Peripheral blood mononuclear cells and regulatory T cells in acute viral hepatitis

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SUMMARY During acute viral hepatitis, we observed a significant decrease in OKT4/OKT8 ratio with a significant increase in the OKT8 positive subset in acute type B and non-A-non-B hepatitis. This altered ratio persisted in type B for a long time until HBsAg antibody became detectable, while it soon returned to normal in type A and non-A-non-B hepatitis. In the majority of acute hepatitis the altered ratio is because of an increase and not to a decrease in the whole T cell population, as described in chronic HBV infection. The number of HNK-1 positive cells remained raised during the recovery phase of type B and non-A-non-B hepatitis, a finding consistent with the hypothesis that NK cells play a role in the host defence against B and non-A-non-B virus infections. Serum β_2 -microglobulin concentrations were increased only in acute hepatitis B and non-A-non-B where immunological mechanisms are suspected to be involved, and showed a good correlation with the population of activated OKIa positive cells.

A number of viruses affect the liver as a part of systemic infections – for example, cytomegalovirus, Epstein Barr virus and yellow fever virus – but the term ‘hepatitis viruses’ usually refers to HAV (hepatitis A virus), HBV (hepatitis B virus) and non-A-non-B agents. Hepatitis A virus seems to be directly cytopathic for infected hepatocytes and never leads to chronic infection. Hepatitis B virus infection may result in either acute or chronic infection. As HBV is not directly cytopathic for infected hepatocytes, host determined immunologic response is considered a likely pathogenetic mechanism in this disease.¹ The cell mediated immune response to component antigens of the virus results in the lysis of infected hepatocytes in acute and chronic active hepatitis while only a poor or absent response has been shown in chronic persistent hepatitis and in healthy carriers.² This immune response to the virus may be regulated by the immunoregulatory network. The importance of a balance between inducer and suppressor cells in maintaining immune homeostasis has recently been illustrated and abnormalities in these T cell subsets have been associated with a number of human diseases.³ Recently, the development of monoclonal

antibodies against T cell subsets has allowed the demonstration of a decreased helper to suppressor cell ratio, because of an increase in suppressor cell concentrations, during acute type B hepatitis.⁴ When this ratio returns to normal the HBsAg is cleared and anti-HBs becomes detectable. Similar abnormalities have been found in HBsAg positive chronic hepatitis.^{4,5} We have used similar techniques to analyse peripheral blood lymphocytes and T cell subsets in patients with acute type A, B, and non-A-non-B hepatitis.

Moreover, β_2 -microglobulin (β_2m) was measured in serum samples from our patients at or after the disease onset. This protein has a molecular weight of 12 000 and is non-covalently linked to glycoproteins bearing Class I histocompatibility antigens on almost all cell membranes, especially lymphocytes. It may be considered a potential marker for evaluating T cell activation *in vivo*.⁶ Recently, several reports^{7,8} have suggested that β_2m is a marker of activity in immune mediated liver diseases and might be useful in the study of pathogenic mechanisms of liver injury.

Methods

PATIENTS AND CONTROL DONORS

Forty five (25 men and 20 women) patients affected

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by acute viral hepatitis (five with type A, 31 with type B and nine with type non-A-non-B) were studied. Ages ranged from 16–68 years. serologic studies of HBV and HAV markers (HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe, anti-HAV IgM, anti-HAV total) and β_2m were carried out by radioimmunoassay using commercially available reagent kits (AUSRIA II, AUSAB, CORAB, HBe/anti-HBe, HAVAB-M, HAVAB, Abbott Laboratories; Phadabas β_2 MICROTEST, Pharmacia). HBV-DNA polymerase (DNA-P) activity was measured in patients affected by acute type B hepatitis, using the method originally described by Kaplan *et al.*⁹ and modified by Zanetti *et al.*¹⁰ Patients negative for HBV and HAV markers and for antibodies to cytomegalovirus and Epstein Barr virus were considered to have non-A-non-B hepatitis. Table 1 shows the changes in ALT and serum viral markers in the patients studied. Peripheral blood mononuclear cell populations, T cell subsets and β_2m levels were evaluated in different phases of the disease. Eighteen patients (three with type A, 11 with type B and four with type non-A-non-B) were studied within 20 days from the onset of symptoms, 19 patients (two with type A, 12 with type B, five with type non-A-non-B) were studied within 21–50 days. In addition, eight patients were evaluated in the recovery phase of acute type B hepatitis (three within 51–90 days from the onset of symptoms and five within 91–120 days). The control population consisted of 22 healthy subjects for characterisation of the T cell subpopulations and of 15 healthy individuals for the serum β_2m levels, matched for age and sex, with no history of viral hepatitis and negative for all HAV and HBV serological markers. Serum creatinine

levels were within the normal range in all controls and patients.

ISOLATION OF CELLS

Peripheral blood mononuclear cells were isolated from heparinised venous blood through Ficoll-Hypaque density gradient centrifugation¹¹ and washed three times in RPMI 1640 (Eurobio, Paris) supplemented with 5% heat-inactivated fetal calf serum (FCS) (Gibco, GI, NY), 25 mM Hepes (Gibco), 100 U/ml penicillin and 100 μ g/ml streptomycin (Eurobio).

LYMPHOCYTE SURFACE PHENOTYPE

A suspension of 5×10^6 cells per millilitre was made. The lymphocyte surface phenotype was established by indirect immunofluorescence, as previously described in detail,⁵ using well characterised monoclonal antibodies (Ortho-Labs and Becton Dickinson) against all peripheral blood T cells (OKT3), T helper cells (OKT4), T suppressor-cytotoxic cells (OKT8), Ia antigens (OKIa), monocytes (OKM1), K and NK cells (Leu 7). At least 300 cells were counted for each sample. Quantitation of peripheral blood mononuclear cell subsets was done under code to avoid the introduction of subjective bias.

STATISTICAL ANALYSIS

Data analysis was performed by means of Student's *t* test for unpaired data to compare patients' and controls' peripheral blood mononuclear cells subpopulation mean values, and the χ^2 test to evaluate the relationships between PMBC and serum ALT, γ -globulins and β_2m concentrations. Results are expressed as means $\pm$ SEM.

Table 1 Serum alanine aminotransferase (ALT) and serum viral markers in each group of acute viral hepatitis patients

Patient group	No	ALT* (IU/l)	HBsAg†	HBeAg†	DNA-P†	anti-HBs†	anti-HAV†IgM†
HBV							
0–20 days	11	1450 $\pm$ 245	100%	45%	82%	/	/
21–50 days	12	460 $\pm$ 228	100%	45%	25%	/	/
51–90 days	3	85 $\pm$ 29	66%	/	/	/	/
91–120 days	5	32 $\pm$ 4	/	/	/	100%	/
HAV							
0–20 days	3	180 $\pm$ 89	/	/	/	/	100%
21–50 days	5	38 $\pm$ 2	/	/	/	/	100%
non-A-non-B							
0–20 days	4	157 $\pm$ 27	/	/	/	/	/
21–50 days	5	95 $\pm$ 30	/	/	/	/	/

HBsAg: Hepatitis B surface antigen; HBeAg: hepatitis B 'e' antigen; DNA-P: DNA polymerase activity; anti-HBs: antibody to HBsAg; anti-HAV IgM: antibody to hepatitis A antigen of IgM class.

*: normal range <50 IU/l; data are expressed as mean $\pm$ 1 SEM.

†: figures indicate the percentages of positive patients in each group; vertical bars are equivalent to 0%.

Results

OKT4/OKT8 ratio was significantly reduced in early phases of all acute viral hepatitis groups, as compared with normal controls (Fig. 1). This ratio soon returned to normal in type A and non-A-non-B hepatitis coincident with improvement in transaminase concentrations, while it was persistently low in type B hepatitis during the entire period of HBs antigenaemia. No difference in OKT4/OKT8 ratio was demonstrated in HBsAg positive patients between DNA-P positive and DNA-P negative cases, or between HBeAg positive and HBeAg negative patients (Table 2). Analysis of peripheral blood mononuclear cells subpopulations (Table 3) showed no significant difference in OKT4 positive cells and an absolute increase in OKT8, OKIa, OKM1 and HNK-1 positive subsets in the early phase of type B and non-A-non-B hepatitis. The concentrations of OKT8 positive cells soon declined to only slight increases in those patients with non-A-non-B hepatitis along with the decrease in transaminase concentrations. In type B hepatitis OKT8 positive cells, as well as OKT3, OKIa, OKM1 and HNK-1 positive cells remained abnormally high along with HBsAg persistence and returned to normal when anti-HBs became detectable; only HNK-1 cells remained persistently raised. Patients with type A hepatitis showed no significant difference in lymphocyte populations and in T cell subsets although a transient expansion in OKT8 positive subsets and a reduction of OKT4 positive cell concentrations were observed. No correlation was observed between ALT or serum γ -globulins and T cell subpopulations.

Increased serum β_2 m concentrations were observed during acute type B and non-A-non-B

Fig. 1 OKT4/OKT8 ratios as a function of time after clinical onset in patients with acute viral hepatitis of different aetiology.

hepatitis but never in type A. These high levels soon returned to normal in non-A-non-B hepatitis while they remained persistently abnormal in type B during the period of HBs antigenaemia (Fig. 2). No correlation was detectable between ALT or γ -globulins and β_2 m, while a significant correlation was found between serum β_2 m and OKIa positive cell percentages ($p<0.025$) (Fig. 3).

Discussion

During acute viral hepatitis we observed a significant decrease in the inducer to suppressor/cyto-

Table 2 Mononuclear cell subsets in acute type B viral hepatitis patients grouped according to HBeAg and DNA polymerase status

	OKT3		OKT4		OKT8		OKIa		OKM1		HNK-1		Ratio
	No	%	No	%	No	%	No	%	No	%	No	%	
HBeAg+ve (6)	1426	63.7	805	35.1	613	28.1	281	13.1	325	15.0	246	10.9	1.31
	±148	±2.1	±129	±2.8	±46	±2.0	±38	±1.9	±56	±3.7	±65	±2.4	±0.18
HBeAg-ve (16)	1431	68.4	829	37.4	617	31.9	345	16.7	377	18.6	249	11.7	1.24
	±108	±1.6	±96	±1.8	±24	±2.3	±40	±2.2	±37	±1.5	±34	±1.6	±0.16
DNA-P+ve (11)	1432	65.9	820	35.4	617	30.9	290	14.0	401	18.6	254	10.5	1.25
	±126	±2.1	±101	±2.1	±37	±2.6	±37	±2.4	±48	±2.5	±46	±1.8	±0.14
DNA-P-ve (11)	1425	68.3	822	38.3	613	30.6	329	17.6	308	16.2	241	12.7	1.39
	±122	±1.8	±121	±2.2	±20	±2.5	±45	±2.3	±31	±1.7	±40	±2.0	±0.20
Controls (22)	1290	70.0	835	44.8	468	25.8	198	10.5	180	9.8	186	10.1	1.76
	±90	±1.0	±55	±0.8	±34	±0.7	±21	±0.4	±22	±0.4	±18	±0.7	±0.05

Note. HBeAg : Hepatitis B 'e' antigen; DNA-P : DNA polymerase. Data expressed as mean ± 1 SEM.

Table 3 Mononuclear cell subsets in acute viral hepatitis of different aetiology

	OKT3		OKT4		OKT8		OKIa		OKMI		HNK-1		OKT4
	No	%	No	%	No	%	No	%	No	%	No	%	OKT8
HAV													
0-20 days (3)	1129	70.6	531	32.7	622	39.1	154	10.3	183	13.1	303	19.1	0.85 ^a
	±385	±4.5	±190	±5.2	±216	±1.8	±26	±1.4	±19	±2.3	±135	±5.7	±0.16
21-50 days (2)	1545	63.5	1083	41.8	503	22.6	139	9.4	468	15.8	295	12.8	1.87
	±299	±1.2	±186	±1.8	±222	±2.3	±28	±3.8	±21	±3.0	±89	±0.9	±0.27
HBV													
0-20 days (11)	1383	68.7	775	35.9	611 ^b	33.2	294 ^c	15.3	378 ^a	18.7	272 ^d	12.2	1.19 ^a
	±138	±1.8	±115	±2.3	±36	±2.8	±38	±2.4	±39	±2.1	±52	±2.1	±0.15
21-50 days (12)	1404	64.7	836	37.5	585 ^d	28.0	305 ^c	15.7	309 ^b	16.2	222 ^c	11.4	1.42 ^c
	±119	±1.9	±97	1.6	±35	±1.8	±46	±2.1	±57	±2.1	±33	±1.5	±0.17
51-90 days (3)	2028 ^c	67.3	991	33.0	1037 ^a	34.7	342 ^c	11.6	293	10.2	595 ^a	19.4	0.96 ^a
	±384	±4.4	±182	±1.6	±193	±3.4	±45	±0.7	±33	±1.4	±153	±1.6	±0.04
91-120 days (5)	1221	70.0	789	45.2	429	24.6	208	11.9	170	9.5	530 ^a	18.9	1.85
	±61	±1.2	±42	±1.1	±28	±0.8	±53	±2.9	±38	±1.7	±98	±1.3	±0.09
non-A-non-B													
0-20 days (4)	1814 ^d	65.4	1115	37.1	784 ^b	26.4	537 ^a	19.3	600 ^a	19.4	324 ^b	11.3	1.42 ^c
	±278	±4.8	±218	±3.7	±155	±3.1	±116	±4.5	±242	±5.6	±62	±0.6	±0.07
21-50 days (5)	1842 ^c	66.4	1180 ^b	42.3	669	24.7	399 ^b	14.3	535 ^a	17.8	350 ^c	11.2	2.01
	±246	±2.8	±115	±2.4	±186	±3.9	±86	±1.6	±94	±3.6	±137	±2.8	±0.51
Controls (22)	1290 ^{cd}	70.0	835 ^b	44.8	468 ^{abd}	25.8	198 ^{abc}	10.5	180 ^{ab}	9.8	186 ^{abc}	10.1	176 ^{ac}
	±90	±1.0	±55	±0.8	±34	±0.7	±21	±0.4	±22	±0.4	±18	±0.7	±0.05

P values a: $p < 0.001$; b: $p < 0.01$; c: $p < 0.02$; d: $p < 0.05$.

Data are expressed as mean $\pm$ SEM.

Fig. 2 Serum β_2 -microglobulin concentrations as a function of time after clinical onset in patients with acute viral hepatitis of different aetiology.

toxic T cell ratio, with a significant increase in the OKT8 positive subset in acute type B and non-A-non-B hepatitis as described in other viral diseases.¹² This altered ratio persisted in type B for a long time until HBsAg antibody became detectable, but soon returned to normal in type A and non-A-non-B hepatitis. Because the immune response is

Fig. 3 Relationship between β_2 -microglobulin concentrations and Ia positive cells in acute viral hepatitis patients.

considered a likely pathogenetic determinant in HBV infection, host immunoregulatory mechanisms might be important in recovery from acute viral hepatitis and in perpetuating hepatocyte injury in chronic liver disease. Suppressor cell function studies in acute and chronic HBV infection have produced conflicting findings.¹³⁻¹⁷ These might be explained by differences in selection of patients and controls and in suppressor cell assay methods. Kakumu *et al*,¹⁸ however, have reported an increased suppression of both IgC and IgM synthesis by PWM stimulated peripheral blood mononuclear cells in patients convalescent from acute type B hepatitis. Therefore, although the OKT8 positive cell population includes both suppressor and cytotoxic T cells, our data suggest that the increase in OKT8 positive cells found during HBs antigenaemia may reflect an increase in suppressor cell influence which might limit the immune response to HBV antigens. The immunoregulatory imbalance returns to normal with complete clinical recovery from acute HBV infection but persisted in chronic carriers, as previously described.⁴⁻⁵ In patients with chronic HBV infection, however, the altered ratio was based primarily on a reduction of the helper (OKT4 positive) subset of lymphoid cells⁴⁻¹⁹ and not on increased OKT8 positive cells number as observed in acute hepatitis. Therefore, in the majority of acute hepatitis the altered ratio is due to an increase and not a decrease in the whole T cell population. Recently, Fahey *et al*²⁰ suggested that homosexual patients with acquired immunodeficiency syndrome (AIDS) and pneumonia or Kaposi's sarcoma (all showing decreased OKT4 positive cells) had a poor prognosis, whereas healthy homosexual men with an expanded OKT8 positive subset, probably reflecting viral infections from cytomegalovirus, HBV or widespread exposure to other infectious agents, had an uncertain but probably better prognosis.

Additional studies on suppressor and helper T cell functions specific for HBV antigens may lead to a better comprehension of the immunoregulatory mechanisms involved in this disease. Moreover, the finding that the subpopulation of inducer cells responsible for maximal helper activity for B cell differentiation is restricted to a minor subset of OKT4 positive cells,²¹⁻²² and that the induction of suppression may require OKT4 positive cells²³ further complicates the interpretation of the findings.

The increased numbers of M1 and HNK-1 cells in acute type B and non-A-non-B hepatitis, even considering patients' age,²⁴ suggest the need for further investigations to evaluate the possible role of K and NK cells in acute viral hepatitis. The

persistence of raised HNK-1 cell numbers during recovery from type B hepatitis, when anti-HBs becomes detectable, is consistent with the finding of increased cytotoxic activity by NK cells against an HBsAg secreting hepatoma cell line in convalescent hepatitis B patients.²⁵ These data support the hypothesis that NK cells play a role in the host defence responses to HBV infection.

The normal peripheral blood mononuclear cell populations and behaviour of T cell subsets in patients with acute type A hepatitis are consistent with the view that immune mechanisms are not involved in the pathogenesis of hepatitis A liver injury. On the other hand, the significant alterations in immunoregulatory cells in the early phase of acute type non-A-non-B hepatitis could reflect the involvement of immunological mechanisms in this disease.

We have shown that serum β_2m concentrations were different in the three types of viral hepatitis as shown by Beorchia *et al*,²⁶ normal values were found in hepatitis A cases, significantly raised concentrations were observed in hepatitis B, and non-A-non-B. Bernier and Fanger²⁷ first demonstrated that β_2m was mainly a product of T lymphocytes as it was produced by PHA but not by PWM stimulated peripheral blood lymphocytes. This has recently been confirmed using a different system.⁶ It is concluded that β_2m release reflects primarily T cell activation. The increase in serum β_2m concentrations in our patients was due to augmented production and not decreased renal catabolism: neither proteinuria nor any evidence of renal insufficiency were present. Serum β_2m concentrations increased only in acute hepatitis where immunological mechanisms are suspected to be involved, and the correlation between β_2m and OKIa positive cell number suggests that activated lymphocytes and not liver cell necrosis is responsible.

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