Activities of 2'-5'Oligoadenylate Synthetase in Peripheral Blood Mononuclear Cells of Patients with Viral Hepatitis, and Chronic Type B Hepatitis during Interferon Therapy

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The activities of the interferon-induced enzyme 2'-5' oligoadenylate synthetase in peripheral blood mononuclear cells were determined in patients with viral hepatitis. Increased levels of the enzyme were found in patients with acute type B hepatitis at the early phase and also in those with chronic type B hepatitis at the active stage. The levels of this enzyme activity were not significantly increased in patients with acute type A hepatitis or in those with acute type non A non B hepatitis. However, in two patients with acute type A hepatitis the levels of this enzyme were elevated shortly at the onset. These results support the hypothesis that an endogenous interferon response in patients with acute type A hepatitis and in those with acute type B hepatitis may be instrumental in the modulation of these types of hepatitis virus infections. Whereas hepatitis non A non B virus replication in patients with acute type non A non B hepatitis may be associated with poor interferon response. The activities of the enzyme in peripheral blood mononuclear cells were determined in patients with chronic type B hepatitis during interferon therapy. Increased levels of the enzyme were found in all patients during interferon therapy. This increase correlated well with the decreased DNA-polymerase activities. The data also showed that assay of this enzyme activity is useful to determine the optimal dosage and means of interferon therapy for chronic type B hepatitis.

Key Words: 2'-5' Oligoadenylate synthetase, Interferon, Human lymphoblastoid interferon, HLB1, Viral hepatitis

Interferon (IFN) plays an important part in the defense system against viral infections. Detection of IFN in serum, therefore, can be a useful diagnostic aid in viral diseases. However, IFN is sometimes undetectable because of the low level of serum IFN and its rapid clearance from the blood. The action of IFN is characterized by the induction of specific antiviral proteins such as 2'-5' oligoadenylate synthetase (2-5AS). In vitro, the activities of this interferon-induced enzyme, 2-5AS, were determined in interferon-treated cells, and were found in lymphocytes from normal

mouse spleen that had received neither exogenous IFN nor its inducers¹⁾. In vivo, increased levels of this enzyme were observed in peripheral blood mononuclear (PBM) cells of patients with naturally occurring viral diseases, and also in those with autoimmune diseases^{2, 3)}. Although IFN was cleared rapidly from the blood, the activity levels of this enzyme remained elevated in PBM cells for a prolonged period⁴⁻⁶⁾. Thus, detection of 2-5AS has been a sensitive indicator of antiviral effect. Our study was designed to investigate the role of IFN in the course of viral hepatitis, and to attempt

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Received for publication October 2, 1985.

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to correlate the activities of 2-5AS in PBM cells with the activities of Dane particle associated DNA polymerase (DNA-P) in sera of patients with chronic type B hepatitis during IFN therapy.

MATERIALS AND METHODS

Patients:

Study 1: Fifty-six patients with viral hepatitis were studied. Five had acute type A hepatitis (AH type A), 16 had acute type B hepatitis (AH type B), 8 had acute type non A non B hepatitis (AH type NANB) and 27 had chronic type B hepatitis. No patient had been treated with antiviral therapy (IFN or IFN inducers, etc.) or corticosteroids. Thirty-three healthy HBsAg-negative adults were studied as a normal control group. Nine patients with chronic type B hepatitis were studied twenty-four hours after the first injections of 3.0×10^6 IU human leukocyte IFN- α (HuLIFN- α) intramuscularly as a positive control group.

Study 2: Four patients with chronic type B hepatitis were studied during therapy of human lymphoblastoid IFN- α (HLBI). HLBI was prepared by Sumitomo Pharmaceuticals (Osaka, Japan). The patients were given HLBI for 4 consecutive weeks intramuscularly in a dose of 3.0×10^6 IU, 6.0×10^6 IU, 9.0×10^6 IU or 12.0×10^6 IU per day.

Preparation of peripheral blood mononuclear cell extracts:

A sample of 5 to 10 ml venous blood was taken from each subject and heparinized. PBM cells were isolated by Ficoll-Hypaque gradient centrifugation immediately. The cells were washed twice in 5 ml phosphate buffered saline (PBS) and counted in a hemacytometer. They were pelleted in a micro-test tube and frozen at -80°C until the protein assay was started. After the cells were suspended at 5×10^6 cells in about 0.1 ml lysis buffer; 20 mM Hepes buffer, pH 7.5, 5 mM MgCl_2 , 120 mM KCl, 1 mM dithiothreitol, 10% (v/v) glycerol containing 0.5% (v/v) Nonidet P-40 (NP-40), lysis was carried out at 4°C and the extract was centrifuged at 10,000 rpm for 15 min. The supernatant contained 1 to 2 mg/ml of protein as measured by the method of the protein assay kit (Bio-Rad, Richmond, Calif.). The supernatant was used either immediately for the enzyme assay, or kept frozen

at -80°C .

Assay for 2'-5' oligoadenylate synthetase activity:

Synthetase activities were determined by the column method as described previously^{1, 3, 7, 8} with slight modifications. Poly (rI): (rC)-agarose beads (PL-Biochemicals), washed in several volumes of reaction buffer A, 10 mM Hepes buffer, pH 7.5, 100 mM KCOOH, 25 mM $\text{Mg}(\text{COOH})_2$ and 1 mM dithiothreitol, were distributed in 25 μl aliquots in Eppendorf Standartips (1–100 μl). Aliquots (50 mg protein) of the supernatants were applied to a column of Poly (rI): (rC)-agarose beads, and then the beads were washed three times with reaction buffer A, Reaction buffer B (20 μl), which contained reaction buffer A, 100 nmol ATP and [^3H]ATP 1.25 μCi , was then added to the beads. Then, the beads were incubated at 33°C for 2 hours. After incubation, 2 ml of 90 mM KCL-20 mM Tris-hydrochloride (pH 7.5) were applied to the column. The eluate was passed through a column of DEAE-cellulose (DE-52; Whatman Inc., Clifton, N.J.) which was equilibrated with this buffer. The columns were washed with 10 ml of the same buffer and with 5 ml of 100 mM KCL-20 mM Tris-hydrochloride (pH 7.5) buffer, and then eluted with 2 ml of 350 mM KCL-20 mM Tris-hydrochloride (pH 7.5) buffer. The effluent was collected in scintillation vials and counted in the [^3H]-channel of a scintillation counter by using 10 ml of PCS solubilizer (Amersham Corp., Arlington Heights). The enzyme activity was represented in nanomoles of polymerized ATP per milligram of protein per hour.

Assay for Dane particle associated DNA polymerase activity:

The activity of DNA-P was measured by the method of Kaplan⁹ with slight modifications. The level of DNA-P activity was expressed as counts (cpm) of tritium-labeled deoxynucleotide triphosphate incorporated into an acid-insoluble form per 3 hours per 0.5 milliliter by hepatitis B virus particles pelleted from 2 ml of serum.

The nonparametric Wilcoxon test was used for statistical analysis.

RESULTS

The results of study 1 are shown in Table 1 and

Table 1. The level of 2-5AS activity in PBM cells of patients with viral hepatitis compared with that of HBsAg negative healthy controls

	Total no. of patients	Enzyme activity level (mean $\pm$ S.D.)	Wilcoxon test
Healthy controls	33	13.8 $\pm$ 9.6	
AH type A	5	34.0 $\pm$ 23.5	NS*
AH type B	16	27.0 $\pm$ 18.9	P < 0.05
AH type NANB	8	9.4 $\pm$ 4.3	NS
CH type B	27	33.1 $\pm$ 38.0	P < 0.01
Positive controls**	9	55.6 $\pm$ 18.4***	P < 0.01

*NS, not statistically significant.

**Patients with chronic type B hepatitis treated with HuLIFN- α .

***Data are the enzyme activity levels at 24 hours after first injection of 3.0×10^6 IU HuLIFN- α .

Figure 1. As previously shown¹⁰⁾, 2-5AS activities were constantly detectable in PBM cells from the healthy controls (13.8 $\pm$ 9.6). The mean level of this enzyme activity was higher in the patients with AH type B (27.0 $\pm$ 18.9) than in the healthy controls ($p < 0.05$), and was higher in patients with CH type B (33.1 $\pm$ 38.0) and in the positive controls treated with HuLIFN- α (55.6 $\pm$ 18.4) than in the healthy controls ($p < 0.01$). Three patients with CH type B had very high levels of this enzyme activity; the values were higher than those of the positive controls. The mean level (9.4 $\pm$ 4.3) of 2-5AS activity in patients with AH type NANB was similar to the control value. Three patients with AH type A had increased activities of the enzyme in the early phase; no difference in the mean level of this enzyme activity was found between patients with AH type A and the healthy controls. The same trend was found in patients with AH type B, but patients with AH type NANB had low levels of this enzyme activity in the early

* Each bar is the mean level.

Fig. 1. Distribution of 2-5AS activity in PBM cells of patients with viral hepatitis. Fifty-six patients were studied: 5 had acute type A hepatitis [AH(A)]; 16 had acute type B hepatitis [AH(B)]; 8 had acute type NANB hepatitis [AH(NANB)]; 27 had chronic type B hepatitis [CH(B)].

Table 2. The level of 2-5AS activity in PBM cells of patients with chronic type B hepatitis during HLBI therapy

	Age	Sex	Dose of IFN (IU/day)	Before	Enzyme activity level during IFN therapy	
					Average	Peak
Case 1	34	male	3.0×10^6	22.7	32.9	44.2
Case 2	40	female	6.0×10^6	13.7	88.2	108.4
Case 3	27	female	9.0×10^6	33.8	172.0	221.5
Case 4	31	male	12.0×10^6	22.7	129.2	159.0

phase (Fig. 2).

Results of study 2 are given in Table 2 and Figure 3. The latter shows the changes of 2-5AS in PBM cells and DNA-P in sera of patients receiving HLBI therapy. The enzyme levels were increased remarkably at 12 hours after the first injection at the onset of therapy, and remained elevated during therapy. Induced 2-5AS levels were associated with the dosage of HLBI. This increase in enzyme levels correlated well with the decreased DNA-P activities. Two weeks later the DNA-P activities in

sera were undetectable in 2 patients who received daily injections of 9.0×10^6 IU or 12.0×10^6 IU HLBI. But 2 patients who received daily injections of 3.0×10^6 IU or 6.0×10^6 IU HLBI had high levels of DNA-P activity at the end of the treatment, though the levels were decreased below 50 percent of the initial values.

DISCUSSION

Since Isaacs et al¹¹⁾ first demonstrated that virus infection induced the activity of interferon, a number of researchers have reported that interferon plays an important part in the inhibition of virus replication, though the mechanism by which it exerts an antiviral effect remains to be elucidated. In recent years the importance of interferon-induced enzyme 2'-5' oligoadenylate synthetase has been more closely evaluated in connection with antiviral function and inhibition of cell growth. Sokawa et al have shown that the activities of both 2-5AS and interferon parallel viral titers during the acute phase of herpes simplex virus infection in the trigeminal ganglia of mice after cheek skin inoculation⁸⁾. Many studies have reported that the levels of this enzyme activity are elevated in PBM cells of patients with viral dis-

Fig. 2. Change of 2-5AS activity in PBM cells of patients with acute type A hepatitis [AH(A)], acute type B hepatitis [AH(B)] or acute type NANB hepatitis [AH(NANB)] during the course of the disease.

Fig. 3. Change in the activity level of 2-5AS (broken line) in PBM cells and DNA-P (solid line) in sera of patients with chronic type B hepatitis during HLBI therapy. The patients were given a dose of 3.0×10^6 IU ($\circ$), 6.0×10^6 IU (Δ), 9.0×10^6 IU (Δ) or 12.0×10^6 IU ($\bullet$).

eases and also in those with autoimmune diseases [Schattner et al²], Fujii et al³]. In a few studies, the levels of 2-5AS activity in PBM cells of patients with viral hepatitis were measured. However, up to now, there has been no study showing that the levels of this enzyme activity in PBM cells of patients with viral hepatitis related hepatitis B virus (HBV) are elevated.

In the present study it has been shown that the activities of 2-5AS are elevated in PBM cells of patients with acute type B hepatitis at the early phase and also in those with chronic type B hepatitis at the active stage. The enzyme activities appear shortly after the onset of acute type B hepatitis, and thereafter decline with the decrease of the level of SGPT. The enzyme activities were elevated in PBM cells of patients with acute type A hepatitis in the early phase; no difference in the mean level of enzyme activity was found between patients with acute type A hepatitis and healthy controls. These results support the hypothesis that an endogenous interferon response in patients with acute hepatitis type A or type B may be instrumental in the modulation of these types of hepatitis virus infections. On the other hand, the enzyme activities were low in patients with acute type NANB hepatitis. This result may be caused by a decreased production of interferon. More data is needed before the clinical implications of these findings become clear.

Since the initial report on experimental interferon therapy for chronic type B hepatitis by Greenberg et al¹², a number of investigators have reported the effect of interferon therapy on chronic type B hepatitis [Weimer et al¹³, Matsushima et al¹⁴]. There have been no studies to determine the levels of 2-5AS activity in PBM cells of patients with chronic type B hepatitis during interferon (HLBI) therapy. We have observed increased levels of enzyme activity in all patients during HLBI therapy and shown that this increase correlated well with decreased DNA-P activities and also with the dosage of HLBI. The present results verify that assay of this enzyme activity in PBM cells is useful to determine the optimal

dosage and means of administration of interferon therapy.

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