STUDIES ON THE EFFECTS OF ESTROGEN ON ANTIBODY RESPONSES IN ASYMPTOMATIC HB VIRUS CARRIERS AND NON-RESPONDERS TO HB VACCINE INOCULATION

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Summary

Antibody-forming cells against trinitrophenylated sheep red blood cells (TNP-SRBC) were induced to similar extents, when peripheral blood mononuclear cells from normal individuals, non-responders to HB vaccine inoculation and asymptomatic HBV carriers were stimulated in vitro with pokeweed mitogen (PWM). Although these antibody responses were significantly augmented by adding estrogen simultaneously with PWM to mononuclear cell cultures prepared from normal individuals, no such augmentation was demonstrable in cultures from asymptomatic HBV carriers and non-responders to HB vaccine inoculation. Interleukin-1 production and DNA synthesis in PWM- and PHA-stimulated mononuclear cells were also increased by estrogen in normal individuals, but not in the other two groups. These observations suggest that asymptomatic HBV carriers and non-responders to HB vaccine inoculation share a similar deficiency, not found in normal subjects, with regard to the modulatory effects of estrogen.

Key Words: Asymptomatic HBV carrier, Non-responder to HB vaccine inoculation, Antibody-forming cells, Estrogen.

Introduction

It has been reported that serum levels of immunoglobulins and antibody responses against different antigens are higher in females than in males\(^1\). The clinical manifestation of autoimmune diseases is also more frequent in sexually mature females than in their male counterparts\(^2,3\). These facts suggest that immune responses may be influenced by the sex of the immunized subject. The capacity for virus elimination and the inducibility of immune responses against viral antigens in HB virus-infected patients also differ between female and male individuals\(^4\). In a previous report, we showed that there were sexual differences in the detection rates of HBs antigen, HBs antibody and some autoantibodies in patients with chronic active hepatitis or liver cirrhosis: the incidence of HBs antigen-positive cases was significantly higher in males, while the detection rates for anti-HBs, anti-nuclear, anti-smooth muscle and anti-liver cell membrane antigen
antibodies were higher in females\(^5\). Although the detailed mechanisms responsible for sexual differences in immune responses largely remain to be elucidated, it is presumed that X-linked genes or sex hormones or both play a decisive role in determining responsiveness\(^6,7\). In this connection, we previously reported that the antibody responses against trinitrophenylated sheep red blood cells (TNP-SRBC) induced by stimulation with pokeweed mitogen (PWM) in human peripheral blood mononuclear cells were significantly enhanced by estrogen added simultaneously with PWM and were inhibited by testosterone. A similar effect of estrogen on PWM-induced lymphocyte proliferation has also been demonstrated\(^8\). In this report, we studied the effects of estrogen on antibody responses in asymptomatic HB virus carriers and non-responders to HB vaccine inoculation.

**Materials and Methods**

1. **Non-responders to HB vaccine inoculation**
   20\(\mu\)g of HB vaccine (The Kitasato Institute) was given subcutaneously, and subsequently after the 4th and 21st weeks. After the 28th week, anti-HBs antibodies were measured by the RIA method (Ausab, Abott Laboratory). The cut-off index in the RIA assay of HBs antibody was decided at below 2.0 which applied to 11 of the 88 subjects. These eleven were selected as non-responders to HB vaccine inoculation.

2. **Preparation of mononuclear cells from human peripheral blood**
   Mononuclear cells were obtained by Ficoll-Conray density gradient centrifugation from the peripheral blood of 22 normal individuals, 18 patients who were asymptomatic HBV carriers and 11 who were non-responders to HB vaccine inoculation. They were washed and suspended in Eagle's MEM containing 10% fetal calf serum at a concentration of 2 \(\times\) 10^6 cells/ml.

3. **Preparation of trinitrophenylated sheep red blood cells (TNP-SRBC)**
   Sheep red blood cells were trinitrophenylated according to the method of Rittenberg et al.\(^9\). After washing twice with Eagle's MEM, the TNP-SRBC were suspended in the same medium at a concentration of 7.5 \(\times\) 10^8 cells/ml.

4. **Estimation of anti-TNP-SRBC antibody-forming cells induced by PWM stimulation**
   The mononuclear cell suspensions (2 \(\times\) 10^6 cells/ml) were incubated *in vitro* with 50 \(\mu\)g/ml of PWM (Difco Co.) at 37°C for 72 hrs in a humidified cell incubator with aeration by 5% CO\(_2\) in air. After incubation, TNP-SRBC were added to each mononuclear cell suspension, and the number of antibody-forming cells produced was counted by the hemolytic plaque assay according to the method of Jerne et al.\(^10\). The effects of estrogen on antibody responses were examined using exactly the same experimental procedure with the exception of the simultaneous addition of estrogen with PWM. In these experiments, we used "combined estrogen" containing sodium salt of estrone sulfuric acid and equilin sulfuric acid (Mochida Pharmaceutical Co.) for estrogen, since it was more soluble than other preparations.

5. **Measurement of lymphocyte transformation**
   The mononuclear cells were incubated with PHA or PWM in the presence or absence of estrogen at 37°C for 48 hrs. 1 \(\mu\)Ci of \(^3\)H-thymidine (specific activity, 5 Ci/mmol) was then added to each cell suspension, and incubation was continued for another 24 hrs. After incubation, the cells were collected on a millipore filter membrane and washed with 10 ml of cold isotonic saline followed by ice cold 5% trichloroacetic acid solution under suction. The radioactivity retained on the filter membrane was subsequently measured by the liquid scintillation spectrometry using a Packard Tricarb