IMMUNOHISTOCHEMICAL EXPRESSION OF INTERLEUKIN 2 RECEPTOR (IL2R) AND FORMATION OF IL2R POSITIVE CELL ROSETTE IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN ASYMPTOMATIC HEPATITIS B SURFACE ANTIGEN CARRIERS*

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Immunohistochemical expression of interleukin 2 receptor (IL2R) is examined by an APAAP method in the peripheral blood mononuclear cells (PBMC) in asymptomatic HBsAg carriers (AsC) and in normal individuals. It is found that the percentage of IL2R-positive cells is significantly lower in PBMC induced by HBsAg or ConA in AsC, but not so by PHA, than that in normal individuals ($P < 0.01$). Moreover, it is found that IL2R-positive cell is surrounded by many IL2R-negative mononuclear cells following induction by HBsAg. This phenomenon is temporarily termed IL2R-positive cell "rosette". Typical rosette formation of IL2R-positive cells is not observed in induction by ConA and PHA. Besides, when the percentage of IL2R-positive cell rosette is counted, the results show that the prevalence of rosette formation is much higher in normal individuals than that in AsC ($P < 0.01$). This new phenomenon of immune response to HBsAg may be of important significance in immunoregulation. The results suggest that the abnormal IL-2R expression and immunoregulation of IL2R-positive cell may be an important mechanism of poor immune response to hepatitis B virus (HBV) in AsC.

Some individuals are infected with hepatitis B virus (HBV), which is not cleared from their serum and they become chronic asymptomatic HBsAg carriers (AsC). The mechanism of abnormal response to HBV in AsC is very complex and unclear.

We found that the kinetics of IL2 production and interleukin 2 receptor (IL2R) expression in peripheral blood mononuclear cells (PBMC) in AsC is abnormal (3). Moreover, it was found that the serum level of soluble IL2R in AsC is much higher than that in normal individuals. The increased serum IL2R level may be responsible for the down-regulation of the IL2-dependent proliferation (4). These findings might suggest an important mechanism of immune tolerance to HBV in AsC.

In this study, the immunohistochemical expression of IL2R in PBMC of AsC and normal individuals stimulated *in vitro* by HBsAg, ConA or PHA is described. In addition, we found that the PBMC induced by purified HBsAg in AsC and in normal individuals formed IL2R-positive cell "rosette". This new phenomenon of response to HBsAg might be related to the transmission and memory of the second signal. It might be of significance in expounding the mechanism of immune tolerance to HBV in AsC and of the immunoregulation of response to HBsAg in normal individuals.

MATERIALS AND METHODS

Subjects: 10 AsC with normal liver function and 16 volunteers with completely negative HBV markers and normal liver function were used. PBMC were

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isolated from freshly heparinized venous peripheral blood of all cases by Ficoll-Hypaque gradient, washed twice in RPMI1640 medium, and resuspended with a concentration of 3×10^6 /ml PBMC in RPMI1640 containing 0.5 mol/L 2-mercaptoethanol (2-Me), 2 mol/L L-glutamine (GL), and 10% heat-inactivated human AB serum. The cells were incubated in 24-well culture plates, and 40 µg/ml purified HBsAg, 20 µg/ml ConA, or 100 µg/ml PHA was added to the suspension. Control wells without antigen or mitogen were included in each experiment. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 48 hr. The cultured cells were then harvested and the cell concentration was diluted with 0.2% EDTA-added bovine albumin. One hundred µl of the diluted cell suspension was added into each well of a 10-well glass slide, and centrifuged (1000 rpm) for 2 min. The supernatant was then carefully removed and the PBMC dried on the glass.

Immunohistochemical procedures: The dried cells were fixed in formaldehyde-acetone for 25 sec, and then washed with PBS-bovine serum albumin (PBSA) 3 times. The primary antibody [mouse anti-human IL-2R monoclonal antibody], 20 µl, was added to the cells, which were then incubated at 37°C in humidified atmosphere for 50 min. After washing with PBSA, the sheep anti-mouse IgG antibody, 20 µl, was added and the cells were incubated at 37°C for 25 min. Mouse anti-alkaline phosphatase antibody-

alkaline phosphatase complex, 20 µl, was added after washing, and the cells were incubated again at 37°C for 40 min. To each well the substrate was added and the slides were kept at 37°C for 30 min for color development. The samples were then washed, counterstained with hematoxylin for 25–30 sec, rinsed in water and blown dry.

Reagents: Purified HBsAg was obtained from the Department of Infectious Diseases, Nanfang Hospital, China. PHA, ConA, GL, 2-ME, RPMI1640, and alkaline phosphatase was purchased from Sigma Co., USA. Mouse antihuman IL2R McAb was from Wuhan Institute for Biological Products. Mouse anti-alkaline phosphatase antibody and anti-mouse IgG antibody were from Peking Institute for Biological Products. Heat inactivated human AB serum, and lymphocyte separation medium were also purchased from Shanghai Chemical and Reagent Co., China.

Statistical evaluation: The number of IL2R-positive cells in 200 PBMC were counted, and IL2R-positive-cell-rosette formation was examined for 200 IL2R-positive cells. Means and standard deviations were calculated for each experimental group, and the data evaluated using Student's t-test.

RESULTS

Immunohistochemical expression of IL2R in PBMC:

Red reaction products were found in the cell mem-

TABLE 1. Percentages of IL2R-positive cells in PBMC induced by HBsAg, ConA or PHA in AsC and in normal individuals

Group	Number	HBsAg	ConA	PHA
Asymptomatic HBsAg carriers	10	2.2 ± 1.2	13 ± 5.3	40.5 ± 3.9
Normal individuals	16	6.0 ± 2*	28 ± 11*	43 ± 4.0**

Data are indicated by means ± standard deviations

* Statistically significant difference from normal individuals ($p < 0.01$)

** No significant difference from normal individuals, ($p > 0.05$)

TABLE 2. Percentages of IL2R-positive cell rosettes in PBMC induced by HBsAg, ConA or PHA in AsC and in normal individuals

Group	Number	HBsAg	ConA	PHA
Asymptomatic HBsAg carriers	10	3.2 ± 1.3	—	—
Normal individuals	16	21 ± 7.0*	—	—

Data are indicated by means ± standard deviations

* Statistically significant difference from normal individuals ($p < 0.01$)

— No typical IL2R-positive cell rosettes are observed

FIG. 1. IL2R-positive-cell-rosette in PBMC induced *in vitro* by purified HBsAg in AsC. Larger cell in the center is an IL2R-positive cell. The blue cells around the IL2R-positive cell are IL2R-negative cells. IL2R-positive cell is closely linked with IL2R-negative cells. $\times 200$

FIG. 2. IL2R-positive-cell-rosette in PBMC induced *in vitro* by purified HBsAg in normal individuals. Many IL2R-negative cells are seen to link around IL2R-positive cells. $\times 200$

FIG. 3. Expression of IL2R on PBMC induced *in vitro* by PHA in AsC. Larger cells are an IL2R positive cell, IL2R-positive-cell-rosette is not observed. $\times 200$

FIG. 4. Rosette formation of IL2R-positive-cell in PBMC induced *in vitro* by ConA is not observed in AsC. $\times 200$

branches of the larger mononuclear cells (Figs. 1, 2). As shown in Table 1, the level of IL2R expression of PBMC induced by HBsAg in AsC is less than that in normal individuals ($p < 0.01$), and the number of ConA-induced IL2R-positive cells in AsC is less than that in normal individuals as well ($p < 0.01$). However, the number of PHA-induced IL2R-positive cells in AsC and in normal individuals is not significantly different ($p > 0.05$). IL2R expression by PBMC is much higher when induced by PHA than by either HBsAg or ConA in the same individuals ($p < 0.01$).

Rosette formation of IL2R-positive cells:

Following *in vitro* stimulation by HBsAg for 48 hr, some larger IL2R-positive PBMC appear to be surrounded by many smaller IL2R-negative PBMC, the cell membranes of the former being closely linked with those of the latter (Figs. 1, 2). This phenomenon

is temporarily termed IL2R-positive cell "rosette". This finding is clearly seen among normal controls. On the other hand, typical IL2R-positive cell rosette is not observed in PBMC induced by ConA or PHA under the same conditions, (Figs. 3, 4). As seen in Table 2, rosette formation is selectively observed in PBMC stimulated by HBsAg, and the number of IL2R-positive-cell-rosette is significantly less in AsC than in normal individuals ($p < 0.01$).

DISCUSSION

The fate of HBV infection is determined by cellular and humoral immunity of the human body (7). It is confirmed that IL2 can enhance the killing activity of macrophages and NK cells, and directly or indirectly regulate T cell as well as B cell differentiation and proliferation (8, 11). The immunoregulation

of IL2 depends on the expression of specific IL2R (6). We have observed previously that the peaks of IL2 production and IL2R expression of PBMC in AsC are both delayed when compared with those in normal individuals. The kinetics of IL2 production of PBMC is often out of step with that of IL2R expression in AsC, and the level of IL2 production in AsC is much lower than that in normal individuals (3, 5); This makes it difficult for the IL2 to regulate the expression of IL2R both timely and quantitatively in AsC, and hence it is difficult to complete the whole course of cell differentiation and proliferation as well as immune response to HBV, which may be one of the important mechanisms leading to the inability to clear out the HBV in AsC.

In this report, we compared the level of IL2R expression of PMBC in AsC and in normal individuals. The results show that the level of IL2R expression of PBMC induced by PHA in AsC is not lower than that in normal individuals. It is suggested that the cells are in a good responsive state of PHA-induced IL2R expression in AsC. But the results also show that the level of IL2R expression of PBMC induced by HBsAg or ConA in AsC is much lower than that in normal individuals. This may be related to the down regulation of IL2R expression by ConA-induced suppressor cells, or that the function of ConA-induced suppressor cells in AsC is higher than that in normal individuals. It was reported that the administration of ConA can result in enhanced activity of the T suppressor cells in mice (1). In this report, however, it is found that the level of IL2R expression of PBMC induced by ConA or HBsAg is too low to initiate an intracellular signal, which may lead to the disability to promote differentiation and proliferation of immune cells. It was demonstrated that the level of P55 component (a subunit) of IL2R can control IL2 responsiveness, IL-2R β subunit (P75) expression and the formation of IL-2/IL2R complex (2). The results of our study suggest that low level of IL2R expression may be one of the important mechanisms of abnormal immune response to HBV in AsC, nevertheless the mechanism of abnormal response to HBV in AsC might be very complex.

In the complicated immune response, the interaction of immune cells, the double signal stimulation and the intracellular transmission of immune second signals may be the most important factor controlling the immune response. It is found that the activation of the T cell depends on the intensity and nature of specific antigen signals and the second signals (9, 10). However, the mechanisms of the interaction of the IL-2R-positive cells and other immune cells and the transmission and memory of the second immune signal in the immune response to HBsAg are still

unclear in AsC as well as in normal individuals. In this report, it is found that during the IL2R expression of PBMC induced by purified HBsAg in AsC and in normal individuals there is mutual regulation between IL2R-positive cells and IL2R-negative cells, namely the IL2R-positive mononuclear cell linking with the cell membranes of many IL2R-negative smaller mononuclear cells. This phenomenon of close linking resembles that of rosette formation during the binding of T-cells with sheep erythrocytes, and is thus temporarily named "the IL2R-positive-cell-rosette". Moreover, it is seen that the number of IL2R-positive rosette is lower in AsC than that in normal individuals, and the typical rosette of IL2R-positive cell is not observed in PBMC stimulation by ConA or PHA. The results suggest that the immune response to HBsAg stimulation relies very much on the mutual regulation between IL2R-positive and IL2R-negative cells, and it is shown that AsC precisely lacks this type of immunoregulation.

IL2R-positive-cell-rosette formation may be a new phenomenon of immunoregulation among immune cells, which is probably of important significance in the immune response to HBsAg: It might be a particular form of regulation among the immune cells, by which they can respond normally to the stimulation of HBsAg. The immune second signal would then be transmitted to multiple immune cells simultaneously by the IL2R-positive cell. Hence, the immune response to HBV may be enhanced effectively. This new phenomenon of response to HBsAg may relate to the immune memory of the second signal. The immune memory signal is transmitted by the IL-2R-positive cells to multiple immune cells simultaneously, so that the immune memory may be effectively expanded by many immune cells. It may be an important mechanism leading to the secondary immune response to HBV infection. The result as mentioned above suggests that the abnormal IL2R expression and abnormal immunoregulation of the IL2R-positive cells, and the incapability of transmitting the immune second signal and immune memory signal effectively may be an important mechanism of the abnormal immune response to HBV infection in AsC.

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