DYNAMICS OF THE ROSETTE-FORMING CELL POPULATION AFTER PRIMARY AND REIMMUNIZATION OF MICE TREATED WITH SEROTONIN PRECURSOR 5-HYDROXYTRYPTOPHAN

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The dynamics of the population of rosette-forming cells in the lymph glands of mice with a normal and increased serotonin concentration was studied in inbred CBA mice immunized with bovine serum albumin (2 mg per mouse, with Freund's complete adjuvant. Serotonin synthesis was modified by a single injection of 5-hydroxytryptophan (30 min before the first immunization in a dose of 100 mg/kg) released slowly from the incomplete adjuvant. Rosette-forming cells were determined every 1-2 days after primary and reimmunization given at an interval of 14 days. In the animals receiving 5-hydroxytryptophan there were significantly fewer rosette-forming cells than in the controls, and the dynamics of the rosette population was disturbed.

An increase in the concentration of active serotonin produced in various ways, as previous experiments showed, leads to inhibition of humoral antibody production, reflected in a decrease in the titers of these antibodies and a change in their dynamics [1, 2].

The object of the present investigation was to study changes in the dynamics of the population of rosette-forming cells, many of which have been shown [11-13] to be antibody-forming cells.

EXPERIMENTAL METHOD

Experiments were carried out on 256 male inbred CBA mice aged 3.5-6 months. The animals were immunized with 2 mg bovine serum albumin with Freund's complete adjuvant subcutaneously in the upper third of the thigh in a dose of 0.1 ml. Reimmunization was carried out with the same dose of antigen after an interval of two weeks. (Preliminary experiments enable the shortest interval between immunizations to yield the typical seondary response to be chosen.) At various times after immunization regional lymph glands were removed from the experimental mice and a suspension of single cells prepared from them. Rosette-forming cells were detected by Shvartsman's method [3], by absorption of heterogeneic red cells conjugated with antigen through bis-diazotized benzidine, on the surface of the immunologically active cell. The preparations were examined under the microscope in a moveable system with floating cover slip, using a phase-contrast immersion optical system. In the cell suspension from each mouse 1000 cells were examined and the number of rosettes recorded. The mean number of red cells absorbed on a single producer cell (the absorption index) was used to quantify the immunological activity of the cells.

The serotonin precursor 5-hydroxytryptophan was injected subcutaneously as a single dose of 100 mg/kg on the day of the primary immunization and 30 min before injection of the antigen, and this led to an increase in serotonin synthesis at the time of immunization [4, 6]. Bearing in mind the rapid inactivation of serotonin, to delay its absorption and to create a constant supply of the substance, it was given in a slowly released form together with Freund's incomplete adjuvant.

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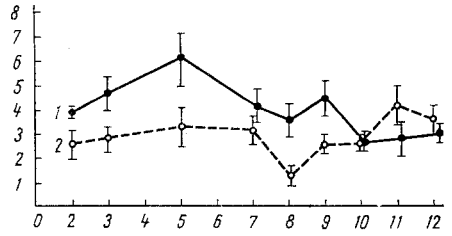

Fig. 1. Dynamics of number of rosette-forming cells during primary response after a single injection of 100 mg/kg 5-hydroxytryptophan: 1) control group; 2) experimental group. Abscissa, days after immunization; ordinate, number of rosette-forming cells per 1000 lymph gland cells examined.

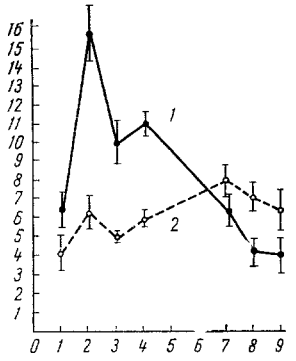

Fig. 2. Development of secondary response in lymph glands of mice receiving 100 mg/kg 5-hydroxytryptophan on day of primary immunization: 1) control group; 2) experimental group. Abscissa, days after immunization; ordinate, number of producer cells per 1000 cells examined.

EXPERIMENTAL METHOD

The experimental results showed that after primary immunization two maxima of the number of rosette-forming cells occurred in the control mice (Fig. 1). The first maximum of the number of rosette-forming cells was observed on the 5th day; the second, which was lower, occurred on the 9th day, this dynamics is typical and agrees with observations described previously [5, 7-10]. By contrast with the controls, the immune response developed slowly and less intensively in the mice receiving 5-hydroxytryptophan; the increase in the number of rosette-forming cells corresponding to the first maximum in the control was essentially absent, while the second maximum, equal in magnitude to the control, was shifted to a later period (the 11th day). So far as the number of rosette-forming cells is concerned, it was reduced as a result of injection of the substance almost throughout the period of observation.

Elevation of the serotonin level in the period of primary contact between recipient and antigen affected not only the primary but also the secondary immune response (Fig. 2). Whereas in the control animals a typical secondary response occurred, in the mice receiving 5-hydroxytryptophan the anamnestic response was weak; on account of the delay in the increase in the number of cells to its maximum, this response was more like the primary response in its character. Just as after primary immunization, the inhibitory effect of serotonin in relation to the number of rosette-forming cells was particularly marked in the period when the immune response in the control mice reached its highest level and the first maximum was formed; the second maximum was a few days later than in the control.

The "adsorption index" was altered much less by serotonin. A decrease in the intensity of adsorption of red cells by cells conjugated with the antigen began to be observed only on the 7th day after primary immunization and on the 2nd-3rd day after reimmunization.

Comparison of the primary immune response with the secondary clearly showed that the inhibitory effect of 5-hydroxytryptophan was more marked in the secondary response although the substance was injected only once, before primary immunization.

It can accordingly be postulated that the process of formation of the cells of immunological memory is particularly sensitive to a change in the serotonin level in the body.

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