Clin. exp. Immunol. (1989) **76**, 222–226

HBsAg-induced antigen-specific T and B lymphocyte responses in chronic hepatitis B virus carriers and immune individuals

A. FERNAN, C. J. R. CAYZER & W. G. E. COOKSLEY
The Liver Unit, University of Queensland Brisbane 4029, Australia

(Accepted for publication 6 January 1989)

SUMMARY

This report describes a study of *in vitro* proliferative and antibody responses to the hepatitis B virus surface antigen (HBsAg) of lymphocytes from chronic HBsAg carriers, subjects with naturally acquired immunity, and responders to the hepatitis B vaccine. Peripheral blood T and B lymphocytes were cultured with a wide range of concentrations of HBsAg (0·025–250 ng/ml). We were unable to detect HBsAg-specific proliferation or antibody synthesis in any of the subject groups studied, despite the use of a range of antigen concentrations, cell ratios and culture periods. The addition of recombinant interleukin 2 (rIL-2) or T cell growth factor at either initiation or day 3 of culture enhanced proliferative responses, but in an antigen-independent manner. In contrast to the proliferation observed following the addition of IL-2, the absence of responses to specific antigen suggest there may be low numbers of HBsAg-specific precursors in the peripheral blood.

Keywords hepatitis B surface antigen chronic HBsAg carriers T and B cell activation

INTRODUCTION

It is believed that immunological responses of the host constitute major determinants of outcome following exposure to the hepatitis B virus (Dienstag, 1984). Immune responses to various antigenic components of the hepatitis B virus have been examined (Sherlock, 1987). Of these antigens, the hepatitis B surface antigen (HBsAg) has been the most studied. Antibodies to this antigen (anti-HBs) are present following immunization with the hepatitis B vaccine and are absent in the chronic HBsAg carrier state. Previous studies by Dusheiko *et al.* (1983) showed that chronic carriers lacked circulating B lymphocytes capable of synthesizing anti-HBs *in vitro*. T lymphocyte function was also abnormal in certain individuals (Dusheiko *et al.*, 1983).

These *in vitro* studies used the non-specific mitogens phytohaemagglutinin (PHA) and pokeweed mitogen (PWM). Subsequent studies by several authors measured T and B lymphocyte responses to HBsAg *in vitro* to explore these immunological responses further. Hellstrom, Sylvan & Lundbergh (1985) and Sylvan, Hellstrom & Lundbergh (1985) found proliferative responses and antibody synthesis in antigen-stimulated cultures, not only from immune individuals but also from chronic carriers. In contrast, Cupps *et al.* (1984) studied lymphocytes from individuals immunized with hepatitis B vaccine and detected anti-HBs synthesis following stimulation with HBsAg, but were not able to detect proliferative responses. Celis, Kung & Chang (1984) detected HBsAg-specific T and B cells in the circulation of some, but not all, hepatitis B vaccine recipients; however, anti-HBs synthesis could only be detected when B lymphocytes were cultured in the presence of HBsAg-specific T lymphocyte clones. Finally, Hanson *et al.* (1984), using lymphocytes from immune individuals which synthesized anti-HBs in response to PWM, were not able to detect anti-HBs synthesis in response to HBsAg. The reason for these differences is unclear. Whether the differences lie in the individuals from whom the lymphocytes were prepared or in the various methodologies employed is uncertain. Since the ability to measure HBsAg-specific T and B lymphocyte responses would enable the immune responses to hepatitis B virus to be studied, we have endeavoured to define the conditions necessary to establish an *in vitro* system to measure HBsAg-specific responses. For this, we have used a variety of individuals, cell ratios, growth factors and antigen concentrations.

Correspondence: W. G. E. Cooksley, Liver Unit, University of Queensland, Brisbane 4029, Australia

MATERIALS AND METHODS

Patients

Blood was obtained from 10 non-immune individuals, 15 individuals with immunity to hepatitis B, and five chronic carriers. The non-immune individuals (five females and five males, mean age 30·9 years, range 25–47 years) were healthy clinical or laboratory staff who were negative for all markers of hepatitis B virus infection. The HBV-immune donors comprised six individuals (two females and four males, mean age 30·7 years, range 25–40 years) with naturally acquired immunity to hepatitis B as

judged by the presence of anti-HBs and anti-HBc and the absence of HBsAg in the serum, and nine healthy individuals (six females and three males, mean age 31·8 years, range 24–50 years), eight of whom had been immunized with the HB-VAX vaccine (Merck, West Point, PA) which does not contain pre-S antigen, and one individual who had been immunized with a vaccine preparation (NIAID, National Institutes of Health, Bethesda, MD) containing HBsAg plus pre-S. All immune donors had detectable anti-HBs in the serum. All of the five chronic carriers were HBeAg-positive, and lacked anti-hepatitis D (delta) and anti-human immunodeficiency virus (HIV) in the serum. None had received immunosuppressive or anti-viral treatment in the previous 12 months. All were caucasian males, and ages ranged from 23 to 55 years (mean age 37·6 years).

The study was approved by the University of Queensland Ethical Committee and informed consent was obtained.

HBsAg

Purified HBsAg (Batch Rx 73186) was a generous gift from Dr J. McAleer (Merck Sharp and Dohme Research Laboratories, West Point, PA). This material was prepared from 22 nm particles purified from the plasma of chronic HBsAg carriers, and had undergone all of the steps involved in the preparation of HBsAg for the HB-VAX vaccine, except for the final alum absorption, and contained no preservative (Hilleman *et al.*, 1978). This preparation was determined to be immunologically reactive with the specific antibody of a commercial radioimmunoassay for the detection of HBsAg (AUSRIA II; Cat. No. 7802–31, Lot No. 1989-M200: kindly donated by Abbott Laboratories Diagnostics Division, North Chicago, IL). Comparison with a set of ad/ay standards of known concentrations (HBsAg sensitivity Panel No. 9108 Lot No. 16728M100—a kind gift of Abbott Laboratories, Diagnostic Division) confirmed the HBsAg concentration.

Preparation of TCGF

Healthy laboratory staff were used as a source of peripheral blood mononuclear cells (PBMC) for the production of T cell growth factor (TCGF) by the method of Bonnard, Yasaka & Maca (1980). Briefly, PBMC were isolated from 100 ml of heparinized venous blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density-gradient centrifugation, resuspended to a concentration of 1×10^7 cells/ml in RPMI 1640 medium (Flow Laboratories, New Haven, CT) supplemented with 25 mM HEPES (Boehringer Mannheim, FRG), 100 U/ml penicillin and 100 μg/ml streptomycin, to which were added 2% fetal calf serum (FCS, Flow Labs.) and 0·5% phytohaemagglutinin (PHA, Wellcome, Australia), and incubated overnight in 50 ml tissue culture flasks (Nunclon, Denmark) in a humidified CO_2 in air environment. The supernatant was carefully removed, the cells washed twice in RPMI 1640, and incubated for a further 48 h in RPMI 1640 alone. The culture supernatant was then harvested, filter-sterilized, and stored at $-20°C$.

Lymphocyte separation

Peripheral blood mononuclear cells were isolated as previously described from 50 ml of heparinized blood. PBMC harvested from the interface were washed extensively to remove cytophilic immunoglobulin. T- and non-T-lymphocyte-enriched fractions were prepared from PBMC using 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cell rosettes repeated once. Monocytes were separated from the non-T-cell-enriched fraction by adherence to plastic Petri dishes at 37°C for 1 h.

Proliferation assay

T lymphocytes (1×10^5) were cultured in duplicate or quadruplicate in 96-well round-bottom culture plates (Linbro Division, Flow Laboratories) in 200 μl RPMI 1640 with 10% heat-inactivated pooled normal human AB serum and 10% autologous monocytes at 37°C in humidified 5% CO_2 in air for 5–8 days. HBsAg was added at day 0 at a concentration ranging from 0 to 40 ng/ml for the duration of the culture. In addition, some cultures were incubated for 7 days supplemented with either 50 U/ml of recombinant interleukin-2 (r-IL2, Cetus Corporation) or T cell growth factor to a final concentration of 15%. Eighteen hours prior to harvest 1 μCi of ^{3}H-thymidine (specific activity 5 Ci/mmol, TRA.120, Radiochemical Centre, Amersham, UK) was added to each well in a volume of 25 μl. Cells were harvested on to glass-fibre filter paper (Titertek, Flow Labs.) using a semi-automated cell harvester (Titertek). Radioactivity in the filters was measured using a liquid scintillation counter (Beckman, USA). Data were analyzed as the means and standard errors (s.e.) of replicate cultures.

B lymphocyte function

B cells ($2·5 \times 10^4$) were cultured in 96-well round-bottom culture plates containing autologous irradiated (2000 rads) helper T lymphocytes (T : B cell ratio 0·5 : 1 to 4 : 1) and 10% autologous monocytes in RPMI 1640 for 4 days supplemented with 20% FCS and HBsAg, followed by a further 6 days with RPMI 1640 and 5% FCS without HBsAg. HBsAg was added to each well to produce a range of concentrations (0, 0·025, 0·25, 2·5, 25 and 250 μg/ml). In addition, rIL-2 (50 U/ml) or TCGF (15% final concentration) was added in cultures carried out in parallel. At the termination of culture, the supernatant was aspirated and anti-HBs was measured using an enzyme linked immunosorbent assay as described by Cupps *et al.*, (1984). Data were expressed in μg/ml as the means and s.e. of triplicates. Standards were kindly supplied by Commonwealth Serum Laboratories, Melbourne, and 1 μg/ml is equivalent to 1 U/ml.

Statistical analysis

Where groups of individuals were studied data were depicted as means $\pm$ s.e. of the group.

RESULTS

HBsAg-induced T cell proliferation

The amount of thymidine incorporated increased with the length of culture period, with maximal incorporation occurring on days 7 and 8 of culture (Fig. 1). No significant difference in amounts of thymidine incorporated was observed among the subject groups studied. There was also no significant difference observed between ^{3}H-thymidine incorporation in lymphocytes from non-immune individuals, responders to the hepatitis B vaccine, individuals recovered from hepatitis B virus infection, and chronic carriers. In addition, ^{3}H-thymidine incorporation was similar in both the presence and absence of HBsAg, regardless of the Ag concentration employed (Fig. 2).

In an attempt to optimize the detection of antigen-specific responses, either rIL-2 or TCGF was added to selected cultures

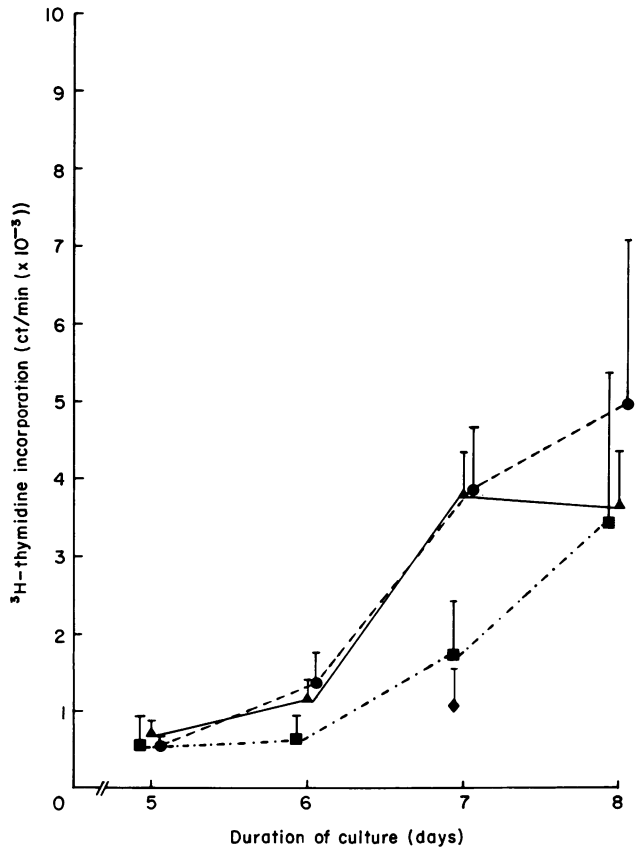

Fig. 1. Time course study of proliferative responses to HBsAg of lymphocytes from chronic carriers (◆), non-immune subjects (●), and vaccinated (▲) and naturally immune (■) individuals. Peripheral blood T cells (1×10^5) were cultured with 1×10^4 autologous monocytes as antigen-presenting cells (APC) per well for 5–8 days in the presence of 5 ng/ml HBsAg. Chronic carriers were studied on day 7 only. The results are expressed as mean ct/min ± s.e.m. of replicate cultures. (Points from different subject groups are offset to prevent overlap).

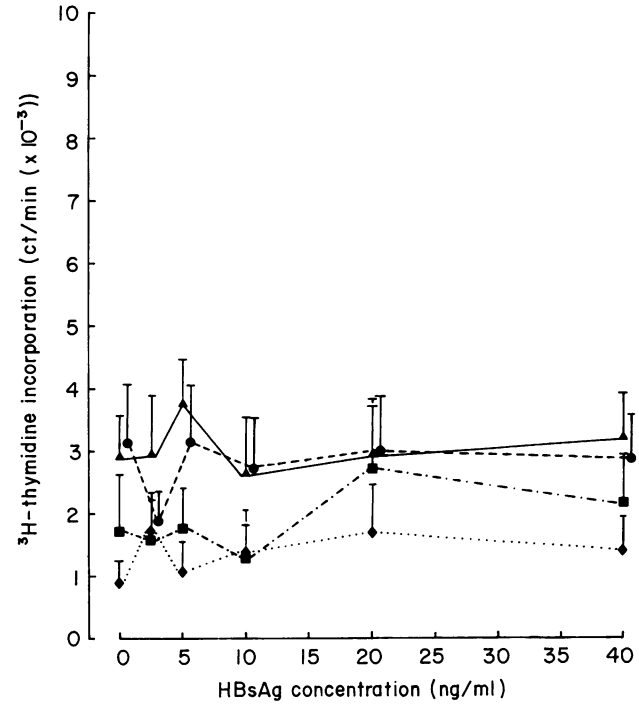

Fig. 2. Effect of HBsAg concentrations on T cell proliferation. T cells (1×10^5) from vaccinated (▲) and naturally immune (■) individuals, chronic carriers (◆) and non-immune subjects (●) were cultured for 7 days with 1×10^4 autologous monocytes as APC in the presence of different concentrations of HBsAg (2·5, 5, 10, 20 & 40 ng/ml). Results are expressed as the mean ct/min ± s.e.m. of replicate cultures.

at initiation or day 3 of culture. In Fig. 3 it can be seen that the addition of rIL-2 did enhance proliferative responses in non-immune individuals and vaccinees, although in an antigen-independent manner. The responses were similar regardless of the time of addition to culture. Identical results were achieved when TCGF was used (data not shown).

HBsAg-induced specific antibody synthesis

Secretion of anti-HBs was not detected in the supernatants of HBsAg-stimulated B cell cultures, regardless of whether carriers, HBV-susceptible, or HBV-immune donors were used as a source of lymphocytes (data not shown). This was despite the use of a range of T:B cell ratios, HBsAg concentrations, and culture periods. The further addition of rIL-2 or TCGF to cultures at initiation or day 3 of culture did not enhance responses.

DISCUSSION

The experiments undertaken in this study were designed to confirm and extend the work of other authors who had reported the successful detection of antibody and proliferative lymphocyte responses to HBsAg *in vitro*. However, we did not demonstrate the presence of HBsAg-induced antigen-specific responses *in vitro* despite the use of a number of manipulations of culture conditions, including a wide range of antigen concentrations, cell ratios and growth factors. Specifically, HBsAg concentrations of 2·5 to 40 ng/ml were utilized for proliferative assays, and 0·025 to 250 ng/ml for anti-HBs assays. For the assay of B lymphocyte function, T:B cell ratios of 0·5:1 to 4:1 were used as these were described by Hellstrom *et al.* (1985) and by Sylvan *et al.* (1985) as optimal for the secretion of anti-HBs. In addition, either rIL-2 or TCGF was added to selected cultures to assess the possibility that growth factors are required for optimal responses.

The studies reported by Hellstrom and co-workers (Hellstrom *et al.*, 1985; Sylvan *et al.*, 1985) showed that lymphocytes were able to respond to HBsAg *in vitro*. We were unable to reproduce their results, although their methods were closely followed. They emphasized that the time of response varies considerably between individuals but the times we studied should have enabled specific responses to have been detected. The antigen used in our laboratory was donated by Merck and was therefore probably free of pre-S antigen (Ohnuma *et al.*, 1986). It is possible that pre-S antigen was present in the HBsAg preparation used by Sylvan and Hellstrom's group, and if so might be expected to increase lymphocyte responses (Einarsson, 1978; 1981). However, in our study, lymphocytes from the individual who had been immunized with vaccine containing pre-S antigen did not show enhanced proliferative or antibody responses *in vitro*, regardless of whether antigen preparations containing pre-S or HBsAg alone were used. In addition, Sylvan

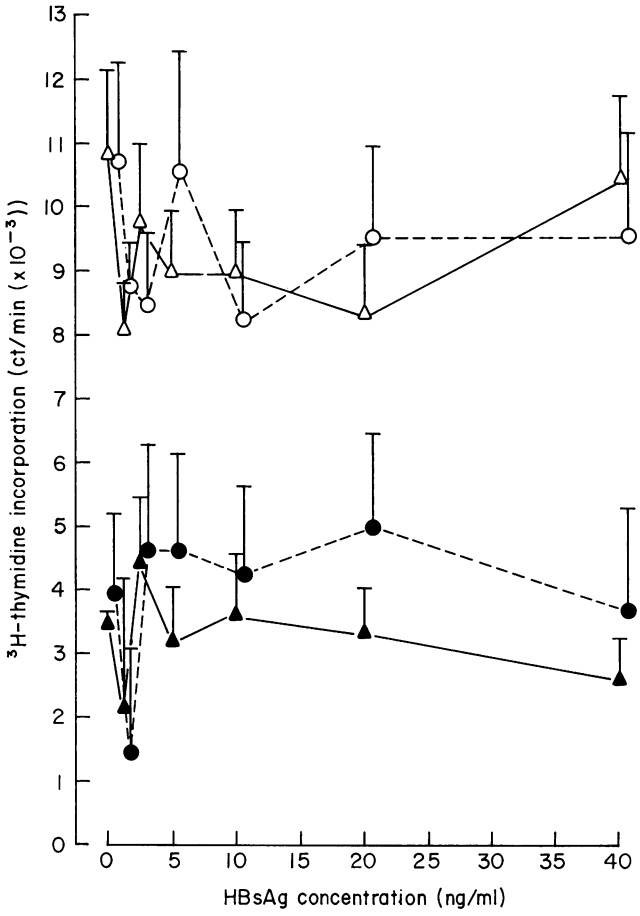

Fig. 3. The effect of recombinant IL-2 (rIL-2) on T cell proliferation in response to varying concentrations of HBsAg (2·5, 5, 10, 20 & 40 ng/ml). T cells (1×10^5) from vaccinated (triangles) and non-immune (circles) individuals were cultured with 1×10^4 autologous monocytes for 7 days in the presence (open symbols) and absence (closed symbols) of rIL-2 (50 U/ml).

et al. (1985) found identical results when HBsAg from Merck was used.

Cupps *et al.* (1984) were also unable to detect a proliferative response to HBsAg. They employed a wide range of antigen concentrations, the presence of cell fractions partially depleted of adherent cells, and the presence of indomethacin. It is of some interest that both Hellstrom *et al.* (1985) and Sylvan *et al.* (1985) found that similar doses of antigen produced optimal proliferative and antibody responses. This is in contrast to the dichotomy of keyhole limpet haemocyanin (KLH)-antigen-induced systems where lower doses of antigen trigger optimal specific antibody production, and larger doses produce strong proliferative responses (Volkman *et al.*, 1984). Nevertheless, despite antigen concentrations ranging from 2·5 μg to 20 000 μg/ml in the experiments of Cupps et al. (1984) and the present study, a proliferative response could not be detected. In the study of Cupps *et al.* (1984) further immunization did not enable the detection of a proliferative response. Indeed, Celis *et al.* (1984) were the only other group who could detect a proliferative response to HBsAg. However, in only one of their subjects immunized with the Merck vaccine was vigorous proliferation observed. This individual responded to HBsAg concentrations of approximately 100 000 ng/ml which was in the range of the study of Cupps *et al.* (1984), but far greater than that of Hellstrom *et al.* (1985) or in the present study. Three further individuals responded to high concentrations of HBsAg if antigen-stimulated cell lines were established in the presence of T cell growth supplement (TCGS) and HBsAg.

Since Celis *et al.* (1984), by using TCGS and HBsAg, were able to detect a proliferative response in 4 of 10 individuals studied, we set up cultures using TCGF or IL-2 but were unable to achieve HBsAg-induced proliferation in any of seven individuals. IL-2 produced a non-specific response with about twice the amount of ^{3}H-thymidine incorporated in the presence of IL-2 at all concentrations of HBsAg. In addition, the use of TCGF or IL-2 to increase the HBsAg-specific helper T lymphocytes to induce antibody synthesis was unsuccessful.

Although the proliferation assay was similar in all studies the methods of measuring anti-HBs varied somewhat. Previous studies (Dusheiko *et al.*, 1983; Celis *et al.*, 1984) used either commercially available immunoassays or their own ELISAs (Hellstrom *et al.*, 1985; Cupps *et al.*, 1984). We used the method of Cupps *et al.* (1984) to measure anti-HBs in culture supernatants due to its high degree of sensitivity.

Cupps *et al.* (1984) were, however, able to detect antigen-induced specific antibody synthesis following immunization. This appeared to be highly dependent on the time after booster immunization at which lymphocytes were obtained. At 2 weeks after immunization, cells appeared that spontaneously secreted anti-HBs and did not respond further to PWM or HBsAg. By 4 weeks the numbers of these cells had declined and B lymphocytes that responded to HBsAg *in vitro* were found. These were no longer detectable by 8 weeks although the cells could still respond to PWM. Celis *et al.* (1984), using cells obtained 1 week after booster immunization did not detect antibody synthesis in response to HBsAg *in vitro*. Although we studied two individuals 4 weeks after booster immunization and were unable to detect antigen-induced specific anti-HBs synthesis *in vitro*, it is noteworthy that Cupps *et al.* (1984) found that the time point at which responses are measured after booster immunization is critical.

Since it was shown that variation in antigen dose-dependency existed, we employed a wide range of antigen concentrations in our cultures. Culture plates identical to those used by Hellstrom and co-workers (Hellstrom *et al.*, 1985; Sylvan *et al.*, 1985) were used in our study, thus the configuration of the wells is not relevant (Lane *et al.*, 1981). Although our study population contained subjects with both natural immunity and immunity acquired after vaccination, whereas Hellstrom and co-workers' subjects all had naturally acquired immunity, the response was stated not to correlate with anti-HBs titre which in some individuals was present in only a titre of 1. It could be proposed that those with naturally acquired immunity had been previously exposed to pre-S antigen which may have enhanced the response; however, our subjects with naturally acquired immunity behaved similarly to the vaccinees.

Of particular interest was the observation by Hellstrom *et al.* (1985) and Sylvan *et al.* (1985) that carriers also possessed sensitized lymphocytes. They suggested that the absence of antibody *in vivo* despite antibody *in vitro* indicated the formation of HBsAg-Ab complexes *in vivo*. This could prevent the antibody being detected in the serum, and also cause down-regulation of specific lymphocyte responses (Uhr & Moller,

1968; Setcavage & Kim, 1980). This finding does not confirm previous data showing that B lymphocytes from chronic carriers were unable to synthesize anti-HBs (Dusheiko *et al.*, 1983; Barnaba *et al.*, 1985).

Attempts by Celis *et al.* (1984) to detect specific anti-HBs were unsuccessful unless they increased the proportion of antigen-specific cells by panning in HBsAg-coated Petri dishes. In addition, HBsAg-specific T cell clones were used to provide help. Despite this, only one out of 13 T cell clones appeared to be capable of anti-HBs synthesis initially. They concluded that both HBsAg-specific T and B cells are at limiting concentrations for cell proliferation *in vitro* and antibody production.

We conclude that HBsAg-specific immune responses to HBsAg *in vitro* are difficult to detect, and may not be detected in all immune individuals. Indeed, Celis *et al.* (1984) could produce a proliferative response in only a minority of individuals, and then only by the establishment of cell lines. The in-vitro production of anti-HBs was also difficult to obtain, although Cupps *et al.* (1984) suggested that booster immunization may allow antibody synthesis to be detected regularly. In contrast, Hellstrom *et al.* (1985) and Sylvan *et al.* (1985) suggested that proliferative responses as well as antibody synthesis can be regularly produced in immune individuals. In addition, they were able to demonstrate proliferative and antibody responses to HBsAg *in vitro* in lymphocytes from chronic HBsAg carriers. Using the same methodology and a wider range of antigen concentrations and culture conditions, we were unable to confirm their results. Consequently, their claim that neither a B nor T cell defect is involved in the pathogenesis of the chronic carrier state cannot be substantiated.

ACKNOWLEDGMENTS

This work was supported by the National Health and Medical Research Council of Australia.

REFERENCES

BARNABA, V., VALENSINI, G., LEVRERO, M., ZACCARI, C., VAN DYKE, A., FALCO, M., MUSCA, A. & BALSANO, F. (1985) Immunoregulation of the *in vitro* anti-HBs antibody synthesis in chronic HBsAg carriers and in recently boosted anti-hepatitis B vaccine recipients. *Clin. exp. Immunol.* **60,** 259.

BONNARD, G.D., YASAKA, K. & MACA, R.D. (1980) Continued growth of functional T lymphocytes: production of human T cell growth factor. *Cell. Immunol.* **51,** 390.

CELIS, E., KUNG, P.C. & CHANG, T.W. (1984) Hepatitis B virus-reactive human T cell clones: Antigen specificity and helper function for antibody synthesis. *J. Immunol.* **132,** 1511.

CUPPS, T.R., GERIN, J.L., PURCELL, R.H., GOLDSMITH, P.K. & FAUCI, A.S. (1984) *In vitro* antigen-induced antibody responses to HBsAg in man: kinetic and cellular requirements. *J. clin. Invest.* **74,** 1204.

DIENSTAG, J.L. (1984) Immunologic mechanisms in chronic viral hepatitis. In: *Viral Hepatitis and Liver Disease* (ed. by G.N. Vyas, J.L. Dienstag & J.H. Hoofnagle) pp. 135–166. Grune & Stratton, Florida.

DUSHEIKO, G.M., HOOFNAGLE, J.H., COOKSLEY, W.G.E., JAMES, S.P. & JONES, E.A. (1983) Synthesis of antibodies to hepatitis B virus by cultured lymphocytes from chronic hepatitis B surface antigen carriers. *J. clin. Invest.* **71,** 1104.

EINARSSON, M., KAPLAN, L. & PERTOFT, H. (1981) A two-step procedure for the purification of hepatitis B surface antigen (HBsAg). *Vox. Sang.* **41,** 91.

EINARSSON, M., KAPLAN, L. & UTTER, G. (1978) Purification of hepatitis B surface antigen by affinity chromatography. *Vox. Sang.* **35,** 224.

HANSON, R.G., HOOFNAGLE, J.H., MINUK, G.Y., PURCELL, R.H. GERIN, J.L. (1984) Cell-mediated immunity to HBsAg in man. *Clin. exp. Immunol.* **57,** 257.

HELLSTROM, U., SYLVAN, S. & LUNDBERGH, P. (1985) Regulatory functions of T- and accessory-cells for hepatitis B surface antigen induced specific antibody production and proliferation of human peripheral blood lymphocytes *in vitro*. *J. clin. Lab. Immunol.* **16,** 173.

HILLEMAN, M.R., BERTLAND, A.U., BUYNAK, E.B., LAMPSON, G.P., MCALEER, W.J., MCLEAN, A.A., ROEHN, R.R. & TYTELL, A.A. (1978) Clinical and laboratory studies of HBsAg vaccine. In: *Viral Hepatitis* (ed. by G.N. Vyas, S.N. Cohen & R. Schmidt) pp. 525–537. Franklin Institute Press, Philadelphia.

LANE, H.C., VOLKMAN, D.J., WHALEN, G. & FAUCI, A.S. (1981) *In vitro* antigen-induced, antigen-specific antibody production in man. Specific and polyclonal components, kinetics, and cellular requirements. *J. exp. Med.* **154,** 1043.

OHNUMA, H., MACHIDA, A., TAKAI, E., TSUDA, F., MIYAMOTO H., TANAKA, T., ODA, K., USUDA, S., NAKAMURA, T., MIYAKAWA, Y. & MAUMI, M. (1986) Translation products of pre S1, pre S2 regions and the S gene of hepatitis B virus. Susceptibility of their antigenic activities to treatment with heat, urea, formalin or pepsin. *Clin. exp. Immunol.* **67,** 709.

SETCAVAGE, T.M. & KIM, Y.B. (1980) Inhibition of the immune response by Fc-receptor-bound specific antibody on T lymphocytes. *J. Immunol.* **124,** 553.

SHERLOCK, S. (1987) The natural history of hepatitis B. *Postgrad. Med. J.* **63** (Suppl. 2), 7.

SYLVAN, S.P.E., HELLSTROM, U.B. & LUNDBERGH, P.R. (1985) Detection of cellular and humoral immunity to HBsAg in asymptomatic HBsAg carriers. *Clin. exp. Immunol.* **62,** 288.

UHR, J.W. & MOLLER, G. (1968) Regulatory effect of antibody on the immune response. *Adv. Immunol.* **8,** 81.

VOLKMAN, D.J., LANE, H.C. & FAUCI, A.S. (1981) Antigen-induced *in vitro* antibody production in humans. A model for B cell activation and immunoregulation. *Proc. natl. Acad. Sci. USA.* **78,** 2528.