DIFFERENTIATION OF IMMUNOCOMPETENT CELLS IN LYMPH GLAND TISSUE CULTURES

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Lengthening of the life span of the population of antibody-forming cells was observed in a culture of lymph gland tissue and was due to the longer preservation of the pool of young, actively dividing forms under the conditions of cultivation.

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Investigations [5, 6, 9] have shown that antibody formation in lymph gland tissue cultivated in vitro continues much longer than in the intact organism. This results from the longer persistence of the population of antibody-producing cells.

The object of this investigation was to determine whether the more prolonged antibody formation in culture takes place through an increase in the life span of differentiated plasma cells or through the more prolonged proliferation of these cells [5, 10].

EXPERIMENTAL METHOD

Rabbits weighing 2-2.5 kg were immunized by two subcutaneous injections of crude diptheria toxoid in doses of 130 Lf into the heel at intervals of 30 days. Some of the rabbits were sacrificed 24 h after reimmunization; the regional popliteal lymph glands were removed, and fragments of them (measuring 3 × 3 mm) placed in revolving tubes with Eagle's medium with the addition of 20% homologous serum and antibiotics. The tubes were kept at 37°. The medium was changed every 3 days. At various times of cultivation pieces were removed for histological treatment (5 pieces at each time). Parallel investigations were made of 4 regional popliteal lymph glands from control rabbits which had been immunized twice. The lymph glands were fixed and embedded in paraffin wax by Sainte-Marie's method [1]. Series sections were stained with methyl green–pyronine, and examined by the direct luminescence-serologic method for antigen and by the indirect method simultaneously for specific antibodies and nonspecific (to the particular antigen) immune globulins [1]. The general morphological picture was assessed, and the number of cells producing specific and nonspecific immune globulins per equal area of section through the lymph gland was determined. Types of plasma cells producing antibodies and nonspecific immune globulins were identified. On the basis of identification of not less than 200 cells from each lymph gland or its fragment their formula was established or the ratio of hemocytoblasts to juvenile and juvenile to mature plasma cells was determined separately.

EXPERIMENTAL RESULTS

Cells producing antibodies and nonspecific immune globulins were found in the regional lymph glands of control rabbits which had been immunized and reimmunized, principally in the region of the medullary cords in the medullary zone. Luminescent cells were located singly or in groups (usually a mixture of cells producing specific and nonspecific immune globulins was found in each group). Before immunization, no

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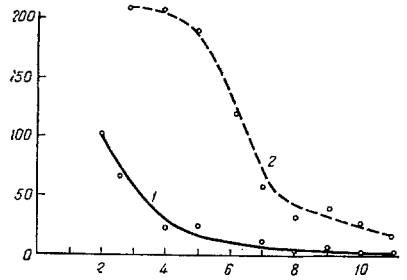

Fig. 1.

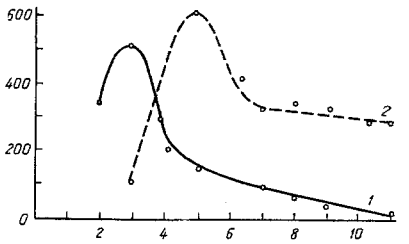

Fig. 2.

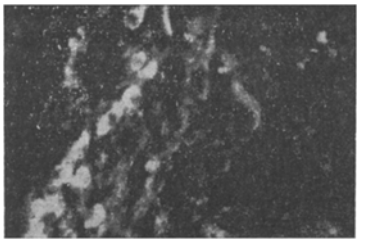

Fig. 3.

Fig. 1. Ratio between hemocytoblasts and juvenile plasma cells in population of antibody-synthesizing cells from regional lymph glands of immunized and reimmunized rabbits in the intact organism (1) and in tissue culture (2). Abscissa, days after reimmunization; ordinate, percentage of hemocytoblasts relative to juvenile plasma cells.

Fig. 2. Ratio between juvenile and mature plasma cells in population of antibody-synthesizing cells from regional lymph glands of immunized and reimmunized rabbits in the intact organism (1) and in tissue culture (2). Abscissa, days after reimmunization; ordinate, percentage of juvenile plasma cells relative to mature.

Fig. 3. Groups of antibody-containing cells in marginal areas of piece of lymph gland 4 days after beginning of cultivation in vitro. Coons' method, 20 ×.

antibody-containing cells were found. They first appeared on the 2nd day. They increased in number to reach a maximum by the 4th day (several thousands of cells per section), becoming much less numerous on the 5th day (hundreds of cells per section). On the 9th-11th days, only single antibody-containing cells could be seen. The formula of these cells (Figs. 1, 2) showed a successive replacement of hemocytoblasts by juvenile, and eventually by mature plasma cells from the early to the latter periods after reimmunization. When considering the dynamics of the total number of cells in this population it must be remembered that practically all plasma cells proceed into the mature stage not later than 5 days after reimmunization. A few cells containing nonspecific immune globulins were also observed before reimmunization (mostly mature forms). Their number increased 24 h after reimmunization on account of the appearance of juvenile forms. In the later stages the dynamics of the total number and population composition of the plasma cells containing nonspecific immune globulins corresponded to the dynamics of the antibody-containing cells.

Extensive degenerative changes were found in the central zone of the fragments of regional lymph glands cultivated in vitro 1-2 days after the beginning of cultivation, and a zone containing morphologically unchanged cells was found only at the periphery of the fragment. In these areas, until the 10th day of cultivation, large number of pyroninophilic cells could be seen, some of them in a state of mitosis. In the first two days, numerous pyroninophilic reticular cells were found. No antigen could be detected 24 h after the beginning of cultivation and, correspondingly, on the 2nd day after reimmunization or at later periods in the lymph gland fragments. Cells containing specific and nonspecific immune globulins were found in the peripheral zones of the pieces of lymph gland tissue (Fig. 3). They were arranged in groups, stretched along reticular bands. On the 2nd day after immunization and, correspondingly, on the first day after the beginning of cultivation of the lymph glands in vitro, globulin-producing cells appeared in very small numbers. On the 3rd-5th day after immunization the total number of cells containing antibodies and nonspecific immune globulins increased, and remained at a high level until the 11th day, whereas in the control animals the antibody-containing cells had virtually disappeared from the lymph gland on the 8th-10th day after immunization. In the formula of antibody-containing cells, a shift from hemocytoblasts to juvenile and from juvenile to mature plasma cells was observed during cultivation. However, compared with the lymph glands of the intact organism, a much longer persistence of the pool of cells capable of active division (hemocytoblasts and juvenile plasma cells; Figs. 1 and 2) was observed in tissue culture among the antibody-containing cells. Similar changes were also observed in the formula of cells producing nonspecific immune globulins. Under tissue culture conditions, therefore, the longer persistence of antibody-containing cells in the lymph glands took place on account of the longer proliferation and differentiation of these cells.

The life cycle of a clone of differentiated plasma cells in the lymphoid tissue of the intact organisms is known to be relatively short (5-5.5 days [4, 8]), and 3-6 h after the second stimulus, synchronous differentiation of the precursors of the plasma cells begins. The question arises: under tissue culture conditions do changes take place in the mode of differentiation of the plasma cells formed immediately after the antigenic stimulus, or do new cells begin to differentiate in the later stages after stimulation in tissue culture? The experiments now described do not provide an unambiguous answer to this question. However, from indirect evidence, it appears that the more probable cause of the increased life span of the population of antibody-synthesizing cells is a change in the mode of differentiation of plasma cells formed soon after the antigenic stimulus, toward an increase in the period which the cells spend in the juvenile stages of development. In cultures of reticulo-endothelial tissues of different types, the release of new cells to begin differentiation is inhibited [3], and the actual process of cell differentiation is distorted [2]. In the present experiments carried out in vitro, a smooth, although slow compared with control, replacement of juvenile forms by mature was observed in the cell formulas (Figs. 1 and 2). In the latter periods, with the release of new precursors of antibody-forming cells for differentiation, an increase in the percentage of hemocytoblasts and of juvenile plasma cells could be expected at these times.

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