

NOTES

Lack of Correlation Between Interferon Production of Mononuclear Cells and Virus Replication in Chronic Hepatitis B Virus Infection

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Interferon production in hepatitis B virus carriers with normal liver functions was preserved, whereas in carriers with chronic hepatitis it was depressed. The lower interferon production in hepatitis B virus carriers with chronic hepatitis appears to be the result rather than the cause of chronic hepatic disease.

The role of interferon (IF) has been well studied in acute viral infections, mainly in animal experiments. The administration of anti-IF enhances infection and increases mortality (7). Moreover, the administration of IF or IF inducer is effective in some viral infections (5).

Recently it was reported that long-term IF administration was effective in chronic active hepatitis due to hepatitis B virus (HBV) (2), but it is not known whether IF plays a role in the pathogenesis of persistent viral infection.

A unique feature of HBV infection is persistent infection with complete virus particles (Dane particles), viral structural protein (HB surface antigen), or both in the blood. Thus HBV infection is a typical chronic viral infection.

In this study we tested the production of IF by mononuclear cells of patients with chronic HBV infection and compared it with that of healthy subjects. Moreover, HBV-associated deoxyribonucleic acid (DNA) polymerase activities in the sera of the same patients were assayed as a marker of virus replication, and IF production was correlated with virus replication.

The subjects of this investigation were 8 HBV carriers aged 31 ± 6.2 years with normal liver functions and 14 HBV carriers aged 30.1 ± 6.5 with histologically proved chronic hepatitis (7 with chronic active hepatitis and 7 with chronic persistent hepatitis); 10 HB surface antigen-negative healthy subjects aged 28.7 ± 5.0 served as controls.

IF production. Peripheral mononuclear cells were separated on a Ficoll-Hypaque gradient. Mononuclear cells (between 5×10^6 and 1.5×10^7 per ml) were suspended in 2 ml of RPMI 1640 with 10% fetal calf serum, and 200 hemag-

glutinating units of Sendai virus per 10^7 cells was added. After 1 h of incubation at 37°C , cells were washed and suspended in the medium in a concentration of 10^7 per ml. The supernatant fluid was collected after incubation overnight at 37°C . For virus inactivation, the pH of each sample was adjusted to 2 for 48 h at 4°C .

The interferon titer was assayed by cytopathic effect inhibition. Briefly, a microplate was seeded with 3×10^4 human foreskin fibroblast cells per well in 0.1 ml of minimal essential medium with 5% fetal calf serum, containing triplicate serial threefold dilutions of IF samples. Each microplate included dilutions of a standard IF. After overnight incubation at 37°C , the medium was removed, and 0.125 ml of vesicular stomatitis virus suspension, diluted in minimal essential medium with 0.5% fetal calf serum to contain between 10^2 and 10^3 50% tissue culture infective doses, was added to each well. After 24 h of incubation, the IF titer was determined by 50% reduction of cytopathic effect and expressed in reference to the standard National Institutes of Health human leukocyte reference IF G-023-901-527.

HBV-associated DNA polymerase activity in the sera of the same patients was assayed by a modification of the method of Kaplan et al. (3). HBV specificity of DNA polymerase activity was confirmed by precipitation with antiserum to HB surface antigen.

The mean IF titer of controls (\pm standard deviation) was $2,100 \pm 840$ U/ml, that of HBV carriers with normal liver functions was $1,900 \pm 800$ U/ml, and that of HBV carriers with chronic hepatitis was 970 ± 400 U/ml (Fig. 1). The IF titers of the controls and HBV carriers with

TABLE 1. DNA polymerase activity in serum and IF titer of mononuclear cells

HBV carriers	DNA polymerase activity (cpm \pm SD ^a)	No. of cases	IF titer (U/ml \pm SD)	<i>P</i> ^b and <i>r</i> ^c
Normal liver functions	Negative (64.1 \pm 22.6)	5	2,005 \pm 506	<i>P</i> > 0.1; <i>r</i> = 0.07
	Positive (368 \pm 131)	3	1,680 \pm 1,250	
Chronic hepatitis	Negative (63.0 \pm 15.8)	10	960 \pm 430	<i>P</i> > 0.1; <i>r</i> = 0.1
	Positive (314 \pm 161)	4	980 \pm 354	

^a SD, Standard deviation.

^b *P* value is calculated by Student's *t* test from the data of IF titer in DNA polymerase-positive and -negative groups.

^c Correlation coefficient between DNA polymerase activity and IF titer.

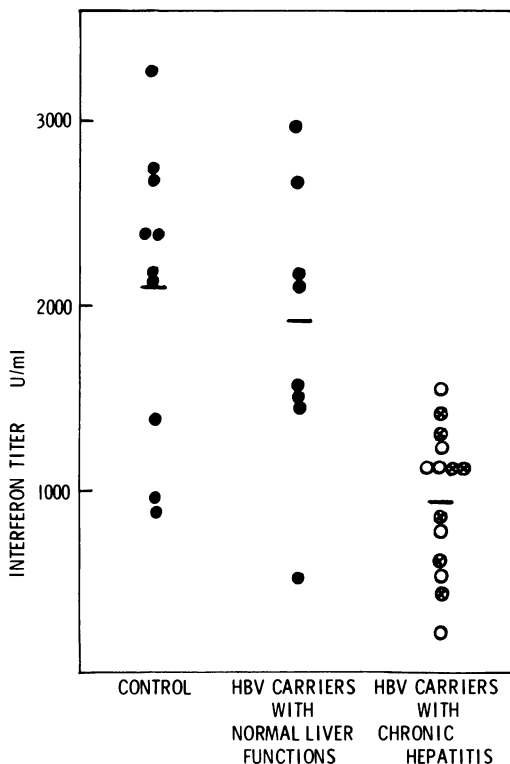


FIG. 1. IF titers in controls, HBV carriers with normal liver functions, and HBV carriers with chronic hepatitis. Bar designates mean values of IF titer. (○) Chronic active hepatitis; (⊗) chronic persistent hepatitis.

normal liver functions were significantly higher than those of HBV carriers with chronic hepatitis (*P* < 0.001 by Student's *t* test). There was no significant difference between controls and HBV carriers with normal liver functions.

Table 1 shows the relation of IF titers to DNA polymerase activities in HBV carriers. There was no correlation between them. In our assay system the mean DNA polymerase activity of HB surface antigen-negative donors was 44.5 \pm

6.3 cpm; more than 150 cpm of DNA polymerase activity was considered to be positive. There was also no difference in IF titer between the DNA polymerase-positive and -negative groups in HBV carriers with normal liver functions and HBV carriers with chronic hepatitis.

HBV carriers with normal liver functions produced almost the same amount of IF as did the controls. This shows that the IF production of mononuclear cells is independent of the HBV carrier state.

HBV carriers with chronic hepatitis had the ability to produce IF, but the titer was lower than in the controls. This is in accordance with the study of HB surface antigen-positive children with chronic hepatitis (6). It seems probable that the lower IF production in HBV carriers with chronic hepatitis is not the cause but the result of chronic hepatic inflammation, since (i) HBV replication as indicated by DNA polymerase activity showed no relation to IF production; (ii) HBV is thought to be a poor IF inducer (1); and (iii) a factor which represses the response of lymphocytes to PHA has been reported in liver disease (4) and a similar factor might have been involved in our study.

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