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Trial of Specific Antigen-Mediated Leukocyte Adherence Inhibition Test in Patients with Chronic Active Hepatitis and Hepatitis B Carrier

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In the pathogenesis of chronic active hepatitis, the importance of cell mediated immunity (CMI) has been emphasized. Leukocyte adherence inhibition (LAI) assay, which is one of the methods for analysis of the reaction of CMI, has been used to analysie the CMI of cancer patients.

The authors tried the specific antigen-mediated LAI assay in 40 patients with chronic active hepatitis, 19 patients carriers of hepatitis B virus, and in the 5 persons who have no anti-HBs in spite of receiving vaccination against HBV, and these were compared with 7 normal control subjects who had been exposed to hepatitis B virus previously.

The results were as follows:

1) The NAI was 52.4±14 (mean ± standard deviation) in 7 normal control subjects who had been exposed to hepatitis B virus previously.

2) The NAI was 20.6±11 (mean ± standard deviation) in 40 patients with chronic active hepatitis. The value was significantly lower than that of the normal control group ($P<0.001$).

3) The NAI was 49.7±17.8 (mean ± standard deviation) in 19 patients carriers of hepatitis B virus. The value was not significantly different from that of the normal control group ($P>0.05$).

4) The NAI was 25.1±11 (mean ± standard deviation) in 5 persons who have no anti-HBs in spite of receiving vaccination against HBV. The value was significantly lower than that of the normal control group ($P<0.05$).

5) In patients with chronic active hepatitis and hepatitis B virus carriers, we checked the LAI assay serially. The value of NAI was increased according to the improvement of clinical symptoms and normalization of transaminase, but the value of NAI was decreased according to the worsening of clinical symptoms and elevation of transaminase.

Key Words : *Leukocyte adherence inhibition (LAI) assay, Nonadherent index (NAI), Hepatitis*

INTRODUCTION

In the pathogenesis of chronic active hepatitis, the importance of CMI has been emphasized. LAI assay, which is one of the methods for analysis of the reaction of CMI, has been used to analysis the CMI of cancer patients.

This LAI assay has been introduced by Halliday and Miller in 1972 for the detection of cell mediated anti-tumor immunity[1,2]. Although there are variations in application, this method has advantages of specificity for the analysing of CMI. This method measures an antigen induced, decreased ability of leukocytes to adhere to glass surfaces when exposed to antigens against which the leukocytes have been sensitized. The authors

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tried the LAI assay to get the relationships between the LAI reactions and the prognosis of patients who have chronic active hepatitis, the carriers of hepatitis B virus and the patients who have no anti-HBs in spite of receiving vaccinations against HBV.

SUBJECTS

The study group consist of the patients with chronic active hepatitis and hepatitis B virus carriers and patients who have no anti-HBs in spite of receiving vaccination against HBV, who have been hospitalized or visited the Internal Medicine department from Dec. 1984 to Oct. 1985 at Chonbuk National University Hospital. The authors tried the LAI assay divided the patients into 4 Groups. Group I consisted of 7 normal control subjects who are asymptomatic but exposed to hepatitis B virus previously. Group II consist of 14 patients who had been diagnosed as having chronic active hepatitis by liver biopsy and 26 patients who were strongly suspected of having chronic active hepatitis by clinical manifestations.

Group III consisted of 19 patients who were positive to HBsAg or HBe Ag or only positive to HBsAg. Group IV consisted of 5 patients who have no anti-HBs for 6 months in spite of receiving HB vaccination 3 times (Table 1).

METHODS

All LAI methods fall into one of three general categories : the hemocytometer, microplate, or tube method. We tried the tube LAI method. Ten ml of blood samples were collected from patients who had fasted for 12 hours and LAI assay was done immediately. Blood samples were diluted to one half by adding phosphate buffer saline (PBS) and leukocyte suspension (8×10^6 cells/ml) was made by adding Ficoll-hypaque. The assay is performed in 20 ml, 16×150 mm glass test tubes (Kimax) in triplicate. To each set of three tubes was added 0.1 ml of either the specific (hepatitis B vaccine) or nonspecific antigen (polio vaccine), and 0.1 ml of the suspended peripheral blood leukocyte (PBL). The tubes are well agitated, laid horizontally and then placed in a incubator at 37 C. Two hours later the tubes are removed and stood vertically and the contents at the bottom were gently agitated with a Pasteur pipette. Samples of cells are placed on a hemocytometer with a specially marked surface, and the cells counted. After we count the number of cells we calculate the NAI to express the magnitude of the LAI reaction.

$$\text{NAI}\ (\%)=\frac{A-B}{B}\times100$$

where A ; a sample of the number of nonadherent cells in the presence of a specific antigen.

B ; a sample of the number of nonadherent cells in the pressence of a nonspecific antigen.

Statistical analysis : The value of NAI was expressed as mean ± standard deviation. Statistical significance of the difference between means was determined by t test.

RESULTS

1) The NAI was 52.4±14 (mean ± standard deviation) in 7 normal control subjects who had been exposed to hepatitis B virus previously (Table 2).
2) The NAI was 20.6±11 (mean ± standard deviation) in 40 patients with chronic active hepatitis. The value was significantly lower than that of the normal control group ($P<0.001$) (Table 3-1, 3-2).
3) The NAI was 49.7±17.8 (mean ± standard deviation) in 19 patients with hepatitis B carrier. The value was not significantly different from that of the normal control group ($P>0.05$) (Table 4).
4) The NAI 25.1±11 (mean ± standard deviation) in 5 persons who had no anti-HBs in spite of receiving vaccination against HBV. The value was significantly lower than that of the normal control group ($P<0.05$) (Table 5).
5) In patients with chronic active hepatitis and hepatitis B carriers, we did the LAI assay serially. The value of NAI increased according to the improvement of clinical symptoms and normalization of the transaminase, but the value of NAI decreased according to the worsening of clinical symptoms and elevation of transaminase (Table 6, Fig. 1).

From the above result, we observed the dramatically decreased value of CMI in patients who have chronic active hepatitis and have no anti-HBs in spite of receiving vaccination against HBV. We also observed that the LAI assay can be used as an index of the prognosis for the hepatitis

B carrier or for the chronic persistent hepatitis cases which are progressing into chronic active hepatitis.

DISCUSSION

Chronic hepatitis is defined as a chronic inflammatory reaction in the liver, as shown by liver function tests and histologic studies, and that continues without improvement for at least 6 months[3].

A group of European histopathologists and clinicians in Zurich in 1968 classified chronic hepatitis into chronic active hepatitis and chronic persistent hepatitis[1].

Table 1. Comparison of the Mean Age, Sex Ratio and Laboratory Data among the Groups

Parameter	Group I	Group II	Group III	Group IV
Mean age(years)	25	32.2	30.7	34.4
Sex (M:F)	6 : 1	36 : 4	15 : 4	4 : 1
sGOT (KU)	18	122	30	35.2
sGPT (KU)	32	259	42	55
HBs Ag	-	+	+	-
HBe Ag	-	+	+ or -	-
Anti-HBs	+	-	-	+

*KU : Karmen unit
Group I : 7 normal control subjects who had been exposed to the hepatitis B virus previously
II : 40 patients with chronic active hepatitis
III : 19 patients with hepatitis B virus carrier
IV : 5 persons who have no anti-HBs in spite of receiving vaccination against HBV

Chronic active hepatitis is marked by chronic inflammatory infiltration involving portal zones and extending into the parenchyma with piecemeal necrosis and formation of intralobular septa. Chronic active hepatitis can be progressing to liver cirrhosis and hepatoma.

Also it occasionally can be progressing toward hepatic failure. The pathogenesis of chronic active hepatitis is still unknown but the person developing chronic hepatitis B would be expected to have some deficiency of cell based immunity. This applies to neonates, many of whom become chronic carriers after developing hepatitis B in the perinatal period[5].

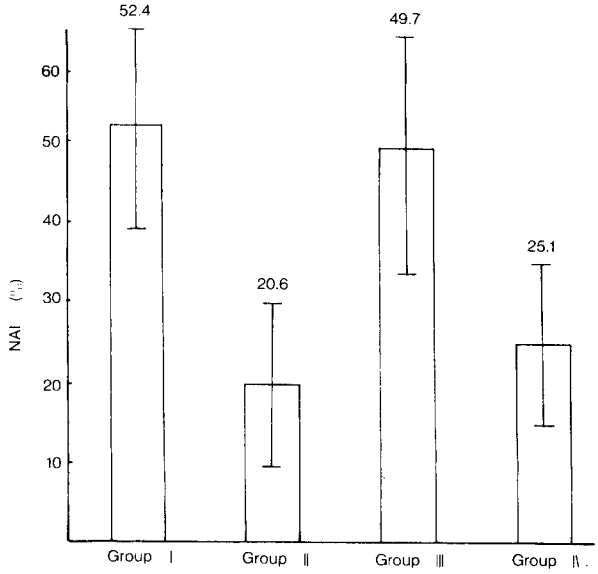

Fig. 1 *Comparison of the value of nonadherent index (NAI) among groups.*
Group I-IV : See Table 1

Table 2. Nonadherent Index (NAI) in Patients who were Exposed to Hepatitis B Virus in the Past

Name	Age	Sex	HBs Ag	HBe Ag	Anti HBc	Anti HBs	NAI (%)
C. S. S	23	M	-	-	-	+	40.6
S. O. U	23	M	-	-	-	+	52.4
S. O. S	23	M	-	-	-	-	77.4
O. Y. I	27	M	-	-	-	+	48.2
L. N. S	26	F	-	-	-	-	32.6
Y. G. H	30	M	-	-	-	+	51.4
L. C. H	23	M	-	-	-	+	64
Mean	25	7					52.4±14

M : Male, F : Female, Ag : Antigen, Anti- : Antibody

It also applies to patients suffering from diseases that depress immunity, such as renal failure, malignant disease or leukemia, and especially those receiving corticosteroids or cancer chemotherapy[6].

It is well known that korea is an endemic area of acute B viral hepatitis and HBs Ag positive carriers. The authors tried LAI assay on patients with HBs Ag positive chronic active hepatitis and HBs Ag positive carriers because these diseases seem to have close relations with a defect of CMI. This LAI assay is methodically simple, specific, rapid, and consists of the tube LAI method, hemocytometer and microplate method[7]. In the LAI technique, there were two methods. One is the direct LAI test and the other is the indirect or two stage LAI test[2,8].

The direct LAI test involves the interaction of sensitized leukocytes with antigens in tumor extracts, so that the glass adherence of these leukocytes is diminished. In the indirect LAI test, the mixtures of cells and tumor extract were incubated for 60 minute at 37 c and centrifuged. The resulting supernatent was tested. The supernatent has the Leukocyte Adherence Inhibition Factor (LAIF) and it inhibits adherence of leukocytes. We used the tube LAI assay and the direct interation of sensitized leukocytes with the antigen (whether mediated via the receptor, which is an integral part oif the cell membrane, or via cell-bound cytophilic antibody) seems to be the mechanism of the tube LAI reaction[2,8]. Human anti-tumor immunity is readily assayed by LAI assay[9,10]. It is based on the phenomenon that leukocytes from cancer patients when incubated in vitro with extracts of tumors arising in the same organ and of the same histogensis lose their property of adherence to glass surfaces[11]. The specificity of the anti-tumor immune response directed at human organ-specific neoantigens (OSN) has been proven unquestionably[12~17]. In the tube LAI assay, patients with early cancer usually have a detectable LAI response, whereas patients with advanced cancer seldom respond. Recently we discovered that if the intracellular nucleotides of the leukocytes from patients with advanced cancer were increased, they regained the specific LAI reactivity to the OSN[18]. Thus, the excess circulating OSN in advanced cancer induces biochemical and physiological changes in most leukocytes that can be quickly reversed by a brief incubation with prostaglandin E_2 or aminophylline[18~21].

This means that we now perform two types of tube LAI assays one without and another with PGE_2 stimulation. Tube LAI assay has been used in

Table 3-1. Nonadherent Index (NAI) in Patients with Chronic Active Hepatitis Diagnosed by Liver Biopsy

Name	Age	Sex	HBs Ag	HBe Ag	Anti-HBc	SGOT	SGPT	NAI (%)
K. J. L	44	M	+	+	+	310	640	25.7
M. K. H	16	M	+	+	−	142	370	9.7
C. S. U	37	M	+	+	−	112	199	16
K. O. S	37	M	+	+	−	98	520	21.7
S. J. H	50	M	+	+	−	111	75	10
K. T. G	30	M	+	+	+	75	130	30
Y. B. O	33	M	+	+	−	180	340	8
Y. B. Y	44	M	+	+	+	65	94	4.5
S. B. Y	28	F	+	+	−	275	364	4.6
K. S. G	32	M	+	+	−	48	94	19.4
S. K. Y	23	M	+	+	+	68	84	19.4
K. M. H	20	M	+	+	−	88	160	25
K. G. S	22	F	+	+	−	198	490	9.1
H. G. Y	30	M	+	+	−	160	520	31.4
Total and Mean	31.9	14				113.5	240	16.8+9.2

M : Male, F : Female, Ag : Antigen, Anti : Antibody
sGOT : Serum oxaloacetic transaminase (Karmen unit)
SGPT : Serum glutamic pyruvate transaminase (Karmen unit)

patients with breast cancer, colorectal cancer, stomach cancer, pancreatic cancer and prostatic cancer[9,22~24].

Especially, initial studies concerned with cell-mediated immunological responsiveness in prostatic cancer relied on the inhibition of leukocyte migration (Ablin[25] 1976). Immunological studies of patients with prostatic cancer, as recently reviewed by Ablin and Bhatti in 1981, have provided evience of a host response to the tumor[26,27]. LAI assay appears to possess a significantly high degree of tissue and disease specificity and can detect cell-mediated tumor-associated immunity in patients with cancer of the prostate. In 79% of the patients with prostatic cancer possessing significant level of reactivity to tumor-associated antigen of malignant prostate accuracy was greater than that obtained with studies employing serum acid phosphatase, where the incidence of sensitivity ranged from 29% to 56% with enzyme immunoassay (EIA)[28,29]. LAI assay has been used in patients with rheumatoid arthritis. Rheumatoid arthritis is a systemic inflammatory disease of connective tissue in which the striking clinical manifestation is the tendency to produce lesions in joints and periaticular structures, and the etiology of rheumatoid arthritis remains unknown[30]. Although there is no firm evidence that primary abnormality in rheumatoid arthritis is an immunological one, there is little doubt that the immunological process plays an essential role in the pathogenesis of the synovitis. LAI assay in patients with rheumatoid arthritis was to analyze

Table 3 2. Nonadherent Index (NAI) in Patients with Suspected Chronic Active Hepatitis that didn't Receive a Liver Biopsy

Name	Age	Sex	HBe Ag	HBs Ag	Anti-HBc	SGOT	SGPT	NAI (%)
K. O. U	G	M	-	-	+	246	285	23.1
L. O. U	43	M	-	-	-	42	82	21.1
H. O. G	38	M	-	+	-	46	122	16.6
O. I. B	45	M	-	+	-	75	116	33.3
K. O. S	36	M	-	+	-	187	620	20
K. O. H	20	M	-	+	-	71	202	38.8
S. O. H	17	M	-	+	+	103	212	30
M. O. G	34	M	+	+	-	150	71	33.3
Y. G. H	29	M	-	+	-	76	157	10.5
Y. O. G	23	M	+	+	+	49	78	33.3
K. G. G	29	M	+	-	+	280	630	33.3
S. I. S	38	M	+	+	-	180	370	12.5
B. C. S	29	M	+	+	-	81	278	8.6
H. H. G	38	M	-	+	+	61	200	21.4
Y. G. S	33	M	+	+	+	148	526	23.5
T. O	52	M	-	+	-	89	152	9.1
Y. L. Y	42	M	-	+	+	43	112	10
L. Y. L	37	M	+	+	+	247	438	18.9
K. G. B	28	M	+	+	-	270	600	28.6
Y. Y. G	45	M	+	+	+	46	140	12
L. Y. G	25	M	+	+	+	290	690	48.3
H. H. D	25	M	+	+	+	153	440	16
S. H. L	41	M	+	+	+	53	110	11.1
G. G. Y	45	M	+	+	-	75	116	13.3
S. S. I	29	M	+	+	+	208	445	17
L. S. M	29	F	+	+	-	22	60	39.3
Total & Mean	32.4	26				126.6	269	22.7±11.3

M : Male, F : Female, Ag : Antigen, Anti : Antibody

rheumatoid synovial membrane antigens to see whether they contained products that were not found in synovial membranes obtained from patients with other arthritic disorders and the stimulating antigens was occasionally derived from the patient's synovial membrane. The results were more specific for subjects with rheumatoid arthritis and the stimulating antigen was always derived from rheumatoid arthritic joints and the control antigen was obtained from osteoarthritic joints[31].

Clinicians have long recognized that the majority of patients with rheumatoid arthritis tend to have a relatively mild course of disease, whereas other rheumatoid arthritis patients become progressively debilitated regardless of therapeutic intervention. A subgroup of rheumatoid arthritis patients who are destined to have a more aggressive illness are usually nonresponsive in the LAI assay[32].

This fact suggests that the LAI responsiveness has a close relationship to CMI. The authors tried the LAI assay in patients with chronic active hepatitis and hepatitis B carriers and persons who have no anti-HBs in spite of receiving HB vaccination because these diseases seem to have

Table 4. Nonadherent Index (NAI) in Carriers of Hepatitis B

Name	Age	Sex	HBs Ag	HBe Ag	NAI (%)
C. H. S	25	F	+	+	90
G. G. L	18	F	+	+	70.7
B. G. O	38	M	+	+	72.6
C. G. G	25	M	+	+	53.6
K. S. P	44	M	+	+	28.9
K. O. H	20	F	+	+	58.2
S. S. S	17	M	+	+	60
B. O. H	22	M	+	+	70
Y. C. H	37	M	+	+	42.8
Y. G. H	29	M	+	+	25
G. M. T	36	M	+	+	27
G. O. G	34	M	+	+	34
L. Y. H	33	M	+	−	48.4
Y. S. G	29	M	+	−	38.5
B. B. B	32	M	+	−	55.7
K. K. K	34	M	+	−	40
K. O. I	46	F	+	−	38.6
K. G. H	23	M	+	−	57.1
K. Y. I	42	M	+	−	44.5
Mean	30.8	19			49.7±17.8

M: Male, F: Female, Ag: Antigen

Table 5. Nonadherent Index (NAI) in 5 Persons who have No Anti-HBs in Spite of Receiving HB Vaccination 3 Times

Name	Age	Sex	sGOT	sGPT	NAI (%)
K. H. G	16	M	21	20	24.6
L. O. C	42	M	18	4.2	26.7
H. N. H	45	M	89	151	6.66
L. S. H	39	M	31	58	35.3
G. G. L	30	F	18	42	32.1
Mean	34.4	5	35.2	55	25.1±11

*Serologic markers of hepatitis B virus are all negative in 5 persons

Table 6. The Changing Pattern of NAI Followed by Increase or Decrease of Transaminase and Negative Conversion of HBe Ag and HBs Ag in Patients with Chronic Hepatitis

Name	Disease	Changes					
		Date	HBs Ag	HBe Ag	sGOT	sGPT	NAI (%)
M. K. H	CAH c̄ biopsy	Apr. 1st	+	+	142	370	9.7
		Apr. 16th	+	+	88	224	15.7
		June. 4th	+	+	22	38	62.1
		Sep. 21th	+	+	155	415	38.1
S. S. O	CAH s̄ biopsy	Mar. 11th	+	+	39	69	30.7
		June. 12th	+	+	18	36	84.38
		Aug. 31th	+	+	71	260	31.03
S. B. Y	CAH c̄ biopsy	June. 4th	+	+	275	364	4.61
		Sep. 14th	+	−	28	42	82.35
K. H. G	CAH s̄ biopsy	June. 12th	+	+	270	600	28.6
		Aug. 31th	+	−	27	41	78.94
K. S. O	CAH s̄ biopsy	Apr. 2nd	+	+	246	285	23.1
		June. 26th	+	−	66	78	80
C.H.S	Carrier	Mar. 11th	−	+	26	43	90
		June. 4th			12	22	147

Note. CAH : Chronic active hepatitis, c̄ : With, s̄ : Without
NAI : Nonadhrenent index

a common defect of CMI. Trial of LAI assay in hepatitis B surface antigen and antibody system as done by Seo and koh[33] in 1984 and in this they observed that NAI in anti-HBs positive patients is higher than that in anti-HBs negative patients. This result is correlated with that of our experiment. For the first time, LAI assay was done by using the hepatitis B vaccine (protein content ; 20ug/ml) as the specific antigen and polio vaccine (protein content ; 17.7ug/ml) as the nonspecific antigen for the hepatitis patient. Hepatitis B vaccine used as the specific antigen and polio vaccine used as the nonspecific antigen have almost the same quantity of protein, and have similar properties as antigen. These two also have the advantages of easy availability. Because we don't have a specific method to predict the prognosis of chronic hepatitis today, LAI assay, if it was done precisely, will be helpful in evaluating the prognosis of a patient. For 6 patients with chronic hepatitis, serologic test for hepatitis B viral marker, liver function test for transaminase and LAI assay were done simultaneously and serially (Table 6).

We observed that negative conversion of HBs-Ag and HBe-Ag and the increasing or decreasing value of transaminase is in inverse proportion to the increasing or decreasing value of NAI.

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