenic and antigenic blastogenesis data of chronically infected patients were compared to those of the acute HBV subjects and healthy controls. Third, significantly decreased IFN-γ. IL-2 levels and IL-2R expression among the chronic HBV subjects as compared to those with acute self-limiting HBV infection. may lead to feedback suppression of the normally functioning cells. So, by comparing the two groups, data of the responders (acute self-limiting HBV infected subjects) would reveal more intact specific and nonspecific blastogenic responses, higher IL-2 and IFN-γ levels and elevated frequency of IL-2 receptor positive cells. T helper:T suppressor balances of the responders were towards the helper effect, while towards the suppressor effect in the nonresponders (chronic HBV infected subjects).

Neither group differed in regard to their proliferative response to HBsAg, whereas the vigorous response to HBcAg was a significant feature of the responders. The responders were followed up 6 months later where 50% were able to come back for further investigations. They were evaluated serologically and immunologically. All proved to be clinically healthy, serologically negative for HBs and their immunological parameters comparable to the healthy controls. So, the recovery rate of our responders was 100% and this was anticipated from the early immunological investigations done for them.

Recommendation

From this study we recommend the need to investigate the role of IFN and or IL-2 as immunotherapy regimens in chronic HBV infections. Antibody responses as well as cellular immune responses, effector and regulatory functions, and soluble mediators of such responses are to be studied. Meanwhile, there is an important need for studies on the various aspects of the immune response to HBV antigens (s, c, e, $pres_1$, $pres_2$) x-gene products and polymerase enzyme in chronic HBV cases and carriers, with emphasis on identifying the responses that contribute to protective immunity and those that may be manifested in immunopathology. Such activities should provide information important to the needs of the WHO vaccine development programme.

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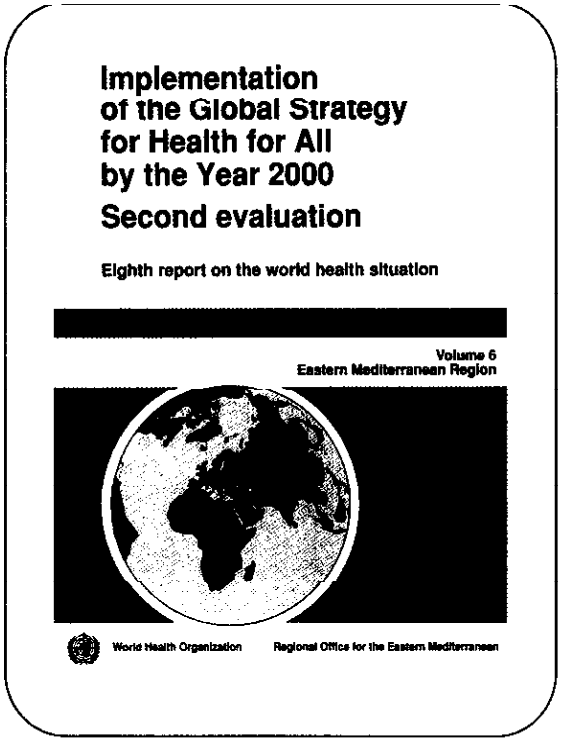

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