The discovery of cross-reactions between CL and PI by some workers and their absence in the experiments of others can evidently be explained by the different spectrum of antibodies against the determinants of the lipid hapten in antisera obtained by different research workers. Important factors determining the spectrum of antibodies against a given hapten in each concrete serum may be the nature of the preparation used for immunization, the method of injection of the antigen, the individual differences in the responses of different animals, and the method used to test serum activity.

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STROMAL FIBROBLASTS OF HEMATOPOIETIC ORGANS AND ANTIBODY FORMATION IN CULTURE

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Stromal mechanocytes of thymic, bone-marrow, and splenic origin obtained from monolayer cultures at the 3rd-6th passage, if added to suspension cultures of rabbit spleen cells by the method of Mishell and Dutton, have a significant effect on the accumulation of antibody-forming cells (AFC) by the 4th day in culture. Their action clearly depends on dose. Stromal mechanocytes of bone marrow origin, in doses of 2.1×10^3-6.25×10^5, caused inhibition of AFC formation in culture. Stromal mechanocytes of thymic origin in doses of 2.75×10^3-8×10^5 caused an increase in the number of AFC, whereas mechanocytes of splenic origin in doses of 2.1×10^3-1.3×10^4 had no significant effect, and in doses of 8×10^4-6.25×10^5 inhibited AFC formation. Many of the living cells and AFC were concentrated in the fraction of nonadherent cells.

KEY WORDS: stromal fibroblasts; antibody formation in vitro.

Stromal mechanocytes (fibroblasts) of hematopoietic tissue play an important role in the creation of the microenvironment for lymphocytes in hematopoietic and lymphoid organs [1-6]. This raises the question of the effect of stromal fibroblasts on the development of immunologic reactions in vitro.

In the investigation described below the effect of stromal fibroblasts on antibody formation in culture was studied. The plan of the investigation was to isolate cell lines of stromal fibroblasts from bone marrow,

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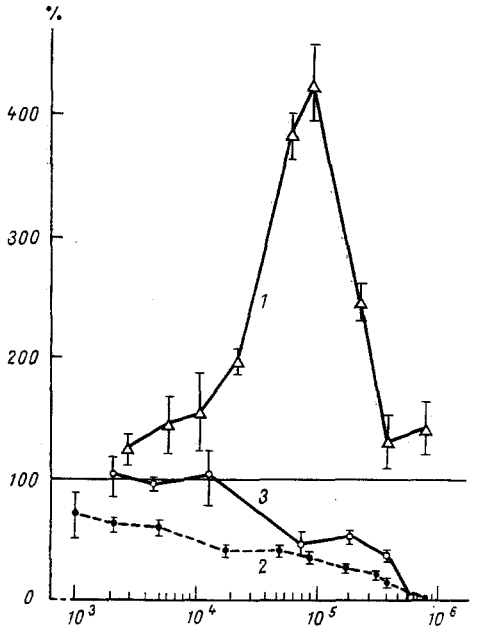

Fig. 1. Effect of fibroblasts of different origin on number of AFC in culture. 1) Fibroblasts of thymic, 2) of bone-marrow, and 3) of splenic origin. Abscissa, number of fibroblasts added to culture; ordinate, number of AFC per culture (in %).

thymus, and spleen and to compare the number of antibody-forming cells (AFC) arising in cultures of spleen cells stimulated by sheep's red blood cells in the presence of stromal fibroblasts of different origin.

EXPERIMENTAL METHOD

Rabbits weighing 1.5-2 kg with a low titer of natural hemolysins were used as donors of the cells for explanation. Suspensions of spleen, thymus, and bone marrow cells were made up in Hanks' solution by the method described earlier [3]. To obtain strains of stromal fibroblasts, bone marrow, thymus, and spleen cells were explanted into Roux flasks with a base area of 42 and 78 cm^2 [3].

The cells were cultured in medium No. 199 with 10% autologous or homologous serum. On the 12th-15th days, when discrete colonies of fibroblasts had formed in the cultures, the cells were removed with trypsin and transferred for further culture to new flasks. These passages were repeated once every 4-6 days.

To induce antibody formation in vitro spleen cells cultured by the method of Mishell and Dutton [8] in the modification of Theis and Thorbecke [9] in minimal Eagle's medium, with the addition of replaceable amino acids, glutamine, vitamins, sodium pyruvate, and 20% heated autologous serum were used. Culture took place in plastic dishes 50 mm in diameter on a revolving platform (7-10 rpm). Each dish contained 2.5×10^7 cells in 2.5 ml nutrient medium; 0.1 ml of a 2% suspension of sheep's red cells was added as the antigen. Mishell and Dutton's [8] nutrient cocktail was added daily to the cultures. When the cultures were set up in them, fibroblasts from bone marrow, spleen, and thymus, obtained from monolayer cultures after the 3rd-6th passage were added in addition to the spleen cells. The number of AFC in the cultures was counted on the 4th day. Cells in the medium were removed with a syringe, and those adherent to the bottom of the dish were removed by means of a siliconized rubber scraper. The number of living cells was determined by the trypan blue exclusion test and the number of AFC by Jerne and Nordin's method of direct local hemolysis in gel [7]. The mean number of living cells and of AFC per culture was determined for each group, and also deviations from the mean. The significance of differences were determined by Student's criterion.

EXPERIMENTAL RESULTS

Although a few macrophages were obtained in monolayer cultures of strains of fibroblasts in the first passage, in the second and subsequent passages macrophages were completely absent, and such cultures consisted of pure strains of fibroblasts, as was confirmed by specific antifibroblast sera [3, 4].

In the presence of antigen the number of AFC in cultures of spleen cells on the 4th day was 257.61 ± 9.21 per culture. The number of "spontaneous" AFC in cultures to which no antigen was added was 26.27 ± 1.35. Within each experiment the number of AFC in cultures with antigen was 4-137 times greater (on the average 10 times) than the number of "background" AFC.

The addition of stromal fibroblasts to the spleen cells had a marked action on AFC production. The character of this effect was determined by the origin and number of fibroblasts added. The corresponding data are given in Fig. 1. On the addition of 2×10^3-1×10^4 fibroblasts of splenic origin the number of AFC did not change significantly; starting with a dose of 8×10^4 fibroblasts per culture the number of AFC fell, so that with a dose of 6.25×10^5 AFC formation was inhibited practically completely.

Fibroblasts of thymic origin invariably had a marked stimulating action on AFC production. A significant stimulating action was noted after the addition of 2.7×10^3 fibroblasts; maximal stimulation of 4-5 times was observed after the addition of 6×10^4-9×10^4 fibroblasts.

Combined culture of spleen cells with fibroblasts of bone marrow origin invariably led to the opposite result: a decrease in the number of AFC. A dose of 2×10^4-3×10^5 bone-marrow fibroblasts reduced AFC formation by about two-thirds, and in a dose of 8×10^5 AFC formation was inhibited practically completely.

The addition of all three types of fibroblasts did not significantly affect the survival rate of the cells in cultures. In fact, on the addition of 1.7×10^3-6.25×10^5 splenic fibroblasts and of 1×10^3-8×10^5 bone marrow fibroblasts the survival rate of the cells was 0.88 and 0.89 respectively of that of cells in cultures to which no fibroblasts were added, whereas after the addition of 2.5×10^3-8×10^5 fibroblasts of thymic origin the survival rate was 0.9 of the corresponding values in the control cultures.

Fibroblasts of different origin exhibited the same intensity of growth in cultures of spleen cells. After the addition of fibroblasts of all three types in a dose of 2×10^3-5×10^3 cells on the bottom of the plastic dish, by the 4th day of culture solitary fibroblasts were distributed in the field of vision; after addition of 1×10^4-5×10^4 cells, an incomplete monolayer of fibroblasts was formed, whereas after the addition of a large number of cells the monolayer was complete. In some experiments the number of living cells and the number of AFC in fractions of freely removable and adherent cells were determined separately. In cultures without the addition of fibroblasts the fraction of nonadherent cells contained 97.92% of all living cells and 96.3% of all AFC, whereas after the addition of 1×10^5 fibroblasts from the bone marrow or spleen and of 1.5×10^4 fibroblasts from the thymus, the number of nonadherent cells was 96.9-94.5% of the living cells and 87-100% of AFC. Among nonadherent cells fibroblasts always accounted for under 1%.

Fibroblasts of different origin thus differed in their action on antigen-dependent AFC formation in cultures of spleen cells: Fibroblasts from thymic origin stimulated, whereas fibroblasts from splenic and bone-marrow origin inhibited it. The action of all three types of fibroblasts clearly depended on dose. If the effect of an equal number of fibroblasts is compared with a dose of, for example, 8×10^5 per culture, it differed by more than 100 times. Cells of noninbred rabbits were used in the experiments and, for that reason, spleen cells were combined in culture with allogeneic stromal fibroblasts. However, in some experiments stromal fibroblasts from the spleen, thymus, and bone marrow were obtained by explantation of cells from the same donor, and these were then combined with the same spleen cells. Under these circumstances the difference in the action of fibroblasts of different origin, as indicated above, on AFC production was manifested completely. This shows that differences in the action of stromal fibroblasts from bone marrow, spleen, and thymus on AFC production is independent on differences in the degree of histocompatibility of the strains of fibroblasts and spleen cells used, and that interaction between stromal mechanocytes and lymphocytes does not require them to be identical with respect to histocompatibility antigens. The effect of stromal fibroblasts on AFC production was quite unconnected with their action on the survival rate of spleen cells in culture, i.e., it was aimed selectively at proliferation and differentiation of AFC or of their precursor cells. These results point to the presence of yet another as yet unstudied level of regulation of antigen-dependent development of antibody-producing cells, connected with the influence of stromal mechanocytes responsible for the microenvironment, in hematopoietic tissue. The point of application of this regulation (T-cells, B-cells, macrophages) and the mechanism whereby it acts (humoral or contact effects) still remain unknown.

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EFFECT OF A HIGHLY PURIFIED FACTOR FROM THE THYMUS ON CELLULAR AND HUMORAL INDICES OF IMMUNITY IN THYMECTOMIZED MICE

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In experiments on thymectomized adult CBA mice the effect of a homogeneous factor of polypeptide nature from the thymus, with mol. wt. about 5000 (thymarin-III) on the cellular and humoral indices of immunity was studied in animals. Thymectomy in animals was shown to sharply reduce the number of T-cells in the spleen. Correspondingly, the ability of the mice to produce both IgM- and IgG-antibody-forming cells and humoral antibodies against a thymus-dependent antigen (sheep's red blood cells) was sharply inhibited in the mice. Subcutaneous injection of thymarin-III in a dose of 1 μg/kg into the animals daily for 7 days completely restored the T-cell population of the spleen and restored the normal immunologic reactivity of the animals.

KEY WORDS: thymus factor; thymectomy; T-lymphocytes; antibody-formation.

It has now been shown that certain factors isolated from the thymus can completely or partly restore the immunologic indices in neonatally thymectomized animals [7, 10]. Thymectomy in adult animals is known to lead chiefly to a reduction in the T_1-population of short-living lymphocytes [5] and to simulate to a considerable degree certain acquired immunodeficiency states. In this connection the possibility of restoring immunologic reactivity in thymectomized adult animals is interesting. The writers previously demonstrated the stimulating action of a highly purified factor from the thymus on the immune response to thymus-dependent antigen in intact animals [1].

The object of the present investigation was to study the effect of thymus factor on the cellular and humoral indices of immunity in adult thymectomized mice.

EXPERIMENTAL METHOD

Experiments were carried out on 105 male CBA mice weighing 16-18 g. The thymus was removed from 75 animals, under superficial ether anesthesia, by a modified method of Galkin and Drobkin [3]. A mock operation was performed on 30 mice, i.e., all stages of the operation except actual removal of the thymus.

Between 1.5 and 2 months after the operation thymus factor was injected subcutaneously into 40 thymectomized animals in a dose of 1 μg/g in 0.2 ml of physiological saline daily for 7 days. The thymus preparation

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