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Circulating Immune Complexes and Cell-Mediated Immunity in Patients with Hepatitis B Virus Associated Liver Diseases

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The prevalence of circulating immune complexes (CIC), their role and their relationship to cell-mediated immunity in patients with hepatitis B virus associated liver disease are still controversial. This study was designed to investigate the prevalence of CIC and their relationship to viral markers, to subsets of peripheral blood T lymphocytes and to suppressor cell activity in patients with hepatitis B virus associated liver diseases. CIC were positive in 29.3% of 41 healthy HBsAg carriers, 37.8% of 88 patients with hepatitis B virus associated liver diseases, and 15% of 41 healthy subjects by the platelet aggregation test (PAT). The prevalence of CIC in patients with acute hepatitis (40.0%) and in those with cirrhosis (61.5%) was significantly higher than in normal controls ($p<0.05$, $p<0.005$ respectively). There was no correlation between the titer of CIC and serum HBsAg titer or the status of HBeAg, and no significant decrease in the peripheral blood lymphocyte CD4/CD8 ratio in healthy HBsAg carriers (1.39 ± 0.31) and in patients with liver diseases (1.40 ± 0.54) compared to the normal controls (1.48 ± 0.31). Concanavalin A induced suppressor cell activity on IgG producing cells was impaired in healthy HBsAg carriers (34.9%) ($p<0.005$) and in patients with liver diseases (25.3%) ($p<0.0001$), and this change was prominent in patients with chronic active hepatitis and cirrhosis ($p<0.0001$). And there was a significant reverse correlation between concanavalin A induced suppressor cell activity on IgG-producing cells and the titer of CIC in PAT positive patients with hepatitis B virus associated liver diseases. In conclusion, it was suggested that defective suppressor cell function may lead to an increased B cell activation and such activity may account for the presence of CIC.

Key Words: Circulating immune complexes, cell-mediated immunity, hepatitis B virus associated liver diseases

Since the hepatitis B virus is not directly cytopathic for infected hepatocytes, the immune response of the host is considered to play a pivotal role in causing liver cell damage (Chisari *et al.* 1981).

After hepatitis B virus infection, immune complexes consisting of viral antigens, liver antigens, their antibodies, and complements may apper in the circulation (Lawley *et al.* 1980). Such complexes have been implicated in the extrahepatic manifestations of this infection, such as prodromal serum sickness syndrome of urticaria, vasculitis and arthralgia (Alpert *et al.* 1971; Onion *et al.* 1971; Wands *et al.* 1975), glomerulonephritis (Combes *et al.* 1971), and neuropathy (Tsukada *et al.* 1983).

Since the observation of immune complexes in the sera of patients with hepatitis by electron microscope (Almeida and Waterson 1969), several studies described the presence of circulating immune complexes (CIC) in patients with hepatitis B virus infection. The CIC were detected with similar frequencies in patients with acute hepatitis, chronic persistent hepatitis, or liver cirrhosis (Abrass *et al.* 1980; Brown *et al.* 1983), but other studies reported that those were detected with higher frequency in patients in the early stage of acute hepatitis (Madalinski and Bragiel 1979), or in patients with chronic active hepatitis (Thomas *et al.* 1978; Araki *et al.* 1982) than in healthy carriers or other types of hepatitis B virus infections. According to the activity or the severity of the disease, some reported there were no differences in the frequencies of CIC (Abrass *et al.* 1980), and others observed a close correlation between the levels of CIC and disease activity (Anh-Tuan and Novak 1981). The fate of immune

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Table 1. Sex and age distributions of controls, healthy HBsAg carriers and patients studied

Groups	Number	Male	Female	Mean age (yr)
Controls	40	23	17	30.5
Healthy HBsAg carriers	41	22	19	34.0
Patients with HBV associated liver diseases	88	59	29	38.2
Acute hepatitis	16	8	8	32.4
Chronic persistent hepatitis	7	6	1	39.4
Chronic active hepatitis	26	21	5	33.3
CAH with cirrhosis	11	6	5	38.3
Cirrhosis	28	18	10	45.6

HBV: Hepatitis B virus
CAH: Chronic active hepatitis

complexes formed in the circulation depends on a number of variables, including the rate of production due to the persistent antigenic stimulation or the occurrences of autoantibodies (Zubler and Lambert 1978), and the removal rate of immune complexes and state of the phagocytic system, especially in the liver (Mannik *et al.* 1971; Thomas *et al.* 1973). As the CIC could be also increased due to antigen-independent enhancement of immunoglobulin synthesis and the occurrence of autoantibodies (Rong *et al.* 1984) resulting from severe impairment of peripheral suppressor T lymphocytes function that may lead to an abnormal B lymphocyte activation (Budillon *et al.* 1983), the relationships between the CIC and peripheral suppressor T lymphocytes should be examined.

The cellular immunity of the host infected with hepatitis B virus remains as an enigma. Many investigators reported that the ratio of helper to suppressor/cytotoxic lymphocytes (the CD4/CD8 ratio) is reduced due to an increased proportion of CD8 positive T lymphocytes (Thomas *et al.* 1982; Alexander *et al.* 1983), and that the activities of suppressor T lymphocytes are also reduced (Kakumu *et al.* 1980; Chisari *et al.* 1981; Nouri-Aria *et al.* 1986). These contrast with other reports that there were no differences in the numbers of peripheral T lymphocytes and CD8 positive T lymphocytes (Regenstein *et al.* 1983; Lee *at al.* 1986; Shin *et al.* 1987), and in the activities of suppressor T lymphocytes (Nardiello *et al.* 1982; Lemm *et al.* 1983) between the patients with hepatitis B virus associated liver diseases and healthy subjects.

So, the present study was designed to investigate the prevalence of CIC, its relationship to viral markers, the subsets of peripheral blood T lymphocytes and the suppressor cell activity in patients with hepatitis B virus associated liver diseases.

MATERIALS AND METHODS

Subjects

The subjects were 41 healthy HBsAg carriers and 88 patients with hepatitis B virus associated liver diseases, including 16 cases of acute hepatitis, 7 cases of chronic persistent hepatitis, 26 cases of chronic active hepatitis, 11 cases of chronic active hepatitis with cirrhosis and 28 cases of cirrhosis, who were admitted to the Department of Internal Medicine, Yonsei University Medical Center. The diagnoses of chronic hepatitis and cirrhosis were proven histologically. Peripheral bloods from patients with acute hepatitis were obtained 1-2 weeks after the onset of the disease. Forth blood donors who were negative for HBsAg and anti-HBc were included in this study as normal controls (Table 1).

Serologic tests for hepatitis B viral markers

Sera from patients and controls were analyzed for the following hepatitis B viral markers: HBsAg, antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), HBeAg, and antibody to hepatitis B envelope antigen (anti-HBe) by radioimmunoassay (Abbott Laboratories, North Chicago, IL). The titer of HBsAg was expressed as ratio unit calculated according to the following formula:

$$\text{Ratio Unit} = \frac{\text{Patient serum (cpm)-Negative control (cpm)}}{\text{Positive control (cpm)-Negative control (cpm)}}$$

Detection of circulating immune complexes

CIC were detected by the platelet aggregation test (PAT). The details of the procedure are given elsewhere (Penttinen 1977). Briefly, the serially diluted

test sera were added to platelets suspended in U-bottom plates. The results were read by the naked eye in dark field illumination after overnight incubation at 5-9°C. Samples showing titer highter than 1:8 are considered as positive in the PAT (Park *et al.* 1985).

Separation of peripheral blood lymphocytes

Peripheral blood lymphocytes (PBL) were isolated from heparinized venous blood of patients and controls. Blood samples were mixed immediately with an equal volume of RPMI 1640 media (Hezleton Research Products Inc., Denver, PA) containing penicillin (100 units/ml) and streptomycin (100μg/ml), and the PBL were separated by density gradient centrifugation over Ficoll-Hypaque solution (1.077g/ml density) (Pharmacia, Piscataway, NJ).

CD4/CD8 ratio of peripheral blood lymphocytes

The CD4/CD8 ratio was determined using the Immunobead (Bio-Rad Co, Richmond, CA) prepared by coupling specific monoclonal antibodies to microbeads.

Suppressor cell function

Lymphocytes separated from peripheral blood were adjusted to a concentration of 2.5×10^6 cells/ml in 10% heat-inactivated fetal bovine serum in RPMI 1640 supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml) and L-glutamine (1.6mM). These lymphocytes were incubated with or without 20 μg/ml concanavalin A (Con A) (Difco Laboratories, Detroit, MI) in 24-well cell culture plates (Linbro, McLean, VA) for 36 hours, then washed three times in RPMI 1640 containing 0.3 M alpha-methyl-D-mannoside (Sigma, St.Louis, MO) to remove Con A, and then washed two times with RPMI 1640 alone. They were reconstituted at 1×10^6 cells/ml in 20% heat-inactivated fetal bovine serum in RPMI 1640. Equal numbers of Con A stimulated and unstimulated lymphocytes were cultured in the presence of 20 μg/ml Pokeweed mitogen (PWM) (Sigma, St. Louis, MO) at 37°C in humidified air containing 5% CO_2. After eight days culture, cells were washed in RPMI 1640 and resuspended at 1×10^6 cells/ml. Fifty microlitres of lymphocytes suspension were added to 50μl sheep red blood cells (33%, v/v) coated with staphylococcal protein A (Pharmacia, Piscataway, NJ), 15 μl rabbit antihuman IgG (100μg/ml) diluted 1:10, and 0.45ml 0.5% agarose. After mixing at 46°C, they were poured into plastic petri dishes (Becton Dickinson, Oxnard, CA) which were covered with agarose already and incubated at 37°C in humidified air containing 5% CO_2 for 4 hours. To each dish was added 0.6 ml of guinea pig complement (Gibco, Grand Island, NY) diluted 1:10 with Kolmer's saline and incubated for 12 hours at 37°C in a humidifier. Discrete concentric areas of hemolysis were identified around each immunoglobulin-producing lymphocyte.

Percentage suppression is the ratio of IgG-producing cells per million viable lymphocytes and is expressed by the formula:

$$\text{Suppression \%} = \left(1 - \frac{\text{No. of IgG-producing plaques with Con A and PWM}}{\text{No. of IgG-producing plaques with PWM alone}}\right) \times 100$$

RESULTS

Prevalence of circulating immune complexes

CIC were assayed by PAT on 41 sera of healthy HBsAg carriers, 82 of hepatitis B virus associated liver diseases (HBVLD) and 40 of healthy controls (Table 2). PAT positive sera of healthy controls were found in 15.0%. Positive cases were noted in 29.3% of patients with healthy HBsAg carriers and 37.8% with HBVLD. CIC were significantly increased in HBVLD ($p<0.05$, Chi-square test) compared to healthy controls. In HBVLD, CIC were detected in 6 out of 15 (40.0%) patients with acute hepatitis, in none of 7 patients with chronic persistent hepatitis, in 5 out of 23 (21.7%) patients with chronic active hepatitis, in 4 out of 11 (36.4%) patients with chronic active hepatitis with cirrhosis, and 16 out of 26 (61.5%) patients with cirrhosis. CIC were significantly increased in patients with acute hepatitis ($p<0.05$) and in patients with cirrhosis ($p<0.005$, Chi-square test).

Distribution of the titer of CIC in PAT positive individuals

In PAT positive individuals, 2 out of 6 (33.3%) healthy controls exhibited a PAT titer of 1:16. However 5 out of 12 (41.7%) healthy HBsAg carriers and 16 out of 31 (56.6%) patients with HBVLD did. In patients with HBVLD, 2 out of 6 (33.3%) patients with acute hepatitis, 2 out of 5 (40.0%) with chronic active hepatitis, 3 out of 4 (75%) with chronic active hepatitis with cirrhosis and 9 out of 16 (56.3%) with cirrhosis exhibited the titer. Neither the healthy controls nor healthy HBsAg carriers exhibited a PAT titer greater than 1:32, but 9 out of 31 (29.0%) patients with HBVLD did (Table 3).

Table 2. The prevalence of circulating immune complexes in patients with HBV associated liver diseases

Groups	Total number	PAT positive (≥1:8)	
		Number	Percent
Controls	40	6	15.0
Healthy HBsAg carriers	41	12	29.3
Patients with HBV associated liver diseases	82	31	37.8*
Acute hepatitis	15	6	40.0*
Chronic persistent hepatitis	7	0	0.0
Chronic active hepatitis	23	5	21.7
CAH with cirrhosis	11	4	36.4
Cirrhosis	26	16	61.5**

HBV: Hepatitis B virus
PAT: Platelet aggregation test
CAH: Chronic active hepatitis
* $p<0.05$, vs controls (Chi-square test)
** $p<0.005$, vs controls (Chi-square test)

Table 3. The distribution of the titer of circulating immune complexes in platelet aggregation test (PAT) positive individuals

Groups	Number (percent)		
	PAT positive (≥1:8)	PAT (≥1:16)	PAT (≥1:32)
Controls	6	2 (33.3)	0 (0.0)
Healthy HBsAg carriers	12	5 (41.7)	0 (0.0)
Patients with HBV associated liver diseases	31	16 (51.6)	9 (29.0)
Acute hepatitis	6	2 (33.3)	2 (33.3)
Chronic persistent hepatitis	0	0 (0.0)	0 (0.0)
Chronic active hepatitis	5	2 (40.0)	1 (20.0)
CAH with cirrhosis	4	3 (75.0)	3 (75.0)
Cirrhosis	16	9 (56.3)	3 (18.8)

HBV: Hepatitis B virus
CAH: Chronic active hepatitis

Table 4. The status of HBeAg and anti-HBe and circulating immune complexes in patients with HBV associated liver diseases

Viral markers		Total number	PAT positive	
			Number	Percent
HBeAg	Positive	36	15	41.7
	Negative	31	15	48.4
Anti-HBe	Positive	25	9	36.0
	Negative	43	19	44.2

HBV: Hepatitis B virus
PAT: Platelet aggregation test

The relationship between HBsAg titer and CIC in patients with HBVLD

There was no correlation between the titer of CIC and serum HBsAg titer ($r=0.02$) (Fig. 1), or the status of the HBeAg or anti-HBe (Table 4) in healthy HBsAg carriers and in patients with HBVLD.

CD4/CD8 ratio of the peripheral blood lymphocytes

The CD4/CD8 ratio of the PBL was 1.48±0.31 in healthy controls, 1.39±0.31 in healthy HBsAg carriers, and 1.40±0.54 in patients with HBVLD (Table 5). However, there was no significant decrease in CD4/CD8 ratio in HBVLD. And there was no signifi-

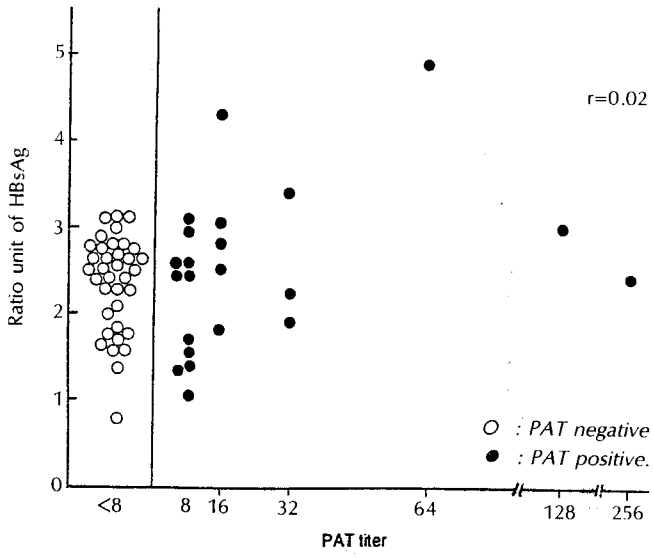

Fig. 1. The relationship between HBsAg titers and circulating immune complexes in patients with hepatitis B virus associated liver diseases. PAT: Platelet aggregation test

Table 5. The peripheral blood lymphocyte CD4/CD8 ratio in patients with HBV associated liver diseases

Groups	Number	CD4/CD8 ratio (Mean±S.D.)
Controls	39	1.48±0.31
Healthy HBsAg carriers	21	1.39±0.31
Patients with HBV associated liver diseases	63	1.40±0.54
Acute hepatitis	13	1.34±0.70
Chronic persistent hepatitis	6	1.36±0.40
Chronic active hepatitis	20	1.48±0.62
CAH with cirrhosis	8	1.38±0.31
Cirrhosis	16	1.40±0.40

HBV: Hepatitis B virus
CAH: Chronic active hepatitis

Table 6. The status of HBeAg and anti-HBe and CD4/CD8 ratio in patients with HBV associated liver diseases

Viral markers		Number	CD4/CD8 ratio (Mean±SD)
HBeAg	Positive	30	1.38±0.46
	Negative	22	1.32±0.38
Anti-HBe	Positive	15	1.45±0.35
	Negative	34	1.35±0.43

HBV: Hepatitis B virus

cant difference in CD4/CD8 ratios between HBeAg positive individuals and anti-HBe positive individuals (Table 6).

Activities of the peripheral blood suppressor T lymphocytes

Suppressor T lymphocytes activity was 53.3±17.5% in healthy controls, 34.9±23.1% in healthy HBsAg carriers, and 25.3±23.6% in patients with HBVLD (Table 7 and Fig. 2). Con A induced suppressor cell activity, therefore, was significantly impaired in healthy HBsAg carriers ($p<0.005$) and in patients with HBVLD

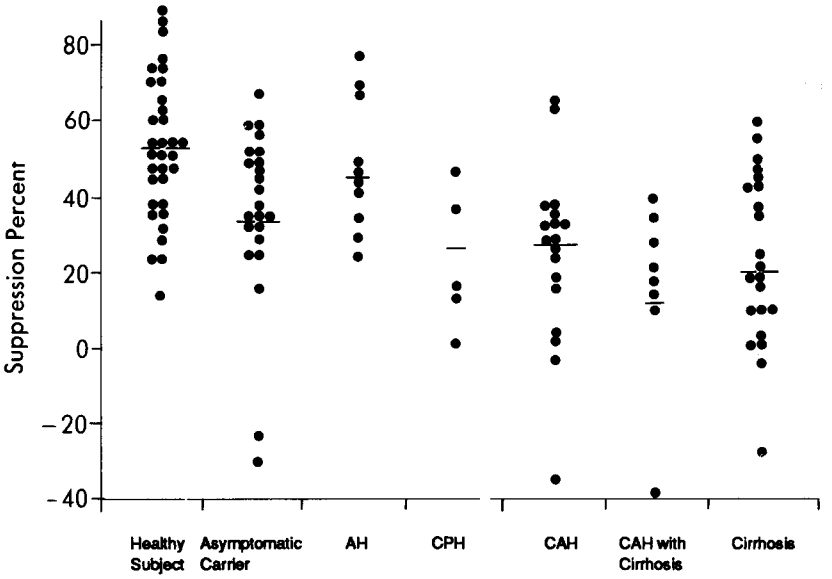

Fig. 2. *The peripheral blood suppressor cell activities in patients with hepatitis B virus associated liver diseases. AH: Acute hepatitis; CPH: Chronic persistent hepatitis; CAH: Chronic active hepatitis. The horizontal bar (–) indicates the mean value of suppression percent.*

Table 7. The peripheral blood suppressor cell activities in patients with HBV associated liver diseases

Groups	Number	Suppression % (Mean±S.D.)
Controls	34	53.3±17.5
Healthy HBsAg carriers	23	34.9±23.1*
Patients with HBV associated liver diseases	63	25.3±23.6**
Acute hepatitis	10	46.1±16.5
Chronic persistent hepatitis	5	23.8±15.6*
Chronic active hepatitis	18	24.1±22.2*
CAH with cirrhosis	8	14.0±22.6*
Cirrhosis	22	21.2±23.6*

HBV: Hepatitis B virus
CAH: Chronic active hepatitis
* $p<0.005$, vs controls (Student's t test)

($p<0.0001$) compared to the healthy controls. In the HBVLD group, it was 46.1±16.5% in patients with acute hepatitis, 23.8±15.6% in chronic persistent hepatitis, 24.1±22.2% in chronic active hepatitis, 14.0±22.6% in chronic active hepatitis with cirrhosis, and 21.2±23.6% in cirrhosis. Suppression was significantly impaired in those with chronic persistent hepatitis ($p<0.005$), chronic active hepatitis with or wihout cirrhosis and cirrhosis ($p<0.0001$), but not in those with acute hepatitis compared to the healthy controls. There was no significant difference in suppressor T lymphocyte activities between those who were HBeAg positive (28.1±23.1%) or anti-HBe positive (24.8±32.3%) (Table 8).

Relationship between CD4/CD8 ratio and suppressor cell activity

There was no correlation between the CD4/CD8

Table 8. The status of HBeAg and anti-HBe and suppressor cell activities in patients with HBV associated liver diseases

Viral markers		Number	Suppression % (Mean±S.D.)
HBeAg	Positive	32	28.1±23.1
	Negative	20	25.3±32.9
Anti-HBe	Positive	14	24.8±32.2
	Negative	38	29.6±25.9

HBV: Hepatitis B virus

Table 9. CD4/CD8 ratio in patients with HBV associated liver diseases according to the positivity of circulating immune complexes

PAT	Number	CD4/CD8 ratio (Mean±S.D.)
Negative	56	1.40±0.48
Positive	27	1.31±0.56

HBV: Hepatitis B virus
PAT: Platelet aggregation test

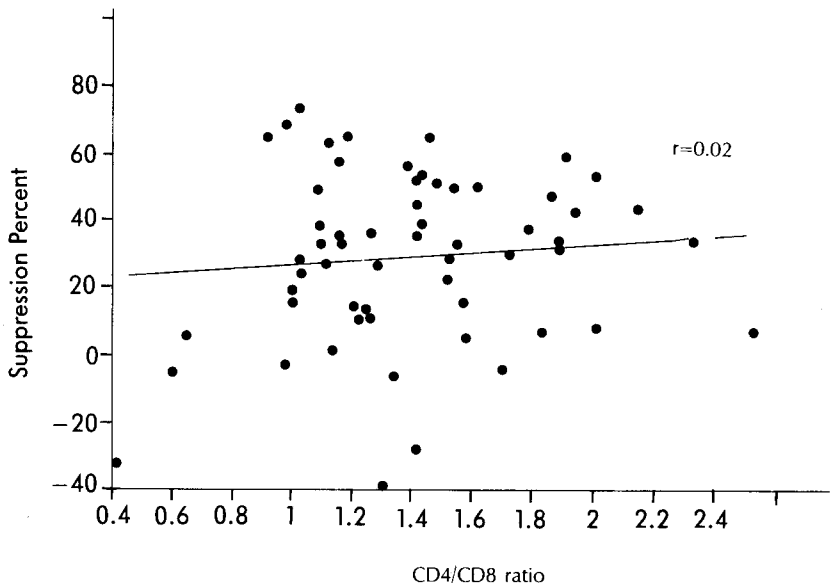

Fig. 3. The realtionship between CD4/CD8 ratio and suppressor cell activity in patients with hepatitis B virus associated liver diseases.

ratio of peripheral blood lymphocytes and the activity of the suppressor T cells (Fig. 3).

Relationship between CIC and CD4/CD8 ratio

In 83 patients with HBVLD, 56 CIC negative patients with HBVLD and 27 CIC positive patients with HBVLD showed CD4/CD8 ratios of 1.40±0.48 and 1.31±0.56, respectively (Table 9). There was no significant difference between the two groups. There was, however, a significant negative correlation between the CD4/CD8 ratio and the titer of CIC in PAT positive patients with HBVLD ($r=-0.47$, $p<0.05$) (Fig. 4).

Relationship between CIC and suppressor cell activity

According to the positivity of CIC in patients with HBVLD, as the suppressor cell activity was 30.0±22.1% in 53 CIC negative cases and 25.8±26.7% in 29 CIC positive cases, there was no significant difference between these groups (Table 10). There was, however, a significant reverse correlation between suppressor cell activity and the titer of CIC in PAT positive patients with HBVLD ($r=-0.42$, $p<0.05$) (Fig. 5).

Table 10. Suppressor cell activities in patients with HBV associated liver diseases according to the positivity of circulating immune complexes

PAT test	Number	Suppression % (Mean±S.D.)
Negative	53	30.0±22.1
Positive	29	25.8±26.7

HBV: Hepatitis B virus
PAT: Platelet aggregation test

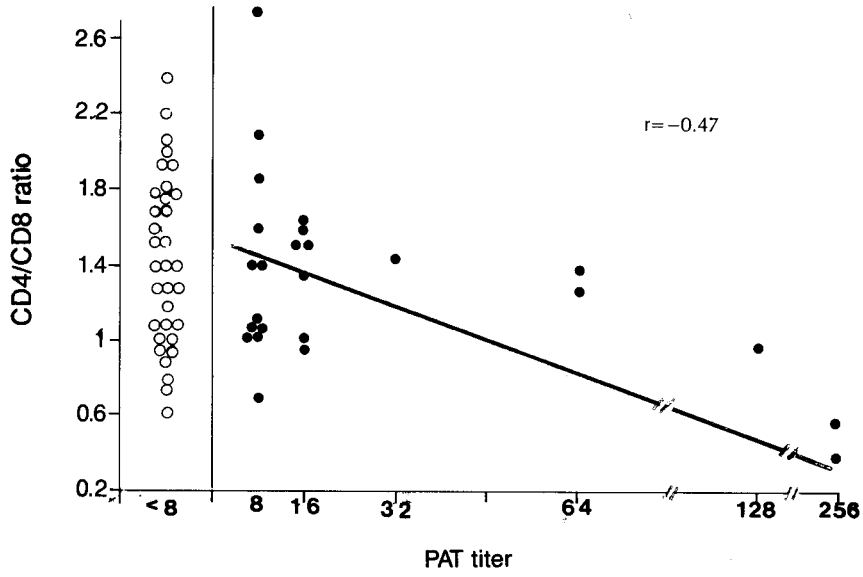

Fig. 4. *The relationship between circulating immune complexes and CD4/CD8 ratio in patients with hepatitis B virus associated liver diseases.*
PAT: Platelet aggregation test
O : PAT negative
● : PAT positive

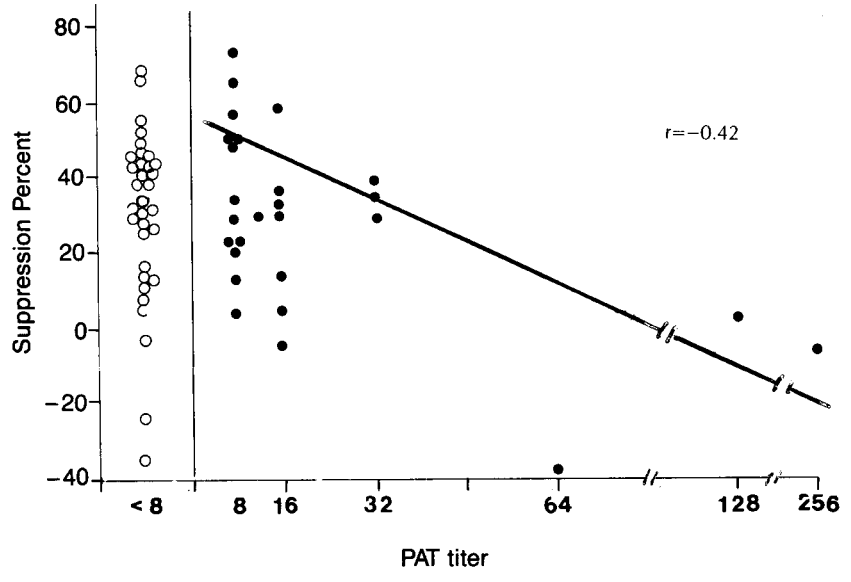

Fig. 5. *The relationship between circulating immune complexes and suppressor cell activity in patients with he-patitis B virus associated liver diseases.*
PAT: Platelet aggregation test
O : PAT negative
● : PAT positive

DISCUSSION

In HBVLD, the presence of CIC has been reported by many authors, but their role in the pathogenesis of this disease is still unclear and the detection frequencies are variable according to the disease spectra. Nydegger *et al.* (1974) reported that CIC were detected more frequently in patients with chronic persistent hepatitis than in normal controls, but in healthy HBsAg carriers the frequency was not different from that in normal controls. Thomas *et al.* (1978) assayed CIC in patients with chronic active hepatitis, chronic persistent hepatitis and healthy controls, and reported that CIC were detected more frequently in patients with chronic active hepatitis than in patients with chronic persistent hepatitis or in healthy controls. Gupta (1982) reported that CIC were detected more frequently in patients with chronic active hepatitis than in patients with acute hepatitis. But Madalinski and Bragiel (1979) reported that the detection frequency of CIC-containing HBsAg (HBsAg-IC) was highest in patients with acute hepatitis and next highest in patients with chronic active hepatitis, in those with chronic persistent hepatitis and in healthy HBsAg carriers. Anh-Tuan and Novak (1981) also reported that HBsAg-IC was detected frequently in patients with acute or chronic hepatitis, but much less frequently in healthy HBsAg carriers, and observed a close correlation between the level of HBsAg-IC and poor prognosis, so that a persistence of HBsAg-IC seems to contribute to predicting a chronic outcome. In patients with chronic hepatitis, the immune reactions may be deficient because they are not effective in eliminating either the virus or the hepatocytes expressing viral antigens (Dudley *et al.* 1972; Aldershvile *et al.* 1977; Dienstag and Isselbacher 1978; Paronetto 1986), but the immune mechanisms and the relation with CIC are still unclear. Sagnelli *et al.* (1983) reported that CIC were significantly increased in chronic active hepatitis compared to that in chronic persistent hepatitis; and in patients with chronic active hepatitis, CIC were detected more frequently in those with cirrhosis that without cirrhosis. In contrast, Abrass *et al.* (1980) and Brown *et al.* (1983) reported that CIC were demonstrated frequently in all patients with liver diseases including those with acute hepatitis, chronic active and persistent hepatitis, and cirrhosis, and that the presense of CIC was not specific for a given type of liver disease and did not correlate with hepatic dysfunction.

In our study, the prevalence of CIC in patients with acute hepatitis and in those with cirrhosis was significantly higher than in the healthy controls or healthy HBsAg carriers (Table 2), so it was compatible with those results of Sagnelli *et al.* (1983). The reasons the patients with acute hepatitis exhibit a high frequency of CIC may be that they cannot eliminate HBsAg effectively in the early stage of the disease, and antibody production may be continued while the source of the antigen persists (Madalinski and Bragiel 1979). The reasons the patients with cirrhosis exhibit a high frequency of CIC may be that firstly, spontaneous porto-caval shunts and a reduction in the number and activity of macrophages in the liver (Thomas *et al.* 1973) may be responsible for this phenomenon since antigens of intestinal origins (food, bacterial, viral and parasitic antigens) may easily circulate under these conditions, with subsequent formation of CIC; and secondly, the hepatic reticuloendothelial system that plays a major role in clearing CIC from the circulation (Mannik *et al.* 1971; Theofilopoulos and Dixon 1980) is impaired in patients with cirrhosis, leading to failure of the host in clearing CIC (Sagnelli *et al.* 1983). So it is suggested that CIC, which may reflect impaired hepatic phagocytic function or the immune clearance of viral antigens or be secondary to autosensitization, play no role in the pathogenesis of liver disease. The almost ubiquitous presence of a variety of complexes in liver diseases of a variety of types including alcoholic hepatitis and primary biliary cirrhosis might suggest that this is so (Thomas *et al.* 1978).

The prevalences of CIC in healthy HBsAg carriers and in patients with liver diseases except acute hepatitis and cirrhosis were not increased compared to healthy controls, and there was no case that exhibited a PAT titer of greater than 1:32 in healthy controls or healthy HBsAg carriers. The result should be interpreted with caution because of the analysis with a small number of patients with chronic persistent hepatitis. It is suggested when the disease activity is low or absent, CIC may be detected but the titer may be not so high.

CIC were not correlated with the titer of serum HBsAg or with the status of the HBeAg or anti-HBe in this study (Fig. 1), and this was compatible with the report that the titer of HBsAg-IC was correlated with the titer of non-specific CIC but not correlated with the titer of HBsAg in serum (Gupta 1982).

In HBVLD, the ratio of helper to suppressor/cytotoxic lymphocytes (CD4/CD8 ratio) in peripheral blood is reduced due to an increased proportion of CD8 positive lymphocytes (Alexander *et al.* 1986) in patients with chronic hepatitis (Thomas *et al.* 1982), in acute and chronic hepatitis (Lemm *et al.* 1983), and in healthy

HBsAg carriers (Alexander *et al.* 1986), and especially in HBeAg positive patients (Thomas *et al.* 1982; Alexander *et al.* 1986). In contrast, some have reported that there was no significant difference in the CD4/CD8 ratio compared to healthy subjects in acute hepatitis or chronic active hepatitis (Regenstein *et al.* 1983; Lee *et al.* 1986), or in healthy HBsAg carriers (Shin *et al.* 1987), and no relationship between the status of HBeAg and the CD4/CD8 ratio in patients with chronic active hepatitis (Regenstein *et al.* 1983; Lee *et al.* 1986). In our study, there was no significant decrease in the peripheral blood lymphocytes CD4/CD8 ratio in healthy HBsAg carriers and in patients with HBVLD compared to healthy controls and no difference according to the status of HBeAg.

With respect to Con A induced suppressor cell regulation of IgG production, Kakumu *et al.* (1980) demonstrated that the suppressor function of T cells of patients with acute hepatitis is normal 1-2 weeks after the onset of the disease but is enhanced after 3-5 weeks. Normalization of the excess suppressor function of T cells was observed when the liver function tests indicated normal ranges. Nouri-Aria *et al.* (1986) reported, however, that the suppressor function was decreased in patients with acute hepatitis. Such contradictory findings may be due to the fact that the populations studied by the above two investigators cannot be correctly matched. In our study, the suppressor function of T cells from patients with acute hepatitis was investigated 1-2 weeks after the onset of the disease, and there was no difference compared to that from healthy controls. Regarding the suppressor cell activities in patients with chronic active hepatitis, some reported there were no differences compared to healthy controls (Nardiello *et al.* 1982), but in general they were reported as decreased (Hodgson *et al.* 1978; Kakumu *et al.* 1980; Chisari *et al.* 1981; Kashio *et al.* 1981; Nonomura *et al.* 1982; Nouri-Aria *et al.* 1986), and those in patients with chronic persistent hepatitis were reported as normal (Kakumu *et al.* 1980; Kashio *et al.* 1981) or as significantly decreased (Nonomura *et al.* 1982; Nouri-Aria *et al.* 1986). The impairment of Con A induced suppressor cell activity on IgG-producing cells could be explained by the altered suppressor cell activity itself or by the presence of an abnormal helper cell population which may have masked suppressor cell activity (Kashio *et al.* 1981). After irradiation of the T cells, known to eliminate suppressor effects, there was, however, no difference in helper cell activity between healthy subjects and chronic active hepatitis (Nonomura *et al.* 1982), suggesting that spontaneous helper activity of chronic active hepatitis was normal. Therefore, the impairment was due mainly to increased suppressor cell activity. The suppressor cell activities were significantly decreased not only in patients with chronic active hepatitis, chronic active hepatitis with cirrhosis, or cirrhosis, but also in patients with chronic persistent hepatitis. However, no relationship could be demonstrated between suppressor cell activity and HBeAg/anti-HBe status in patients with HBVLD. Similar results were observed by Alexander *et al.* (1986).

In the present study, significant defects in suppressor cell function were demonstrated in patients with HBVLD, but there was no comparable alteration in T cell proportions (Fig. 3). This finding could be explained by the fact that a functional defect need not necessarily be accompanied by an alteration in T cell proportion (Alexander *et al.* 1983), or that although there was no change in the total number of peripheral blood lymphocytes reacting with CD8 consisting of suppressor T lymphocytes and cytotoxic T lymphocytes (Reinherz and Schlossman 1980), it is possible that any reduction in the number of suppressor cells may well have been obscured by a concurrent increase in cytotoxic cells (Alexander *et al.* 1986). It is known that cytotoxic T lymphocytes are able to destroy hepatocytes expressing HBsAg and HBcAg on their cell membranes (Alberti *et al.* 1977; Dienstag and Bhan 1980; Eggink et al. 1982; Mieli-Vergani *et al.* 1982; Alexander *et al.* 1986), so cytotoxic T lymphocytes could be involved in the pathogenesis of liver damage in patients with HBVLD (Eggink *et al.* 1982; Thomas *et al.* 1982; Colucci *et al.* 1983; Pape *et al.* 1983). It may, therefore, be important to investigate the lymphocytes cytotoxity to autologous hepatocytes in the study for the immune mechanism in patients with HBVLD.

The relationship between CIC and cell mediated immune response in HBVLD, the severe impairment of T cell function in patients with HBVLD may lead to an abnormal B cell activation (Budillon *et al.* 1983), and the increased B cell activity may account for the presence of CIC and the variety of autoantibodies (Dienstag and Isselbacher 1978) or hyperglobulinemia (Rong *et al.* 1984). Increased CIC may induce the subsequent loss of the suppressor T cell function. However, it was suggested that increased CIC may decrease the suppressor cell function (Hotta *et al.* 1981), and CIC may possibly decrease hepatic cell necrosis by suppression of cell mediated immunity (Araki *et al.* 1982). In the present study, there was a significant reverse correlation between suppressor cell activity and the titer of CIC in PAT positive patients with HBVLD (Fig. 5). Increased CIC seemed to be a

result of impaired suppressor T cell function. But further study would be required to understand the influence of the CIC to suppressor T cells.

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