T- AND B-LYMPHOCYTES OF GUINEA PIGS SENSITIZED WITH RAGWEED POLLEN

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The number of T and B rosette-forming cells (T- and B-RFC) in guinea pigs was determined after sensitization with ragweed (Ambrosia artemisifolia L.) pollen. The number of B-RFC in the regional lymph nodes in the early stages of sensitization was shown to be 4 times greater than the number of B-RFC in normal animals. The functional capacity of the T-cells, assessed by the rosette-formation test, showed a more marked change after sensitization than the corresponding capacity of the B-cells.

KEY WORDS: ragweed pollen; sensitization; T- and B-lymphocytes.

Recent work has shown that for the immune response to take place interaction is required between three cell systems: B-lymphocytes, T-lymphocytes, and macrophages [2]. However, the question of the part played by these cell populations in pollen sensitization receives scant mention in the literature. In the few works which have been published the results of a study of the reaction of T- and B-cells to pollen antigens and to certain protein antigens are described [8,11,12]. To assess the functional state of the T- and B-systems of immunity, methods of spontaneous and complementary rosette formation are used [9,13]. Lymphocytes are known to be capable of forming rosettes on account of receptors located on their surface membrane. Since immunocompetent cells take part in the rosette-formation test, this method can be used to judge immunologic reactivity at the cellular level.

The object of this investigation was to study the functional activity of T- and B-lymphocytes of sensitized guinea pigs.

EXPERIMENTAL METHOD

Experiments were carried out on 84 albino guinea pigs weighing 250-300 g. The animals were sensitized by intradermal injection of a 5% suspension of ragweed (Ambrosia artemisifolia L.) pollen in incomplete adjuvant into each footpad. The ability of the T- and B-lymphocytes of the blood, regional lymph nodes, and spleen to form rosettes was studied at intervals in the course of sensitization. Investigations have shown that rabbit red cells can be used as markers for guinea pig T-cells and sheep's red cells as markers for B-cells [3,7,10].

Obtaining the Lymphocytes. Lymphocytes were isolated from heparinized blood by centrifugation in a Ficoll — Urografin or Ficoll — Urotrast density gradient (density 1.077), as in Boyum's method [6]. The lymphocyte suspension contained not more than 1-2% of dead cells. The spleen and regional lymph nodes (axillary and inguinal) were shelled out of their capsules and minced with the addition of medium 199 to obtain cell suspensions, which were filtered through Kapron gauze and washed 3 times with medium 199. The cell residue was treated with 0.5 ml medium 199 containing 10% calf serum. The lymphocytes were counted in a Goryaev chamber.

Rosette-Formation Tests with T-Lymphocytes. A modified method of Andersson et al [3] was used. Rabbit's red cells, kept in Alsever's solution for not more than 2 weeks after taking, were used as markers for the T-cells. The red cells were washed 3 times with Hanks' solution and a 1% suspension was prepared. Next, $4 \cdot 10^5$ lymphocytes were added to 0.2 ml of the 1% red cell suspension. The mixture of cells was incubated for 30 min at 37°C, then centrifuged for 3 min at 1000 rpm and allowed to stand for 2 h in a refrigerator. The residue was then carefully resuspended and films were made, dried under a fan, fixed for 10 min in methanol, and stained with azure II-eosin. The number of rosettes in the stained films was counted. A lymphocyte

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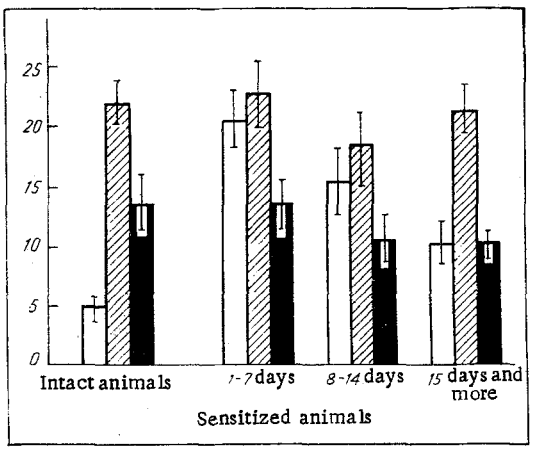

Fig. 1. Number of B-RFC in intact and sensitized animals. Ordinate: number of B-RFC (per 100 cells). Here and in Fig. 2: unshaded columns: lymph nodes; obliquely shaded: spleen; black: blood.

with three or more red cells adherent to it was taken to be a rosette. Altogether 200 lymphocytes were counted.

Rosette-Formation Test with B-Lymphocytes. Sheep's red cells kept in Alsever's solution were used. To the 1% red cell suspension 1 ml of rabbit antiserum against sheep's red cells in a subagglutinating titer was added and the mixture was incubated for 30 min at 37°C. The sensitized red cells were washed twice, the residue was resuspended in 1 ml Hanks' solution, and 1 ml of fresh mouse complement in a dilution of 1:10 was added. After incubation for 30 min at 37°C the sensitized red cells were washed twice and resuspended in 2 ml of Hanks' solution. After addition of $4 \cdot 10^5$ lymphocytes to 0.2 ml of the sensitized red cells the mixture was incubated for 10 min. It was then centrifuged for 3 min at 1000 rpm and allowed to stand for 2 h at room temperature. Films were then prepared from the residue and the number of "rosettes" was counted in the light microscope.

EXPERIMENTAL RESULTS

The investigation began with a study of the ability of lymphocytes from the blood, spleen, and axillary and inguinal lymph nodes of normal guinea pigs to form rosettes. Experiments were carried out on 15 animals. The results are illustrated in Figs. 1 and 2. The results obtained with intact animals agreed with those described by Wilson and Coombs [12] but differed from those obtained by Boxel and Rosenstreich [5].

The next stage of the investigation was concerned with studying the B-system of immunity in guinea pigs in the course of sensitization with ragweed pollen. B-Lymphocytes play an important role in the development of immune reactions of humoral type and, for that reason, the quantitative characteristics of this population are of definite interest. Immunoglobulin receptors, by means of which the cells are recognized by the antigen, are known to be present on the surface of B-cells. On the other hand, interaction between antigen and the surface receptors of the B-cells plays an important role in differentiation and maturation of antibody-producing cells with the participation of thymus-dependent lymphocytes and macrophages.

The experiments showed (Fig. 1) that the number of rosette-forming B-cells (B-RFC) in the regional lymph nodes in the early stages of sensitization (from 1 to 7 days) was more than 4 times greater than the number of B-RFC of the intact animals. This was evidently connected with an increase in the proliferative activity of the B-cells in the regional lymph nodes. Later, with an increase in the duration of sensitization, the number of B-RFC fell, but it still was higher than their number in normal animals.

Todome et al. [11] found an increase in the number of B-cells in the regional lymph nodes of guinea pigs immunized in the footpads with guinea pig serum albumin conjugated with p-aminobenzenesulfonic acid on the 3rd day after immunization.

Ability of the B-cells of the spleen to form rosettes did not differ significantly from that of the B-cells of normal guinea pigs at any time during sensitization (Fig. 1). According to some workers [1,4], after

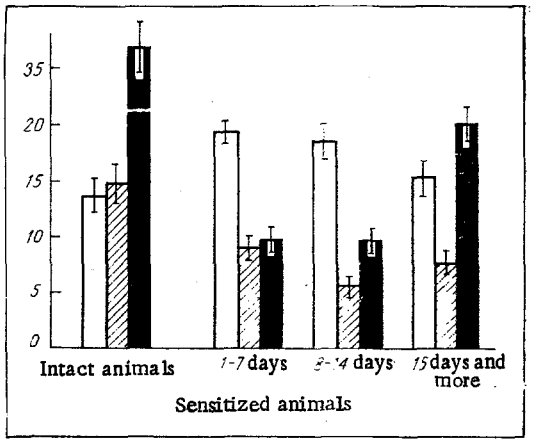

Fig. 2. Number of T-RFC in intact and sensitized animals. Ordinate: number of T-RFC (per 100 cells).

intradermal or subcutaneous immunization the number of RFC in the spleen, unlike their number in the regional lymph nodes, does not increase.

In the early periods of sensitization, the B-cells of the blood form rosettes with about the same intensity as the B-cells of intact animals. Later, this ability of the blood B-cells of sensitized animals decreases somewhat (Fig. 1).

In guinea pigs surviving after anaphylactic shock, the ability of their peripheral blood B-cells to form rosettes is considerably increased (24.5 ± 4.4).

Besides the increase in the number of B-RFC in the early periods of sensitization, there was also a small increase in T-RFC activity in the regional lymph nodes, reflected in a larger than normal number of T-RFC (Fig. 2). Subsequently during sensitization the number of T-RFC in the regional lymph nodes fell slightly, although not down to the level characteristic of normal animals. Particularly marked changes occurred in the number of T-RFC in the peripheral blood. There was a considerable decrease in their number during the first days of sensitization. With an increase in the period of sensitization some increase took place in T-RFC activity in the peripheral blood, but not up to the level characteristic of normal animals (Fig. 2).

In the early stages of sensitization of guinea pigs with ragweed pollen the ability of the B-cells of the regional lymph nodes to form rosettes thus rises sharply. With an increase in the period of sensitization this ability decreases somewhat. The functional capacity of the T-cells of the regional lymph nodes in the course of sensitization, determined by the rosette-formation test, changes to a less marked degree than that of the B-cells.

Consequently, sensitization of guinea pigs with ragweed pollen is accompanied by increased activity of the B-system of immunity in the experimental animals.

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