*The Seoul Journal of Medicine*
*Vol. 29, No. 4:361-371, December 1988*

# HBsAg Mediated Leukocyte Adherence Inhibition (LAI) and Its Immunologic Mechanism

**Jeong Kee Seo**

*Department of Pediatrics, College of Medicine, Seoul National University, Seoul 110-744, Korea*

=Abstract=The leukocyte adherence inhibition (LAI) was studied in the hepatitis B viral infection and its immunologic mechanism was assessed.

Leukocytes from 34 subjects with antiHBs in their serum (group A), leukocytes from 52 normal control subjects without any hepatitis B serologic markers (group B) and leukocytes from 26 subjects with HBsAg in their serum (group C) were tested against HBsAg as a specific antigen and BSA as a nonspecific antigen. The mean Non Adherence Index (NAI) of group A was significantly higher than that of group B or of group C ($P < 0.001$). The NAIs of 29 subjects (85.3%) among 34 subjects in group A were over 50 while only two subjects (3.8%) in group B and only one subject (3.9%) in group C were over 50.

To determine whether soluble mediators such as leukocyte adherence inhibition factor (LAIF) or immunoglobulin produced by sensitized lymphcytes were involved in the LAI response, supernatants collected from the peripheral blood leukocytes (PBL) after 24 hour-incubation at 37°C with HBsAg (200 ug/ml) were assessed for adherence inhibition activity on normal PBL. The supernatant collected following incubation of HBsAg with leukocytes obtained from anti HBs (+), LAI (+) subjects markedly reduced the adherence of the normal leukocytes.

To determine the cell subfractions involved in the LAI assay, T and B cell enriched subpopulations were tested for LAI reactivity to HBsAg. The T cell enriched subpopulation gave positive LAI reactivity while the B cell enriched subpopulation gave no detectable LAI reactivity.

To investigate serum factors of immune donors, the LAI-nonreactive leukocytes from normal control subjects or HBsAg carriers were preincubated with serum from LAI-reactive, antiHBs (+) subjects or with hepatitis B immune globulin (HBIG), and were tested for LAI. They showed positive LAI reactivity to HBsAg.

The present study showed specific HBsAg mediated LAI assay and suggested that both two mechanisms, one dependent on specific antibody and the other on LAIF may be operated in hepatitis B virus antigen-antibody system.

Key works: ***Leukocyte adherence Inhibition, Hepatitis B surface antigen-antibody system, Immunologic mechanism***

## INTRODUCTION

LAI (leukocyte adherence inhibition) assay was first introduced by Halliday and Miller in 1972. It has been used successfully to detect the cell breast Ca, colon Ca, lung Ca, stomach Ca, pancreatic Ca, hepatoma, cervix Ca and laryngeal Ca. (Armistead and Gowland 1975; Flores *et al.*, 1977; Grosser and Thomson 1976; Halliday *et al.* 1977; Lopez *et al.* 1978· Marti and Thomson 1976; Maluish 1979; Rutherford *et al.* 1977; mediated immune response in patients with

Received 14/10/88, revised 14/11/88; accepted 6/12/88

Tataryn 1978; Tatáryn *et al.* 1979). Inhibition of leukocyte adherence (LAI) has also been noted in immunity of purified protein derivative (PPD) and dermatophytic fungi. (Creemers 1977; Walters *et al.* 1974).

The LAI assay is based on the phenomenon that peripheral blood leukocytes of the normal healthy control adhere to glass surface of the test tube, but in the presence of specific antigen, peripheral blood leukocytes from the sensitized subject lose its adherence to glass surface. (Halliday *et al.* 1975; Halliday and Miller 1972). The mechanism of LAI has not clearly been explained yet.

Using the hemocytometer method of the LAI assay, some investigators have demonstrated that the phenomenon of LAI is mediated by a soluble factor (LAIF) and T cells are actively involved in the assay. (Creemers 1977; Koppi *et al.* 1979). Others showed that the monocyte is antigen-responsive indicator cell in the LAI assay and that the monocyte interacts with antigen via cytophilic antibody. (Grosser *et al.* 1976; Marti *et al.* 1976; Tong *et al.* 1979).

In view of the apparent differences between the results of several investigators, additional study is indicated. This discrepant findings may be in part due to the fact that most of these studies have been performed with poorly purified tumor extracts. Comparing with tumor antigen-antibody system, HBsAg-Ab system has some benefits to assess the mechanism of the LAI assay. Hepatitis surface antigen can be available in more purified form than tumor antigen, and Hepatitis B surface antigen and antibody can be accurately checked in serum of the patient by radioimmunoassay while tumor antigen or antibody can't be easily checked in cancer patients.

The LAI in hepatitis B viral infection system to measure specific immune responses to HBsAg has not been studied yet. In the present paper HBsAg mediated LAI in hepatitis B viral infection and its mechanism were studied.

## MATERIALS AND METHODS

Experiments were done to see the HBsAg mediated LAI in the clinical subjects and to elucidate the immunologic mechanism of LAI using HBsAg-anti HBs system.

### A. HBsAg mediated leukocyte adherence inhibition in hepatitis B viral infection

**Donors of leukocyte:** Heparinized blood samples were collected by venipuncture from experimental subjects. Peripheral blood leukocytes were immediately prepared from donors and tested for the leukocyte adherence inhibition by the tube LAI assay which were performed on coded specimen. At the same time, one ml of blood samples from the same donors were tested for Hepatitis B surface antigen and antibody by RPHA or RIA.

Most of normal volunteers were medical staffs, nurses in our hospital and parents of patients with hepatitis B.

Experimental subjects were divided into three groups according to the presence of HBsAg and anti HBs in their serum. The group A were 34 subects who had anti HBs in their serum. The group B were 52 healthy subjects who had neither HBsAg nor anti HBs in their serum. The group C were 26 subjects who had HBsAg in their serum.

**Antigen:** To determine the leukocyte adherence inhibition in the HBsAg-anti HBs sytem, HBsAg at a concentration of 100 ug per ml was used as the specific antigen and BSA(bovine serum albumin) at a concentration of 100 ug per ml was used as the nonspecific antigen.

**Preparation of peripheral blood leukocytes:** The following is a brief description on the procedure. A 20 ml sample of heparinized venous blood was collected in a glass cylinder. The blood was mixed with an equal volume of Hank's balanced salt solution (HBSS). Ten ml of the blood mixture was layered on top of each 15 ml conical centrifuge tube of Ficoll-Hypaque, taking care not to disturb the bottom dense layer.

Then the tube was centrifuged at 450 ×g for 30 minutes at 20°C. Viable lymphocytes which accumulated as a white layer on the Ficoll-Hypaque/media interface, were transferred to a fresh centrifuge tube using a Pasteur pipette.

Certain amounts of HBSS was added and they were centrifuged at 250 ×g for 5 minutes at room temperature. If the supernatant was not clear, centrifugation was tried again. After decanting the supernatant, HBSS was added to the resultant pellet to resuspend and wash the lymphocytes. Centrifugation, resuspension and cell washing were repeated, and the cell concentra-

tion was adjusted to 1 × $10^7$ cells/ml with a Neubauer hemocytometer. Viability was assessed by exclusion of 0.2% trypan blue.

**Antigen-induced tube LAI assay:** The tube LAI assay was performed as described previously (Grosser and Thomson 1976).

Antigen-induced LAI was performed in 20 ml Kimax test tubes (16 × 150 mm). In each experiment three test tubes were prepared.

Aliquots of 0.1 ml of a PBL suspesnion (1 × $10^7$ cells/ml) were placed in three test tubes. Then 0.1 ml of Dulbecco's modified Eagle's medium(DMEM) or 0.1 ml of HBs Ag (10 ug) or 0.1 ml of BSA(10ug) was added to one of the three tubes. The mixture was trought to a final volume of 0.5 ml by the addition of DMEM.

The suspension in each tube was agitated, and the tubes were then incubated horizontally, so that the contents covered at least four-fifths of the length of the lower surface of each tube.

The tubes were incubated at 37°C in a humidified atmosphere of 5% $CO_2$-95% air. After 1 hr of incubation, the tubes were placed upright, and the 0.5 ml of medium at the bottom of the vertical tube was gently aspirated twice with a 10 ul pipet. A sample was placed immediately on a hemocytometer, and the nonadherent cells in 4 squares were counted. Each tube was handled in a precisely uniform manner, and each experiment was done in duplicate.

The results were expressed as NAI(Non Adherence Index).

$$NAI = (A-B)/B \times 100$$

Where A is the number of non-adherent cells in the presence of specific antigen (HBsAg), and B is the number of non-adherent cells in the presence of nonspecific antigen (BSA). NAI's of >50 were positive and those of <50 were negative. This value was chosen arbitrarily on the result of the previous studies (Flores *et al.* 1977; Grosser and Thomson 1976; Lopez and Thomson 1977; Marti and Thomson 1976; Thomson 1978).

### B. Assessment of the mechanism of LAI using HBsAg-anti HBs system

**Donors of leukocytes:** Healthy volunteers who had anti HBs in serum and constantly showed marked leukocyte adherence inhibition on the preliminary studies were used as the source of leukocytes. Blood samples were also collected from normal controls and HBsAg carriers.

**Indirect LAI assay:** PBL were prepared as for the direct LAI assay from volunteers with serum anti HBs. These PBL suspension were then incubated with the same volume of HBsAg (200 ug/ml) or without HBsAg at 37°C in a humidified atmosphere of 5% $CO_2$-95% air. After 24 hour incubation, supernatants were obtained by centrifuging the tubes at 250 ×g for 7 minutes and assessed for adherence inhibition activity on PBL from another subject with negative or positive LAI. From PBL of normal cotrols supernatants were collected after 24 hour incubation with HBsAg (200 ug/ml). All supernatants were stored at −70°C. Then, tube LAI assay was performed as described above except that the aliquot of 0.1 ml supernatant was used instead of the specific or nonspecific antigen.

**Preparation of T and B cell enriched subpopulations:** To the PBL prepared as described above, one drop of thrombin (100 units/ml) was added. Then T and B lymphocytes were separated by nylon wool column. The following is a brief description for this.

The purified lymphocytes in 0.5 ml McCoy's media were added to the top of a nylon wool column preheated to 37°C and allowed to move all the way into the wool. The column was laid flat and 0.2 ml media was added into the horizontal column to prevent drying of the wool. Incubation was done at 37°C for 30 min. The column was placed vertically over a 5 ml tube. The nonadherent cells were collected by allowing 5 ml of 37°C media to drip through the column. It was repeated with a second 5 ml tube.

Then the B-rich adherent cells were recovered in a third 5 ml tube by adding 1.5 ml media to the column and repeatedly squeezing the straw vigorously at the level of the wool while the media passes through. The procedure was repeated twice. The cells were transferred to Fisher tubes and washed twice with standard McCoy's.

**Arming experiment with hepatitis B immune globulin or antiHBs (+) serum:** The PBL from normal controls or subjects with serum HBsAg who showed negative LAI assay to HBsAg, were preincubated with antiHBs (+) serum from LAI-reactive subjects or with hepatitis B immune globulin (HBIG) for 2 hours. After washing, then, direct tube LAI assay was performed in a standard fashion. 100 ul of HBIG per ml of PBL

Table 1. Nonadherent cells (%) from Group A, Group B and Group C patients

| | % nonadherent cells in presence of | | |
|---|---|---|---|
| | no antigen | HBsAg | BSA |
| Group A (34 subjects c̄ antiHBs) | 4.52±0.57* | 12.53±1.13 | 5.64±0.64 |
| Group B (52 normal controls) | 6.03±0.68 | 6.31±0.66 | 6.05±0.65 |
| Group C (26 HBsAg carriers) | 6.42±1.01 | 6.89±1.28 | 6.96±1.27 |

*mean±S.E

p value no Ag vs HBsAg HBsAg vs BSA BSA vs no Ag
In Group A: ($P<0.001$) ($P<0.001$) ($P>0.4$)
In Group B: No significant difference between three data
In Group C: No significant difference between three data

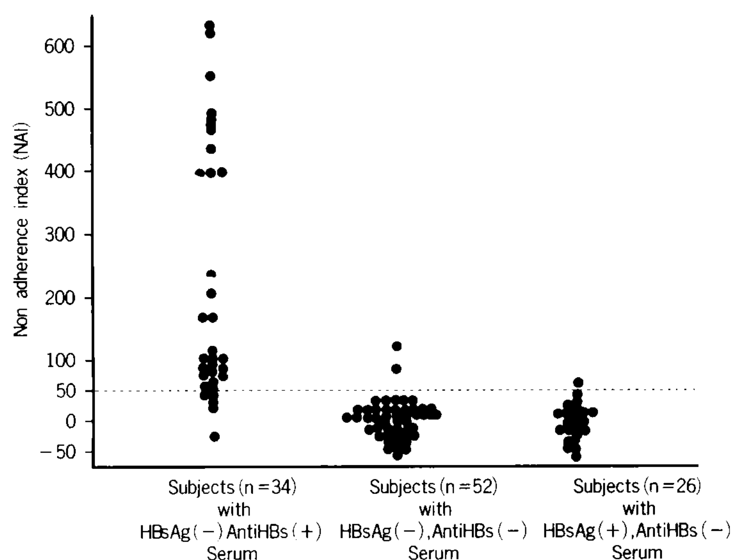


Fig. 1. LAI test by $HB_sAg$ in the subjects with anti HBs(+) or HBsAg(+) serum.

suspension or the same volume of diluted serum (1:1) was mixed and preincubated with LAI-nonreactive PBL.

**Statistical analysis**: Statistical analysis was made by Student's t-test and ANOVA.

## RESULTS

### A. HBsAg mediated leukocyte adherence inhibition in hepatitis B viral infection

Table 1 showed the results of the tube LAI assay in 34 subjects (group A) with antiHBs, hepatitis B surface antibody in their serum and in 52 normal control subjects (group B) and in 26 subjects (group C) with HBsAg in their serum.

These data showed that HBsAg markedly reduced the adherence of leukocytes in group A patients, but not in group B or group C patients. Figure 1 clearly indicated that the NAIs of group A were significantly higher than those of group B or of group C ($p < 0.001$). There was no significant difference between the NAIs of group B and group C ($p > 0.1$). In group A, the NAIs were over 50 except five; 85.3% of them were over 50. In group B, the NAIs of only two subjects (3.8%) were over 50. In group C, the NAI of only one subject (3.9%) among 26 HBsAg carriers was over 50.

### B. Assessment of the mechanism of LAI using HBsAg-antiHBs system

To determine whether soluble mediators such as leukocyte adherence inhibition factor (LAIF) or immunoglobulin produced by sensitized lymphocytes were involved in the LAI resposne, supernatants collected from the PBL after 24 hour-incubation at 37°C with HBsAg (200 ug/ml) were assessed for adherence inhibition activity on PBL from normal controls (Fig. 2). The supernatant(a) and the supernatant(b) were collected following incubation of HBsAg with PBL obtained from two different antiHBs (+), LAI (+) individuals. The supernatant (c) were collected following incubation of HBsAg with PBL obtained from an HBsAg (−), antiHBs (−), LAI (−) individual. Three experiments were shown on Fig. 2.

Results of the experiment 1, in which the leukocyte donor was a HBsAg (−), antiHBs (+) subject, revealed that the percentage of nonadherent cells in the presence of specific HBsAg was significantly higher than that with no antigen. Furthermore, the experiment 1 clearly showed the positive indirect LAI with supernatant(a). The superantant(a) markedly reduced the leukocyte adherence, so the number of nonadherent cells in the presence of supernatan-

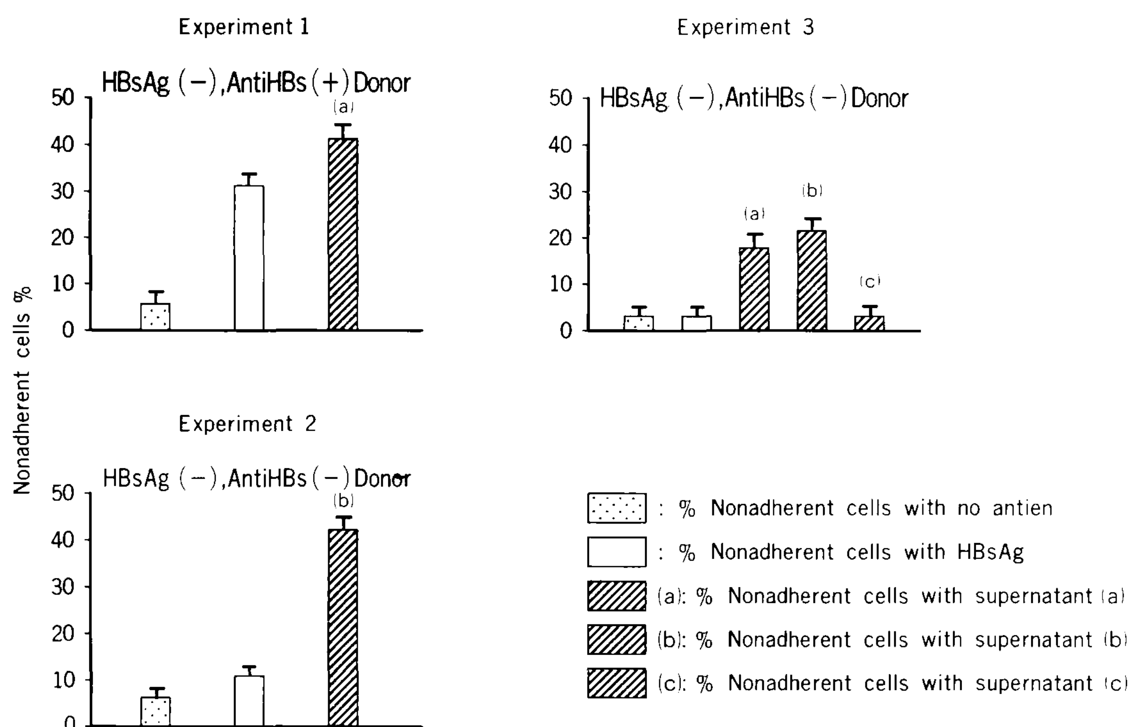


**Fig. 2.** HBsAg Specific LAIF Production by Anti HBs (+) Immune Leukocytes: Supernatant (a) and supernatant (b) were collected following incubation of HBsAg with PBL obtained from anti HBs (+), LAI (+) individuals. Supernatant (c) were collected following incubation of HBsAg with PBL obtained from an HBsAg (−), anti HBs (−), LAI (−) individual.

t(a) were significantly higher than those with no antigen. The NAI calculated as previous description, where A is the number of nonadherent cells in the presence of supernatant instead of specific antigen and B is the number of nonadherent cells without antigen, was 523. 23.

Results of the experiment 2, in which the leukocyte donor was a HBsAg(−), antiHBs(−) subject, clearly showed positive indirect LAI assay with supernatant(a). This donor showed negative direct assay previously. The NAI calculated from the data using the number of nonadherent cells with the supernatant and without was 562.74.

Results of experiment 3, in which the leukocyte donor was another HBsAg(−). antiHBs (−) subject, showed that indirect LAI assay with supernatant(a) or supernatant(b) was positive and bycontrast, indirect LAI assay with supernatant(c) was negative. Direct LAI assay with HBsAg in this subject was previously negative.

With the observation that supernatant(a) or supernatant(b) markedly reduced the adherence of normal leukocytes, but supernatant(c) didn't, it seemed likely that soluble factors, either immunoglobulin in nature or lymphokine like LAIF were produced not by nonsensitized lymphocytes, but only by sensitized lymphocytes. The NAI(a) with supernatant(a), the NAI(b) with supernatant(b) and the NAI(c) with supernatant(c) were 548.22, 702.98 and 30.43, respectively.

T lymphocyte enriched subpopulation and B lymphocyte enriched subpopulation were tested for leukocyte adherence inhibition (LAI) reactivity to HBsAg to determine the cell subfractions involved in the LAI assay. The results were presented in Figure 3. The original cell population (PBL) obtained from the antiHBs (+) subjects who showed highly active responses on the previous LAI assay, was separated into T cell enriched and B cell enriched subpopulations by nylon-wool column according to the method previously described.

The T cell enriched subpopulations from an antiHBs (+) donor gave positive LAI reactivity to HBsAg (100 ug/ml) specifically. The NAI was 90.30; However, B cell enriched subpopulation gave no detectable LAI reactiviy. The NAI was 10.56.

To identify which types of cells were the responder cells in the LAI assay, indirect LAI assay were done on the T cell enriched and the B cell

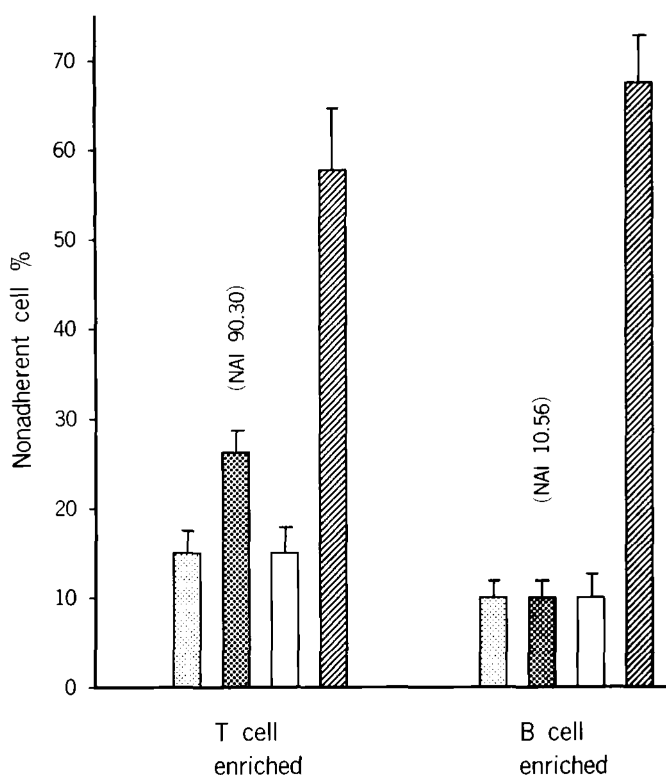


Fig. 3. LAI reactivity of the antiHBs (+) donor with the use of mononuclear cell subpopulations: nonadherent cell % with no antigen (▨), with HBsAg (▩), with BSA (□), and with supernantant (▨).

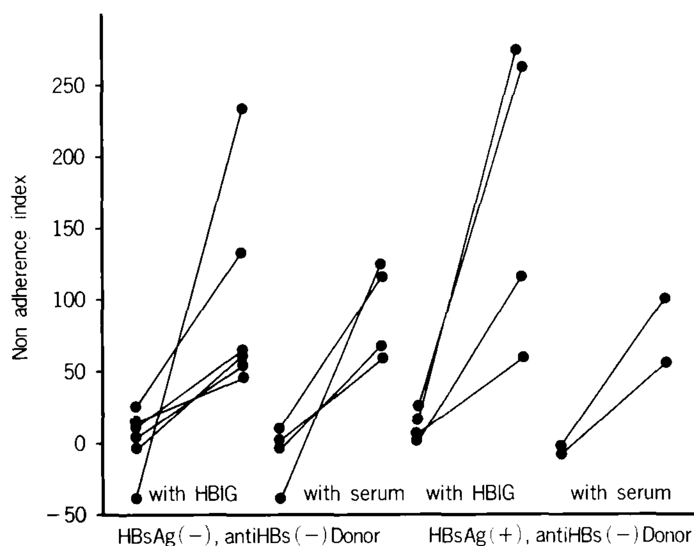


Fig. 4. Specific arming of LAI(−) leukocytes with HBIG or antiHBs (+) serum.

enriched subpopulation with the use of supernatants(b) previously described.

Results of this experiment presented on Figure 3, showed that both T and B cell subpopulations were highly susceptible to adherence inhibition by the supernatant containing LAIF; More than half of the T cell and B cell subpopulations were nonadherent (57.6% and 68.3% respectively). The action of the supernatant thus appears to be quite unselective.

To investigate serum factors of immune donors in the mechanism of LAI reactivity, which are able to arm naive cells to be specifically responsive, the LAI-nonreactive PBL were preincubated with serum from LAI-reactive, antiHBs (+) subjects or with hepatitis B immune globulin (HBIG).

The preincubated PBL were washed twice with DMEM by centrifugation at 200 ×g for 5 minutes. Then the PBL were assessed for the leukocyte adherence inhibition using HBsAg (100 ug/ml) as a specific antigen and BSA (100 ug/ml) as a nonspecific antigen. The results were presented on Table 2 and Fig. 4.

The PBL from ten leukocyte donors whose serologic markers for HBsAg and antiHBs were all negative, were preincubated for 2 hours with HBIG (100 ul of HBIG per ml of PBL suspension) or with the same volume of diluted serum (1:1) from LAI-reactive, antiHBs (+) subjects. The NAI of these 10 donors (from No. 1 to No. 10 on Table B) became positive after arming.

The PBL from the remaining six leukocyte donors, who were HBsAg carriers, were preincubated for 2 hours with HBIG or serum from LAI-reactive, antiHBs (+) subjects. The NAI of these 6 donors (from No. 11 to No. 16 on Table B) became positive after arming.

## DISCUSSION

Application of leukocyte adherence inhibition has not been done yet to evaluate the immunity of hepatitis B virus infection. The present study showed specific HBsAg mediated LAI assay. Peripheral blood leukocytes of 29 of 34 subjects with antiHBs in thier serum responded to the HBsAg by a decrease in adherence to a glass tube surface when using NAI of 50 as a cut-off point, whereas only 2 of 52 normal control subjects showed a similar decrease in adherence. The present study indicated that HBsAg carrier who had large antigen burden like the patients in advanced cancer showed negative LAI reactivity. In LAI assay as the concentration of antigens increases, LAI with sensitized leukocytes increase up to a maximum and then antigen suppress the reaction.

Results of the previous studies indicated that

Table 2. Specific arming of LAI (−) leukocytes with HBIG or anti HBs (+), LAI (+) serum

| No. of donor | Serologic marker HBsAg/antiHBs | Leukocytes preincubated with | % Nonadherent cells in presence of | | | NAI |
|---|---|---|---|---|---|---|
| | | | No Antigen | HBsAg (100 ug/ml) | BSA (100 ug/ml) | |
| 1 | (−)/(−) | HBIG | 12.61 | 33.94 | 14.45 | 13.87 |
| 2 | (−)/(−) | HBIG | 10.86 | 14.97 | 10.05 | 48.95 |
| 3 | (−)/(−) | HBIG | 4.60 | 7.60 | 2.30 | 120.43 |
| 4 | (−)/(−) | HBIG | 22.20 | 34.50 | 21.20 | 62.73 |
| 5 | (−)/(−) | HBIG | 10.02 | 15.25 | 9.75 | 56.4 |
| 6 | (−)/(−) | HBIG | 8.25 | 18.75 | 11.25 | 66.66 |
| 7 | (−)/(−) | Serum | 21.80 | 45.50 | 20.10 | 121.64 |
| 8 | (−)/(−) | Serum | 15.50 | 26.70 | 11.89 | 124.55 |
| 9 | (−)/(−) | Serum | 17.00 | 29.37 | 17.37 | 69.10 |
| 10 | (−)/(−) | Serum | 7.00 | 21.50 | 13.50 | 61.10 |
| 11 | (+)/(−) | HBIG | 9.93 | 19.26 | 8.85 | 117.62 |
| 12 | (+)/(−) | HBIG | 5.46 | 17.60 | 4.25 | 305.88 |
| 13 | (+)/(−) | HBIG | 13.07 | 45.30 | 5.28 | 756.60 |
| 14 | (+)/(−) | HBIG | 9.71 | 18.28 | 10.64 | 71.80 |
| 15 | (+)/(−) | Serum | 6.38 | 30.40 | 18.10 | 67.90 |
| 16 | (+)/(−) | Serum | 19.54 | 28.14 | 13.90 | 102.40 |

with small tumor burdens antitumor immunity was present, but in patients with widespread metastases, it·was not detectable and most leukocytes from patients with advanced cancer did not display LAI activity (Thomson *et al.* 1979). Some investigators suggested that the surface of the LAI-reactive cells in advanced cancer is already coated with TSA (tumor specific antigen) in vivo, so the leukocytes mainfest the property of increased nonadherence to glass when incubated with either the specific or nonspecific cancer antigens(Grosser and Thomson 1975, 1976; Lopez and Thomson 1977).

Results of some investigators are not consistent with the hypothesis to explain the reason why LAI assay becomes negative in advanced cancer. Experiments in the present study didn't indicate that PBL from HBsAg carrier incubated with HBsAg or BSA exhibit a greater mean nonadherence to glass than PBL from control subjects. Moreover, in these 26 HBsAg carriers the percentage of nonadherent leukocytes incubated with HBsAg or BSA was not significantly higher than the percentage of nonadherent leukocytes incubated without antigen. These results of present experiment means that above hypothesis is not applicable to explain the reason why sensitized PBL from HBsAg carrier with large antigen burden show negative LAI reactivity.

The non-reactive PBL from HBsAg carrier became LAI reactive to HBsAg if they were preincubated and armed with HBIG. It may be that to show LAI reactivity, even sensitized PBL needs sufficient amounts of antibody to arm the receptor on the cell surface with.

If PBL are armed with heptitis B virus antibody, then the HBsAg is specifically bound to PBL's cell surface by the cytophilic hepatitis B virus antibody which alters their ability to adhere to glass when the PBL of HBsAg carrier are incubated with the sensitizing antigen.

The author experienced interesting results in two subjects. One subject had both HBsAg and antiHBs in her serum. Results of LAI assay in the subject revealed strong LAI reactivity to HBsAg. Another subiect showed positive LAI reactivity, despite absence of HBsAg and antiHBs in her serum. Her past medical history revealed that she had suffered from serologically confirmed hepatitis B virus infection 2 years ago.

The reason why cells stick to glass surfaces and the mechanism why they stick less firmly in an LAI reaction, have not been clearly understood, although there was a substantial literature in the field of cell adherence (Pegrum and Marounds 1975; Weiss 1967) and substantial

papers on the antigen-specific LAI. The prognostic and diagnostic potential of this assay will not be fully realized until the immunological mechanisms by which the response occurs is understood. Several investigators have been working in trying to make LAI more respectable by finding out how it operated. One of the major reasons why many investigatiors couldn't agree on the mechanism of LAI may be that they could not use highly purified tumor specific antigens. In the present study the author assessed the mechanism of the LAI using highly purified HBsAg instead of crude tumor extracts.

The original hemocytometer LAI assay was first developed by Halliday and Miller in 1972. There have been 2 major modifications of the original hemocytometer or slide LAI method. These involve the use of tube and wells in plates as the surfaces for cell adherence. Patrick Holt (1975) first developed the well method using microtest plates and visual counting of adherent cells. Holan *et al.* (1974) deviced the tube LAI method. Using these 3 different techniques, over one hundred studies were published by more than 20 different laboratories until now.

However, there is considerable disagreement concerning the mechanisms of the LAI assay. The points on which there is profound disagreement are as follows; whether the LAI reactivity is mediated through a lymphokine like soluble mediator (LAIF) or immunoglobulin, which cell types are involved in the mechanism of reactivity and what is their role in antigen-induced adherence inhibition.

According to Halliday's statement, there was no doubt that a lymphokinelike, soluble mediator of T-cell origin was produced and involved in LAI in his mouse tumor system. In Halliday's study, LAIF appeared rapidly in response to antigen, and incubation time of 30 to 60 min were adequate to prepare active supernatants. Holt *et al.* (1975) also demonstrated this independently and called the mediator LAIF. However, Holan *et al.* (1974) and Grosser *et al.* (1976) using tube LAI technique didn't find the existence of LAIF. Instead, they suggested the importance of cytophilic antibody on the monocytes. The possible reasons for disagreement over the existence of LAIF have not been discovered yet. Some investigators suggest that the difference between the tube and the hemocytemeter LAI assay technique appear to result in the measurement of a different reactive cell population which lead to different results and conclusions.

After observing that differences between believers and nonbelievers of LAIF is in the use of serum in the system, Halliday(1979) suggested enzymatic (protease?) activity in tumor extracts might inactivate LAIF, and that serum might have a protective function. This idea was supported by his subsequent experiments. High levels of serum protein reduce cell adherence, yet LAIF reduced it further. In tube LAI assay where serum is not used, so that protease activity to inactivate LAIF was not protected by serum. If Haliday's assumption that protease activity in the crude tumor extract inactivate LAIF is correct, LAIF might be produced even in tube LAI assay by using highly purified antigen which is free of protease activity instead of crude tumor extracts, and discrepancy between above results and the result of other workers can be accounted for.

In this paper the author applied LAI assay to hepatitis B virus system. Using purified HBsAg free of possible protease activity as a specific antigen, the author assessed the mechanism of LAI and observed the LAI inducing soluble mediator, the nature of which is thought to be either immunoglobulin or lymphokine-like LAIF. The supernatants were prepared from PBL of LAI (+), antiHBs (+) subjects and evaluated for the ability to induce adherence inhibition of normal adherent cells. The result in the experiment of present paper clearly showed that leukocytes from LAI (+) subjects previously sensitized to a hepatitis B virus surface antigen, produced a soluble factor, and it was able to modify normal leukocytes so that they adhere less readily to the glass surfaces.

With the observation above, the author suggested that even in serum-free medium, LAIF may be demonstrated if purified antigen free of tissue protease is used instead of the crude tissue extract in the tube LAI assay. It may be that if tissue protease can't be excluded, serum-free conditions in the tube LAI favor an alternate LAI mechanism where by Fc receptor-bearing cells can maintain a coating of cytophilic antibody without competition from or displacement by other plasma proteins. Both two mechanisms, one dependent on LAIF and the other on speci-

fic antibody may thus be possible. In this paper using hepatitis B virus antigen-antibody system the author showed that soluble mediators rather than direct cellular contact were involved in the tube LAI mechanism. The soluble mediators seem to be immunoglobulin in nature or LAIF.

LAI reactive cell types have been investigated by a couple of workers (Grosser *et al.* 1976; Grosser and Thomson 1976; Holt *et al.* 1975; Marti *et al.* 1976; Russo *et al.* 1978). Holt *et al.* (1975) separated mouse splenic cells on an albumin gradient and showed that the PPD-specific LAI occurred only in fractions containing lymphocytes.

A variant of the LAI, the tube LAI, described by Holan *et al.* (1974) and by Grosser *et al.* (1976) suggested that the variant method is independent of lymphokine, that the critical cell type is a monocyte, and that it requires an antibody with cytophilic properties.

Goldrosen *et al.* (1979) observed in progressively growing MCA-38 tumor model that the reactor cell undergoing specific antigen-induced adherence was identified as a monocyte and programming of normal monocytes by MCA-38 sensitized B cell occurs through a soluble mediator and not by direct contact. They suggested the soluble mediator appeared to be immunoglobulin in nature.

Goldrosen's results contrast with the supernatant studies in the mammary tumor virus system by Creemers (1977) who observed that supernatants of sensitized T cell (LAIF) could induce LAI of normal adherent cells. In Creemer's study supernatant were harvested after 24 hr incubation as the experiment in this paper. Whereas in Goldrosen's study supernatants were harvested after 1 hr incubation. By some investigator's obervation, to produce adequate LAIF, more than 24 hr of incubation is needed.

Many discrepant findings of the several investigagors may be concerned with difference of animal species, difference of immunization regimens, difference of antigen type, difference of purification degree of extract, and difference of technique in relation to use of serum.

Results of arming experiment on Table B are consistent with the observations of Holan *et al.* who have contended that monocyte armed with cytophilic antibody appear to be affected directly by the corresponding antigen so that their adherent properties are reduced.

The experiment in the present study to identify which types of cells are involved in the mechanism of LAI showed only populations containint T cells were active. However the target of soluble mediators are both T and B cell enriched subpopulations and this observation is consistent with the previous reports of Koppi *et al.* (1979). These results mean that the type of antigen-reactive cells is not the same as the type of indicator cells.

Role of monocytes in the mechanism of LAI can't be exactly mentioned in the present study. It is possible that very small numbers of monocytes may be contaminated in the T and B cell subpopulations after purification precedures. Results of the arming experiment on Table B indicated that monocytes seemed to be actively responsible for the LAI reaction in hepatitis B virus antigen-antibody system although they may not be the only participant in the LAI assay. In view of the fact that as many as 50% of the T and B cell lost their properties of adherence and the mononuclear cells seperated by density gradient of Ficoll-Hypauqe included about 20 to 30% monocytes on an average, it is reasonably assumed that monocytes are not solely responder cells in the LAI assay. Above observations are consistent with observations of several investigators previously reported.

From the above experiments, it is assumed that both two mechanisms, one dependent on specific atibody and the other on LAIF may be operated in hepatitis B virus antigen-antibody system, and T cells probably with monocytes are actively involved predominant cell type in LAI reactivity although T cells, B cells and possibly monocytes are responder cells.

## ACKNOWLEDGEMENT

I wish to express my thanks to prof. K. W. Ko for his helpful remarks and support, and to Dr. J. S. Seo for his excellent technical advice.

## REFERENCES

**Armitstead PR, Gowland G.** The leukocyte adherence inhibition test in cancer of the large bowel. Br. J. Cancer 1975, 32: 568-573

**Creemers P.** The role of leukocyte subpopulations in the indirectleukocyte adherence inhibiton assay in the mammary tumor virus system. Eur. J. Immunol.

1977, 7: 48-53

**Flores M, Marti JH, Grsser N, MacFarlane JK, Thomson DMP.** An overview: Antitumor immunity in breast cancer asayed by tube leukocyte adherence inhibition. Cancer 1977, 39: 494-505

**Goldrosen MH, Russo AJ, Howell JH, Leveson SH, Holyoke ED.** Cellular and humoral factors involved in the mecanism of the microleukocyte adherence inhibition reaction. Cancer Res. 1979, 39: 587-592

**Grosser N, Marti JH, Proctor JW, Thomson DMP.** Tube leukocyte adherence inhibition assay for the detection of anti-tumor immunity. i. monocyte is the reactive cell. Intern. J. Cancer 1976, 18: 39-47

**Grosser N, Thomson DMP.** Tube leukocyte (monocyte) adherence inhibition assay for the detedtion of anti-tumore immunity, III. "Blockade" of monocyte reactivity by excess free antigen and immune complexes in advanced cancer patients. Intern. J. Cancer 1976, 18;58-65

**Halliday WJ.** Leukocyte adherence inhibition. historical background and aspects of the mechanism of leukocyte adherence inhibition. 1979, 39: 558-563

**Halliday, WJ, Maluish AF, Little JH, Davis NC.** Leukocyte adherence inhibition and pseicifc immunoreactivity in maliganant melanoma. Intern. J. Cancer 1975, 16: 645-658

**Halliday, WJ, Maluish AE, Stephenson PM, Davis NC.** An evaluation of leukocyte adherence inhibition in the immunodiagnosis of colorectal cancer. Cancer Res. 1977, 37: 1962-1971

**Halliday WJ, Miller S.** Leukocyte adherence inhibition. A simple test for cell-mediated tumor immunity and serum blocking factors. Intern. J. Cancer 1972, 9: 477-483

**Holan V, Hasek M, Bubenik J, Chutna, J.** Antigen--mediated macrophage adherence inhibition. Cell. Immunol. 1974, 13: 107-116

**Holt PG, Roberts LM, Fimmel PJ, Keast D.** The LAI microtest: A rapid and sensitive procedure for the demonstration of cell mediated immunity in vitro. J. Immunol. Methods 1975, 8: 277-288

**Koppi TA, Maluish AE, Halliday WJ.** The Cellular mechanism of leukocyte adherence inhibition. J. Immunol. 1979, 123: 2255-2260.

**Lopez MJ, O'Connor R, macfarlane JK, Thomson DMP.** The natural history of anti-tumour immunity in human breast cancer assayed by tube leukocyte adherence inhibition. Br. J. Cancer 1978, 38: 660-674

**Lopez MJ, Thomson DMP.** Isolation of breast cancer tumour antigen from serum and urine. intern. J. Cancer 1977, 20: 834-848

**Mauish AE.** Experiences with leukocyte adherence inhibitin in human cancer. Cancer Res. 1979, 39: 644-648

**Maluish AE, Ames RS, McCoy JL.** Monocyte requirement in production of human leukocyte adherence inhibition in reponse to PPD. Proc. Am. Assoc. Cancer Res. 1980, 21: 248-252

**Maluish AE, Halliday WJ.** Quantitation of anti-tumorcell-mediated immunity by a lymphokine-dependent reaction using small volumes of blood. Cellular Immunol. 1975, 17: 131-140

**Marti JH, Grosser N, Thomson DMP.** Tube leukocyte adherenceinhibition assay for the detection of anti-tumour immunity. II. Monocyte reacts with tumour antigen via cytophilic anti-tumour antibody. Intern. J. Cancer 1976, 18: 48-57

**Marti JH, Thomoson DMP.** Anti-tumour immunity in malignant melanoma assay by tube leukocyte-adherence inhibition. Brit. J. Cancer1976, 34: 116-133

**Pegrum SM, Marouhds NG.** Early events in fibroblast adhesion to glass. Exptl. Cell Res. 1975, 96: 416-422

**Powell AE, Sloss AM, Smith RN.** Leukocyte adherence inhibition: A specific assay of cell-mediated immunity dependent on lymphokine-mediated collaboration between T lymphocytes. J. Immunol. 1978, 120: 1957-1966

**Russo AJ, Howell JH, Leveson SH, Holyoke ED, Goldrosen MH.** Micro-Leukocyte adherence inhibition; Cellular basis of the mechanism of reactivity. J. Immunol. 1978, 121: 1914-1919

**Rutherford JC, Walters BAJ, Cavaye G, Halliday WJ.** A modified leukocyte adherence inhibition test in the laboratory investigation of gastrointestinal cancer. Intern. J. Cancer 1977, 19: 43-48

**Tataryn DN, Macfarlane JK, murray D, Thomson DMP.** Tube leukocyte adherence inhibition (LAI) assay in gastrointestinal(GIT) cancer. Cancer 1979, 43: 898-911

**Tataryn DN, MacFarlane JK, Thomson DMP.** Leukocyte adherence inhibition for detecting specific tumour immunity in early pancreatic cancer. Lancet 1978, 1: 1020-1021

**Taylor IV SG, Saffold PC, Braun DP, Harris JE.** Tg cell involvement in the leukocyte adherence inhibition pheomenon. JNCI 1982, 68: 554-549

**Thomson DMP.** Antigens of human breast cancer. Methods Cancer Res. 1978, 15: 345-398

**Thomson DMP, Tataryn DN, Schwartz R, MacFarlane JK.** Abrogation of the phenomenon of leukocyte adherence inhibition by excess circulating tumore antigen. Eur. J. Cancer 1979, 15: 1095-1110

**Tong AW, Burger DR, Finke P, Barney C, Vandenbark AA, Vetto RM.** Assessment of the mechanism of the leukocyte adherence inhibition test. Cancer Res. 1979, 39: 597-603

**Walters BAJ, Chick JED, Halliday WJ.** Cell-mediated immunity and serum blocking factors in patients

with chronic dermatophytic infections. Intern Arch. Allergy Appl. Immunol. 1974, 46: 849-857
**Weiss L.** The cell periphery, metastasis and other contact pheonomena. Amsterdam: North-Holland Publishing Co., 1967.

=국문초록=

## HBsAg 항원 특이성 백혈구 접착억제 및 그 기전에 관한 연구

서울대학교 의과대학 소아과학교실

서 정 기

1. 백혈구 접착 억제 검사의 HBsAg 항원 특이성을 조사하기 위하여 HBsAg (100 ug/ml)을 특이항원으로, BSA를 비특이 항원으로 하여 백혈구 접착 억제 검사를 시행한 결과 혈청내 B형 간염 바이러스 표면 항체를 가진 실험대상자 34명의 NAI (non adherence index)는 29명(85.3%)에서 50 이상이었다. 반면 표면항체가가 없는 정상대조군 52명 및 바이러스 보균자군 26명에서는 각각 2명(3.8%) 및 1명(3.9%)에서만 NAI가 50 이상이었다.

2. 백혈구 접착억제의 면역학적 기전을 알아보기 위하여 시행한 실험결과를 요약하면

a) B형 간염 표면 항체를 가진 혈액내의 감작 백혈구를 B형 간염 표면 항원과 함께 24시간 배양한 후 이 상청액내에 백혈구의 시험관 접착을 저지하는 인자가 있는가 알아보기 위하여 상기 상청액으로 간접백혈구 접착억제 검사를 한 결과 B형 간염 표면항원 및 표면 항체가 모두 없는 사람으로부터 얻은 백혈구의 시험관 접착이 현저히 억제되었다.

b) 백혈구 접착억제에 관여하는 말초혈액 백혈구의 세포집단을 알아보기 위하여 B형 간염표면항체를 가진 사람의 말초혈액 T임파구, B임파구에 대하여 B형 간염 표면 항원으로 백혈구 접착억제를 시행한 결과, T임파구는 B형 간염 표면 항원 (100 ug/ml) 존재시 시험관 접착이 억제되었으나 B임파구에서 시행한 백혈구 접착 억제는 음성 소견을 나타내었다.

c) 혈청내에 B형 간염 표면항원 및 표면항체가 모두 없는 사람 또는 B형 간염 바이러스 보균자의 말초혈액 백혈구는 B형 간염 면역 글로불린과 배양후 재차 백혈구 접착억제 검사를 시행하면 백혈구 접착억제 검사가 양성으로 전환되었다.

이상의 결과로 보아 B형간염바이러스 감염에서의 백혈구접착억제는 HBsAg 항원특이성을 가지며, 면역학적 기전으로는 임파구에서 생산되는 백혈구 접착억제인자와 함께 B형간염표면항체가 주요한 역할을 할것으로 생각된다.