

## Regulation of the neutralizing anti-hepatitis B surface (HBs) antibody response *in vitro* in HBs vaccine recipients and patients with acute or chronic hepatitis B virus (HBV) infection†

W. O. BÖCHER, S. HERZOG-HAUFF, W. HERR, K. HEERMANN\*, G. GERKEN, K.-H. MEYER ZUM BÜSCHENFELDE & H. F. LÖHR I. Department of Internal Medicine, Johannes-Gutenberg University, Mainz, and \*Department of Medical Microbiology, Georg-August-University, Göttingen, Germany

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### SUMMARY

Antibodies directed to the HBs antigen indicate viral clearance and the development of life-long immunity in patients that recovered from HBV infection. In HBs antigen vaccine recipients anti-HBs antibodies provide protective immunity. However, little is known about the regulation of this HBs-specific antibody response. The existence of anti-HBs-secreting B cells was demonstrated using the highly sensitive ELISPOT technique compared with conventional ELISA in serum and cell culture supernatants. In the peripheral blood of patients with acute self-limited hepatitis B, HBs-specific B cells were demonstrated with a high frequency despite undetectable anti-HBs serum antibodies. HBV-immunized patients that had recovered from infection and vaccine recipients had significantly lower frequencies, whereas chronic HBV carriers and negative controls showed no anti-HBs-secreting B cells. Coculture experiments of isolated B and T cells revealed that the anti-HBs antibody response was restricted to the presence of T helper cells, but not to identical HLA class II molecules. Allogeneic T cells derived from vaccine recipients or chronic HBV carriers stimulated the HBs-specific B cell response in HBs vaccine recipients. Otherwise, isolated T helper cells could never provide sufficient help to induce the HBs-specific B cell response in chronic HBV carriers. Furthermore, peripheral blood mononuclear cells (PBMC) of six out of 10 vaccine recipients, one out of five HBV-immunized patients, but of no chronic HBV carrier showed a proliferative response to different HBs antigen preparations. This study demonstrated a high frequency of circulating anti-HBs-producing B cells in the early phase of acute HBV infection, but a lower frequency of HBs-specific B cells years after resolution of HBV infection. In chronic HBV carriers, however, deficient HBs-specific T and B cell responses were observed.

**Keywords** anti-HBs antibodies ELISPOT cellular response immunoregulation HBs vaccine

### INTRODUCTION

The outcome of acute HBV infection might depend on the host immune response to HBV antigens. Cytotoxic T cells recognize intracellularly processed peptide antigens that are presented by HLA class I molecules of the MHC on infected hepatocytes. These cells may be crucial for the hepatocellular damage and virus elimination in acute hepatitis B [1–3]. Extracellular viral antigens are bound by anti-HBs antibodies, indicating acquired immunity after viral clearance in the case of self-limited disease. The chronic carrier state, however, is characterized by virus persistence, a

lower frequency of cytotoxic T cells to HBV structural antigens, and the lack of anti-HBs antibodies in the serum [2,4,5].

Vaccination with the recombinant HBV surface antigen (rHBs) leads to the development of protective immunity in more than 90% of vaccine recipients with the occurrence of neutralizing anti-HBs antibodies and HBs-specific T cells [6–8]. The mechanisms preventing the development of a sufficient immune response in chronic HBV carriers and non-responders to HBs vaccine are not known. For non-responders an association with the MHC haplotypes HLA-DR3,7, in particular, with the extended haplotypes HLA-B8,SC01,DR3 or HLA-B44,FC31,DR7 was demonstrated [9,10]. In addition, an association of the HLA class II allele DRB1\*1302 with protection against persistent HBV infection was shown [11]. Recent studies have suggested defective T and B cell functions [12,13]. A general problem of these studies was the low sensitivity of the *in vitro* assays used to detect HBs-specific

† Dedicated to the 80th birthday of Professor P. Schoelmerich, Mainz, Germany.

Correspondence: Dr H. F. Löhr, I. Department of Internal Medicine, Johannes-Gutenberg University, Langenbeckstraße 1, 55131 Mainz, Germany.

antibody production [12]. Thus, the aims of our study were: (i) to establish a sensitive *in vitro* assay for the detection of anti-HBs-producing B cells; (ii) to analyse the HBs-specific humoral immune responses in vaccine recipients, HBV-immunized individuals and patients with acute or chronic HBV infection; (iii) to correlate HBs-specific humoral and cellular immune responses; and (iv) to study the T cell dependence of anti-HBs production *in vitro*.

## PATIENTS AND METHODS

### Patients

Lymphocytes were taken from 11 healthy blood donors, who had responded to vaccination with yeast-derived recombinant HBsAg (Engerix-B;  $3 \times 20 \mu\text{g}$  intramuscular doses; SmithKline Beecham, München, Germany) more than 2 years ago (primary anti-HBs levels  $>500 \text{ U/l}$ , anti-HBc<sup>-</sup>). In addition, five chronic HBV carriers (HBsAg<sup>+</sup>, anti-HBs<sup>-</sup>, anti-HBc<sup>+</sup>), four patients with acute HBV infection (HBsAg<sup>+</sup>, anti-HBs<sup>-</sup>, anti-HBc-IgM<sup>+</sup>) and seven HBV-immunized patients who had resolved HBV infection more than 1 year ago (HBsAg<sup>-</sup>, anti-HBs<sup>+</sup>, anti-HBc<sup>+</sup>), four of them with additional markers of chronic HCV infection, were also enrolled in this study. Four persons without markers of HBV infection or vaccination served as negative controls (HBsAg<sup>-</sup>, anti-HBs<sup>-</sup>, anti-HBc<sup>-</sup>). Chronic hepatitis B or C were diagnosed by histological, virological and clinical data. HBV and HCV markers were determined using commercially available enzyme immunoassays (Abbott GmbH, Wiesbaden, Germany). Anti-HBs antibodies in serum and cell culture supernatants were quantified using the Abbott Ausab assay according to the manufacturer's instructions, and results were expressed as U/l. Serum autoantibodies (ANA, AMA, LKM, SLA) were detected by specific radioimmunoassays and immunoblots to exclude patients with autoimmune hepatitis [14]. All patients were negative for serological markers of HIV infection and gave informed consent to the experiments according to the Helsinki Declaration of ethical guidelines.

### HBV antigens

One HBsAg preparation (pHBs) containing HBs, preS1 and preS2 sequences was purified from the plasma of patients with chronic HBV infection of adw subtype, that is known to be most common in Western Europe [15]. A recombinant HBsAg preparation (rHBs) derived from yeast cultures containing exclusively HBs was kindly provided by B. Hoffstedt (MSD, Sharp & Dohme, München, Germany). In addition, a recombinant preS1-antigen (kindly provided by W. Gerlich, Gießen, Germany) and a 24 amino acid long preS2-peptide had been used to stimulate B and T cells [4,16].

### Mononuclear cells, separation of B cell and T cell fractions

Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll density gradient centrifugation [17]. After standard rosetting procedure of PBMC with sheep erythrocytes, CD4<sup>+</sup> T cells were isolated from the rosette-forming cells (E<sup>+</sup>) using immunomagnetic beads coupled with anti-CD4 antibodies (DynaL, Malmö, Sweden). B cells were separated from the non-rosetted fraction of PBMC (E<sup>-</sup>) using immunomagnetic beads coupled with anti-CD19 antibodies (DynaL). After incubation, the beads were removed from separated cell populations using a commercially available 'Detach a bead-kit' according to the manufacturer's instructions (DynaL).

Flow cytometry analysis revealed that the separated B cell

population contained  $<2\%$  CD3<sup>+</sup> T cells, whereas separated T cell fractions contained about 95% CD4<sup>+</sup> T cells.

### Anti-HBs ELISPOT

To analyse anti-HBs-secreting B cells quantitatively *in vitro*, the ELISPOT technique was used as described, with some modifications [18]. Ninety-six-well microtitre plates with hydrophobic PVDF membrane bottoms (Millipore Multiscreen IP; Eschborn, Germany) were coated overnight with  $100 \mu\text{l/well}$  of a solution with  $5 \mu\text{g/ml}$  rHBs antigen in a coating buffer (sodium bicarbonate, sodium carbonate and sodium azide in distilled water). Medium controls or wells coated with irrelevant antigen (ovalbumin  $1 \mu\text{g/well}$ ) were used as negative controls. The plates were washed four times with distilled water followed by post-coating with RPMI 1640 medium supplemented with 3% bovine serum albumin (BSA) for 1 h at 37°C. Triplicates of mononuclear cells were seeded at different concentrations (PBMC:  $5 \times 10^5$ ,  $2.5 \times 10^5$  and  $1.2 \times 10^5$  per well; B cells:  $3 \times 10^4$ ; T cells:  $1.5 \times 10^5$  per well) in RPMI 1640 medium containing 3% BSA and  $5 \mu\text{g/ml}$  pokeweed mitogen (PWM; Seromed, Wertheim, Germany). After incubation overnight at 37°C and 5% CO<sub>2</sub>, the plates were washed in PBS containing 0.25% Tween and incubated with a horseradish peroxidase-linked polyvalent murine anti-human IgG, IgA, IgM antibody (1:1000; Dako, Hamburg, Germany) or monovalent anti-human IgG antibody (1:1000; Dako) for 1 h at room temperature before the dacarbazole-containing substrate solution was added. After 20 min the reaction was stopped by rinsing water and only granular dark red spots were automatically enumerated using an electronic computer-assisted imaging system (Leitz, Wetzlar, Germany) [19]. In addition, the results were checked by eye using a dissection stereomicroscope (Zeiss SV-6; Oberkochen, Germany). All results were expressed as means of triplicates from numbers of spot-forming cells (SFC) per  $10^6$  PBMC, or as SFC per  $10^5$  B cells where indicated.

### Anti-HBs secretion in cell culture supernatants

To analyse anti-HBs antibody secretion *in vitro*  $1.5 \times 10^6$  PBMC were washed twice and cultured in the presence or absence of  $5 \mu\text{g/ml}$  PWM (Seromed) in 1 ml RPMI 1640 supplemented with 5% fetal calf serum (FCS), 1% glutamine, 0.1% HEPES buffer and 0.1% gentamycin in 24-well flat-bottomed plates (Falcon/Becton Dickinson, Heidelberg, Germany) for 8–10 days in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C [20,21]. The supernatants were harvested, filtered and stored at  $-20^\circ\text{C}$  before anti-HBs antibodies were determined using the commercially available assay (AUSAB EIA; Abbott Labs, Wiesbaden, Germany). All results are given as means from triplicates.

### PBMC proliferation assay

Proliferative T cell responses were determined using a standard <sup>3</sup>H-thymidine uptake assay [22,23]. In detail,  $1 \times 10^5$  PBMC were incubated in triplicates together with different antigen concentrations in 96-well microtitre plates (Greiner, Frickenhausen, Germany) for 6 days. The antigen concentrations were: 0.05, 0.1 and 0.2  $\mu\text{g/well}$  for pHBs/rHBs antigens, 0.1, 0.5 and 2.5  $\mu\text{g/well}$  for preS1 antigen, and 3, 10 and 30  $\mu\text{g/well}$  for preS2 peptide. Then the cultures were pulsed with 0.25  $\mu\text{Ci}$  <sup>3</sup>H-thymidine, and 18 h later the incorporated radioactivity was measured by liquid scintillation technique. The test was performed in RPMI 1640 (Gibco, Eggenstein, Germany) medium supplemented with 5% heat-inactivated human AB serum, 1% glutamine, 0.1% HEPES buffer, 0.1%

streptomycin and 0.1% gentamycin. Medium served as negative, phytohaemagglutinin (PHA)-induced cultures as positive controls. The HLA restriction of the T cell response was assessed by blocking experiments using anti-HLA-DR antibodies. Results are expressed as mean stimulation indices (SI = (ct/min stimulated cells)/(ct/min unstimulated cells)) of triplicates  $\pm$  s.d. SIs higher than three times the medium controls were regarded as positive.

#### Statistical analysis

All results are given as means from triplicates and s.d. *P* values were calculated using Student's *t*-test for impaired data. *P* < 0.05 was regarded as statistically significant.

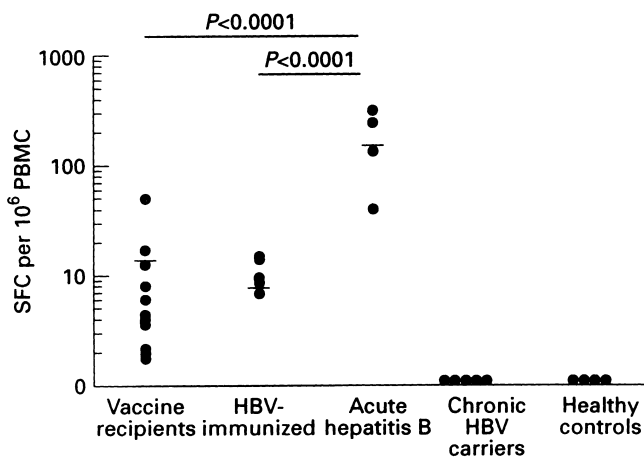
## RESULTS

Using the ELISPOT technique, anti-HBs antibody secretion by specific B cells was quantitatively assessed. PBMC from 11 of 11 vaccine recipients, seven of seven HBV-immunized patients, and four of four patients with acute self-limited hepatitis B, but none of five chronic HBV carriers or four healthy blood donors showed anti-HBs antibody SFC within the peripheral blood (Fig. 1).

The numbers of HBs-specific SFC were significantly higher in four of four patients with acute hepatitis B infection ( $184.5 \pm 103$  SFC/ $10^6$  PBMC) than in HBV-immunized patients years after clearance of the HBV infection ( $8.4 \pm 4.4$  SFC/ $10^6$  PBMC; *P* < 0.0001) and in HBs vaccine recipients ( $10.2 \pm 13.4$  SFC/ $10^6$  PBMC; *P* < 0.0001; Table 1).

To analyse the specificity of the ELISPOT assay, anti-HBs antibody levels were studied in the sera of patients and controls. All anti-HBs antibody-seropositive vaccine recipients (11/11) and HBV-immunized patients (7/7), but none of four anti-HBs-seronegative controls (0/4) showed HBs-specific SFC *in vitro*. However, serum antibody levels did not correlate with the antibody-producing cells *in vitro*. In contrast, the four patients with acute self-limited hepatitis showed high frequencies of HBs-specific SFC *in vitro* although they were still anti-HBs-seronegative (Fig. 2).

Numbers of SFC with the anti-HBs ELISPOT assay in selected



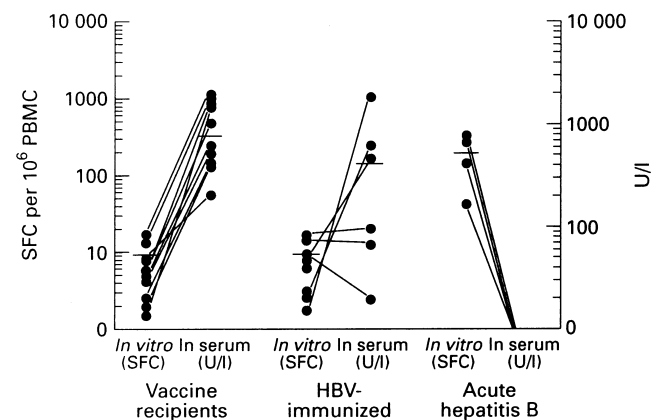
**Fig. 1.** Frequency of anti-HBs-producing B cells within peripheral blood mononuclear cells (PBMC) measured by ELISPOT technique and expressed as spot-forming cells (SFC) per  $10^6$  PBMC in HBs vaccine recipients, HBV-immunized individuals and patients with acute or chronic HBV infection.

individuals were compared with anti-HBs antibody secretion in the cell culture supernatants determined by ELISA. Two from five vaccine recipients but none of three patients with acute hepatitis B infection showed anti-HBs antibodies in the cell culture supernatants, although they showed significant numbers of anti-HBs antibody-producing B cells in the ELISPOT assay (Table 2). No differences in anti-HBs antibody levels could be demonstrated between PWM-stimulated or non-stimulated cultures of the two positive vaccine recipients (15 versus 16 U/l and 6.5 versus 6.7 U/l).

The regulation of anti-HBs-producing B cells *in vitro* was studied by culturing separated T and B cells under different conditions. Isolated T or B cell cultures from vaccine recipients or chronic HBV carriers showed no HBs-specific SFC *in vitro*. However, when cultured together, the T lymphocytes from five vaccine recipients could stimulate the autologous or allogeneic anti-HBs-producing B cells derived from five vaccine recipients irrespective of a match or mismatch in the HLA-DR locus (Fig. 3).

Furthermore, in three patients the T cells from vaccine recipients did not stimulate anti-HBs production by B cells derived from chronic HBV carriers. Otherwise, T cells from chronic carriers were able to stimulate anti-HBs production of B cells derived from five vaccine recipients (Fig. 4).

To analyse whether T cell-dependent anti-HBs antibody production was related to HBV-specific T cell stimulation, the proliferative response of PBMC to a panel of different HBs antigen preparations was studied. PBMC from six of 10 vaccine recipients and from one of five HBV-immunized patients showed a detectable proliferative T cell response (SI > 3) to the recombinant HBsAg preparation (SI  $9.5 \pm 12$  and  $2.0 \pm 1.4$ ) and, in the majority, to the plasma-derived HBs antigen preparation (SI  $6.0 \pm 7.0$  and  $6.6 \pm 10.7$ ). One HBV-immunized patient was also responsive to the recombinant preS1 antigen preparation (SI 9.0). PBMC from two of four patients with acute hepatitis B reacted with the plasma-derived HBs antigen (SI 3.5 and 3.3), but not with the recombinant HBs antigen preparation. One of these PBMC also responded to the recombinant preS1 antigen. Neither the three chronic HBV carriers nor the four healthy blood donors showed a proliferative T cell response to any of the HBs antigen preparations (Fig. 5, Table 1).



**Fig. 2.** Comparison of anti-HBs production *in vivo* (serum levels in U/l) and *in vitro* expressed as spot-forming cells (SFC/ $10^6$  peripheral blood mononuclear cells (PBMC)) in individual vaccine recipients, HBV-immunized individuals and patients with acute HBV infection.

**Table 1.** Patient characteristics of different groups and summary of *in vitro* data

Characteristics	HBs vaccine recipients	HBV-immunized individuals	Acute hepatitis B	Chronic hepatitis B	Healthy controls
Sex (M/F)	4/7	4/3	2/2	5/0	3/1
Age (years; mean $\pm$ s.d.)	30.5 $\pm$ 7.1	50.1 $\pm$ 19.3	28 $\pm$ 15	37.2 $\pm$ 13.8	48.8 $\pm$ 14.1
Serum anti-HBs (U/ml, mean $\pm$ s.d.)	665 $\pm$ 406	284 $\pm$ 367	Neg.	Neg.	Neg.
ALT (u/l, mean $\pm$ s.d.)	NT	39.8 $\pm$ 31	331 $\pm$ 204	50.2 $\pm$ 23	NT
No. with positive B cell response to HBs <i>in vitro</i>	11/11	7/7	4/4	0/5	0/5
Mean numbers of spot-forming cells (SFC/10 <sup>6</sup> PBMC $\pm$ s.d.)	10.2 $\pm$ 13.4	8.4 $\pm$ 4.4	184.5 $\pm$ 103	0	0
<i>P</i> (Student's <i>t</i> -test)	$P < 0.0001$				
No. with positive T cell response (SI > 3) to	$P < 0.0001$				
pHBs	4/10	1/5	2/4	0/3	0/4
rHBs	6/10	1/5	0/4	0/3	0/4
preS1	0/10	1/5	1/4	0/3	0/4
preS2	0/10	0/5	0/4	0/3	0/4
Stimulation index (means $\pm$ s.d.) of					
pHBs	6.0 $\pm$ 7.0	6.6 $\pm$ 10.7	2.3 $\pm$ 1.0	1.7 $\pm$ 0.6	2.3 $\pm$ 0.3
rHBs	9.5 $\pm$ 12	2.0 $\pm$ 1.4	1.0 $\pm$ 0.3	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1
preS1	1.3 $\pm$ 0.7	2.9 $\pm$ 3.1	1.6 $\pm$ 1.1	1.0 $\pm$ 0	1.0 $\pm$ 0.2
preS2	1.4 $\pm$ 0.4	1.8 $\pm$ 0.7	1.1 $\pm$ 0.1	1.0 $\pm$ 0	1.5 $\pm$ 0.4

NT, Not tested; SI, stimulation index.

## DISCUSSION

The pathogenesis of acute and chronic hepatitis B is thought to be mediated by HBV-specific cellular immune responses. It has been shown that HLA class I-restricted cytotoxic T cells recognize intracellularly processed viral antigens and lyse infected cells [2,5]. The occurrence of anti-HBs antibodies in the course of acute HBV infection indicates the development of protective immunity. Accordingly, high-titred anti-HBs antibodies demonstrate the successful immunological response in HBs vaccine recipients. In contrast, non-responders and chronic HBV carriers show no anti-HBs antibody response.

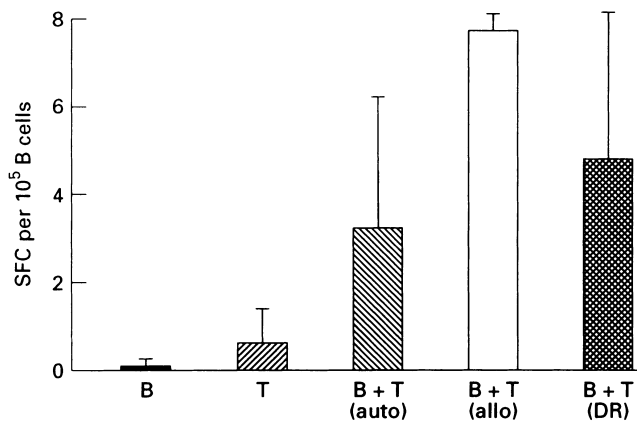
In vaccine non-responders, several immunological mechanisms have been discussed resulting in a deficient anti-HBs production. The failure of antigen presentation, lack of specific T cell help, suppression by specific CD8<sup>+</sup> T cells, or lack of costimulatory

signals were suggested [9,24]. In chronic HBV carriers, however, the etiology of the lack of anti-HBs production is unknown. In addition, an immunogenetic background was demonstrated in vaccine non-responders, but not in chronic courses of hepatitis B [10,11].

To analyse the regulation of anti-HBs antibody formation *in vitro* we established a modified ELISPOT technique and detected significant numbers of anti-HBs antibody-producing B cells in all anti-HBs<sup>+</sup> vaccine recipients and HBV-immunized patients, but not in any of the tested anti-HBs<sup>-</sup> chronic HBV carriers or controls. Additionally, anti-HBs antibodies were only detectable in the cell culture supernatants of two from five ELISPOT-positive vaccine recipients with high serum anti-HBs levels (> 1000 U/l), but in none from three ELISPOT-positive acutely infected patients by ELISA. These data reflect the specificity and sensitivity of the HBs-specific ELISPOT assay *in vitro* despite the low frequencies

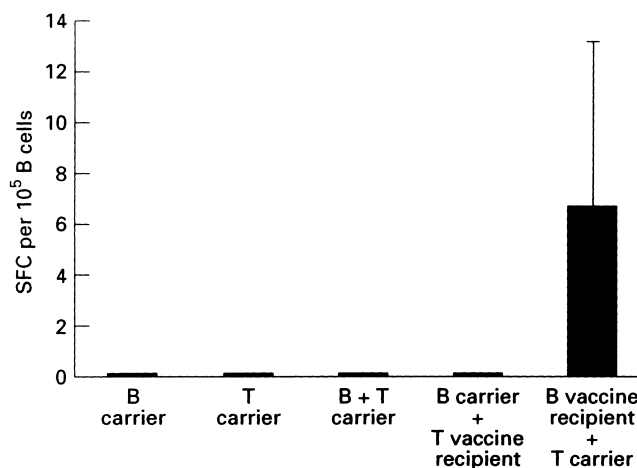
**Table 2.** Specificity and sensitivity of *in vitro* anti-HBs antibody assays: mean anti-HBs antibody levels  $\pm$  s.d. measured by ELISA in sera and cell culture supernatants from patients with acute hepatitis B and HBs vaccine recipients. Alternatively, anti-HBs-producing B cells (expressed as spot-forming cells (SFC)) were determined quantitatively by ELISPOT technique

Group	Number of tested patients	Anti-HBs in serum (U/l) (mean $\pm$ s.d.)	Anti-HBs <i>in vitro</i> (n) (ELISPOT)	SFC per 10 <sup>6</sup> PBMC (mean $\pm$ s.d.)	Anti-HBs <i>in vitro</i> (n) (ELISA)	Anti-HBs level in supernatants (mean $\pm$ s.e.m.)
HBs vaccine recipients	<i>n</i> = 5	467 $\pm$ 430	5/5	5.2 $\pm$ 4.9	2/5	5.4 $\pm$ 5.3 U/l
Acute hepatitis B	<i>n</i> = 3	Neg.	3/3	200 $\pm$ 115	0/3	0.8 $\pm$ 0.1 U/l

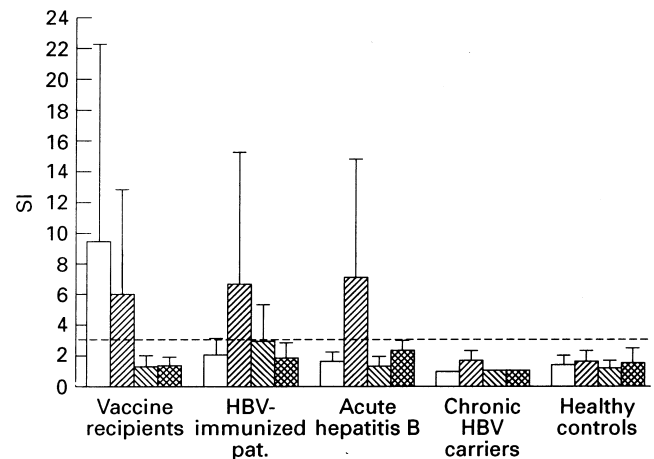


**Fig. 3.** Coculture experiments demonstrating T cell-dependent and HLA II-unrestricted anti-HBs production *in vitro*. Results are expressed as spot-forming cells (SFC)/10<sup>5</sup> B cells. Separated B cells ( $3 \times 10^4$  per well) of vaccine recipients were cocultured with  $1.5 \times 10^5$  separated autologous (auto), allogeneic (allo) or HLA-DR-matched (DR) CD4<sup>+</sup> T cells per well. Anti-HBs antibodies were produced by B cells from vaccine recipients in the presence of DR-matched and mismatched T cells from other vaccine recipients. Data represent means  $\pm$  s.d. of five vaccine recipients.

of anti-HBs-producing B cells in the peripheral blood. In contrast to recent studies, the frequencies of anti-HBs-producing B cells did not correlate with anti-HBs titres quantified by ELISA in the sera of individual patients [12,13,25,26]. This apparent discrepancy could be explained by the enumeration of actually anti-HBs-secreting B cells *in vitro* using the ELISPOT assay, whereas anti-HBs antibodies persist in the sera for a long time. Furthermore, it must be noted that the main sites of antibody production after acute viral infection are the lymphoid tissues or the bone marrow, where specific antibody-producing B cells were shown to



**Fig. 4.** Coculture experiments of B cells derived from chronic HBV carriers together with T cells from HBs vaccine recipients and *vice versa*. Isolated B cells never secreted anti-HBs antibodies. B cells from vaccine recipients could be stimulated to produce anti-HBs in the presence of T cells from chronic HBV carriers. B cells from chronic HBV carriers could never be stimulated by T cells from HBs vaccine recipients. Data represent means  $\pm$  s.d. of three chronic HBV carriers and five vaccine recipients.



**Fig. 5.** Mean proliferative T cell responses (stimulation index (SI)  $\pm$  s.d.) to different HBs antigen preparations in HBV-immunized or -infected patients, vaccine recipients and healthy controls. □, Recombinant HBsAg; ▨, plasma-derived HBsAg; ▩, recombinant preS1 antigen; ▤, synthetic peptide derived from the preS2 region of HBV subtype adw. SI > 3 were regarded as positive (broken line).

be enriched after acute viral infections [27]. Recently it was shown by ELISPOT that oral vaccination with the cholera toxin B (CT-B) evokes a long-lasting enrichment of anti-CT-B-producing B cells in the intestinal lymphoid tissue, whereas in the peripheral blood lower specific B cell frequencies were detectable for a short time [28]. In concordance with these data we demonstrated a high frequency of anti-HBs-producing B cells shortly after the onset of acute hepatitis B, and low frequencies years after viral clearance or vaccination [21–29]. Future studies will have to clarify whether patients with acute self-limited HBV infection can be distinguished from chronic HBV carriers in the early phase of hepatitis by the numbers of SFC.

To study the regulation of the HBs-specific B cell response, cell populations were separated and cultured under various conditions. Single T cell preparations, unlike isolated B cells, showed very low numbers of HBs-specific SFC, suggesting the presence of residual B cells. Cocultures of B cells derived from vaccine recipients together with autologous or allogeneic HLA-class II-matched or -mismatched T cell preparations resulted in a strong increase of HBs-specific SFC. Thus, it could be confirmed that anti-HBs antibody formation is dependent on the presence of T cells but not restricted to identical HLA molecules [12,13,25,30]. It can be speculated that this T cell help is mediated by alloreactive T cell stimulation followed by the release of cytokines or the expression of adhesion molecules. Previous studies have also suggested that *in vivo* preactivated B cells might require only antigen-independent T cell help [22,31]. Moreover, since T cells from chronic HBV carriers could stimulate B cells from vaccine recipients, but not *vice versa*, it can be suggested that in chronic HBV carriers a functional B cell defect or very low B cell frequency in the peripheral blood might exist, whereas HBsAg-independent T cell help is provided to B cells. The etiology of this defect is not known, but may be due to a lack of HBs-specific B cell prestimulation *in vivo* as a consequence of insufficient antigen presentation or T cell help.

To characterize HBsAg-specific T cell reactivity the proliferative T cell response to a panel of different HBs antigen preparations was studied. The PBMC of most HBs vaccine recipients and some

patients with acute hepatitis B or complete recovery from HBV infection, but none of the chronic HBV carriers, showed HBs-specific T cell responses [3,8,22,26,27]. The low rate of HBs-responsive PBMC in patients with acute HBV infection might indicate a low frequency of HBs-specific T cells in the peripheral blood. An enrichment of the T cells in the liver tissue has to be discussed [2,4]. Furthermore, the sensitivity of the conventional <sup>3</sup>H-thymidine uptake assay may be too low.

Vaccine recipients showed higher stimulation with recombinant HBsAg preparations than with plasma-derived HBsAg, whereas HBV-immunized patients preferentially recognized the plasma-derived HBsAg and, in part, the preS1 antigen. These data might indicate that HBs-specific T cells in vaccine recipients and HBV-immunized patients recognized different epitopes on the HBV surface antigen.

Since the HBs-specific T cell response did not correlate with the B cell response, and T cells from chronic HBV carriers could provide help to B cells from vaccine recipients, it has to be discussed that T cells with other antigen specificities may be responsible for the T cell help [16,32,33]. In addition, the B cells could have been primed *in vivo*, so specific antibody production could be induced by HBs-independent T cell mechanisms, as indicated by our coculture experiments. However, to study different mechanisms of T cell help in vaccine recipients and HBV-infected patients, functional analysis of cloned HBV-specific T cells and B cells will give further insights.

In conclusion, high numbers of anti-HBsAg-producing B cells were detectable in the early phase of acute self-limited HBV infection. It could be demonstrated that the humoral immune response to HBsAg is dependent on the presence of T cells. This helper cell activity was not HBsAg-restricted. In chronic HBV carriers, however, the B cells could not be stimulated by T helper cells derived from vaccine recipients. Thus, in chronic HBV carriers the possibility of defective B cell stimulation must be admitted. Furthermore, T cells from vaccine recipients and patients after clearance of recent HBV infection may recognize different T cell epitopes on the HBs antigens.

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