

Cell-mediated immunity in HBeAg-positive homosexuals with chronic liver disease

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SUMMARY Chronic liver disease developing after infection with the hepatitis B virus may be due to impairment of cell-mediated immunity (CMI). In a study of CMI by the lymphocyte transformation test to two mitogens and hepatitis B surface antigen (HBsAg) the response to the mitogens, phytohaemagglutinin (PHA) and purified protein derivative (PPD), was normal in all of 15 male homosexuals with HBsAg-positive chronic liver disease, indicating a normal non-specific cellular immune response. By contrast, only one of the 15 patients showed a response to HBsAg, which may explain the high prevalence of chronic HBsAg carriage in these patients.

Introduction

About 5% of homosexual men are hepatitis-B-surface-antigen-(HBsAg)-positive and many have serious asymptomatic chronic liver disease.¹ The pathogenetic events responsible for hepatocellular injury in chronic hepatitis B virus (HBV) infections have not yet been identified. Because the virus does not appear to be cytopathic for infected liver cells attention has been focused on host-determined genetic and immunological factors, particularly cell-mediated immunity (CMI). The presence of the HBsAg in the serum of many of these patients may indicate a generalised impairment of host immune response to the HBV.² Studies of CMI using lymphocyte transformation to a range of mitogens including HBsAg have not given consistent results,³ but most of these reports do not take account of the HBe antigen/antibody status, except for one recent investigation of non-specific CMI.⁴

The aim of this study was to investigate the CMI response to different mitogens, including purified HBsAg, in a group of Caucasian homosexual men all of whom had high titres of HBsAg in serum, were hepatitis B e antigen (HBeAg)-positive, and had abnormal liver histology.

Patients and methods

Fifteen Caucasian male homosexuals (mean age 29.7 years) were studied. All were sexually active with 30-50 different contacts a year and were asymptomatic chronic HBsAg-carriers of between 12 and 96 months' duration (table). All were HBeAg-positive and anti-HBe-negative by radioimmunoassay. None had any physical signs of liver disease, but all had abnormal results to liver function tests. None was receiving drug treatment.

As controls, we also studied six normal healthy HBsAg-negative and anti-HBsAg-negative subjects and six patients who had recently recovered from an attack of acute type-B hepatitis with return of normal liver function, clearance of HBsAg from serum, and conversion to anti-HBs.

SEROLOGY

The presence of HBsAg was determined by reversed passive haemagglutination (Hepa test, Wellcome). HBeAg and anti-HBe were detected by radioimmunoassay (Abbott).

HISTOLOGY

All 15 patients has at least one liver biopsy performed. Histological diagnoses (table) were chronic active hepatitis (10 patients) and chronic persistent hepatitis (five patients). The Shikata (orcein) stain for HBsAg in liver cells gave a positive result in each case.

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TABLE Clinical, virological, and immunological data on the 15 patients studied

Age (years)	Duration of HBsAg carriage (months)	HBsAg titre	Liver histology	Lymphocyte transformation*		
				PHA	PPD	HBsAg
27	16	1/128 000	CPH	+++	+++	-
38	13	1/64 000	CAH	+++	+++	-
37	19	1/64 000	CAH	+++	++	-
30	96	1/16 000	CAH	+++	+++	-
40	72	1/128 000	CPH	+++	++	-
40	36	1/16 000	CAH	+++	+++	-
19	15	1/128 000	CPH	+++	+++	-
18	15	1/64 000	CPH	+++	++	-
25	18	1/256 000	CAH	+++	+++	-
30	15	1/32 000	CAH	+++	+++	+
25	14	1/64 000	CAH	+++	++	-
28	14	1/32 000	CAH	+++	++	-
26	18	1/256 000	CAH	+++	+++	-
24	16	1/8000	CAH	+++	++	-
32	9	1/128 000	CPH	+++	++	-

*Difference between stimulated and control cultures: +++ = >10 000 cpm; ++ = >5000 cpm; + = >1000 cpm; - = <1000 cpm. CPH = chronic persistent hepatitis; CAH = chronic active hepatitis; PHA = phytohaemagglutinin; PPD = purified protein derivative; HBsAg = hepatitis B surface antigen.

LYMPHOCYTE TRANSFORMATION

The in-vitro lymphocyte transformation was measured by uptake of ^3H thymidine and recorded as counts per minute (cpm) above background counts. The mitogens used were phytohaemagglutinin (PHA, Wellcome Laboratories) in concentrations of 0.5-4 $\mu\text{g}/\text{ml}$, purified protein derivative (PPD; Central Veterinary Laboratory) in concentrations of 0.1-100 $\mu\text{g}/\text{ml}$, and HBsAg from chimpanzee plasma (London School of Hygiene and Tropical Medicine) purified by caesium chloride gradient ultracentrifugation and used in concentrations of 0.6-80 $\mu\text{g}/\text{ml}$ of protein. All concentrations of the mitogens were used for the patients and the controls. Each experiment was performed in triplicate and a single preparation of type AB serum used in all the cell cultures.

Mononuclear cells were separated from sterile heparinised blood by density gradient centrifugation as described by Boyum.⁵ The cells collected at the interphase were washed three times with Hank's balanced salt solution (Flow Laboratories). Prepared lymphocytes were suspended at a final concentration of $10^6/\text{ml}$ in RPMI 1640 medium supplemented with 20% AB serum, L-glutamine, penicillin, and streptomycin. The cells were placed in microtitre plates and mitogens and antigen at different concentrations were added. The plates were incubated at 37°C in a moist 5% $\text{CO}_2/95\%$ air atmosphere for five days and pulsed with 0.5 $\mu\text{Ci}/\text{well}$ of ^3H thymidine (Radiochemical Centre, Amersham, England) for the last 18 hours; the cultures were then harvested on glass-fibre filter paper using an automatic cell harvester (Flow Laboratories) and ^3H thymidine uptake was measured on a β -liquid scintillation counter.

Results

The responses to the different mitogens are shown in the table. In every case there was a strongly positive response to PHA and PPD indicating normal T-cell function, and no significant difference in this response was evident between the patients and the controls. With purified HBsAg, however, a response occurred in only one of 15 patients, but three of the six controls who had recovered from acute type B hepatitis responded. The counts were between 5 000 and 10 000 cpm in two patients and more than 10 000 cpm in the third. None of the six healthy HBsAg-negative and anti-HBs-negative controls responded to this antigen.

Discussion

This study shows that in this well-defined group of male homosexual patients with high titres of serum HBsAg, detectable HBeAg, and abnormal liver function a normal non-specific cellular immune response to PHA and PPD occurred, despite a previous report to the contrary.³ A specific CMI response to HBsAg develops in the recovery phase of acute type-B hepatitis in some, but not all, patients and there appears to be a specific defect in this response in asymptomatic healthy carriers. Conflicting results, however, have been obtained previously in patients with chronic HBsAg-positive liver disease.⁶ This may have been due to the heterogeneous nature of the patients investigated. In the present study we have shown that more than 90% of the patients failed to respond to purified HBsAg.

This lack of response may explain the chronic carrier state and allow continued viral replication. If CMI has a role in the pathogenesis of the liver damage it does not seem to be directed against HBsAg; other viral antigens, such as hepatitis B core antigen (HBcAg) or host liver protein, may possibly be implicated.

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