INHIBITION OF MIGRATION OF SPLEEN CELLS IN VITRO AS A TEST OF SENSITIZATION TO GRAFTS

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UDC 612.6.02.017.3-087.5 : 612.112.4

Details of a test based on inhibition of the migration of spleen cells in the presence of specific antigens are described. The use of direct and indirect versions of this test revealed sensitization of spleen and lymph gland cells to antigens of donors of allogeneic and xenogeneic skin grafts after transplantation in mice, rats, and rabbits.

Inhibition of migration of macrophages in vitro in the presence of specific antigens is used as a test for hypersensitivity of the delayed type observed during sensitization by certain antigens [6, 7, 10-12] and by skin grafts [1, 5, 8, 9]. The investigation to be described below, as well as others [2-4], has shown that this test is specific and sensitive and that its results agree on the whole with those of skin tests. If the cells of an exudate or pure macrophages are used, the migration test is carried out in capillary tubes [2, 10], while if pieces of spleen are used, it is carried out in special chambers [10, 11].

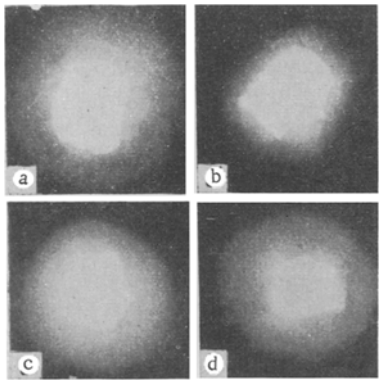

Fig. 1. Inhibition of migration of spleen cells of recipient rats after addition of antigens from donor mice: a) piece of spleen of recipient rat without antigen; b) the same with mouse antigen; c) piece of spleen of control rat without antigen; d) the same with mouse antigen.

Inhibition of migration of spleen cells in vitro has been used by the writers as a test for sensitization of the cells of animals receiving allogeneic or xenogeneic skin grafts.

Skin grafts (12 × 6 mm) from mice of line B10D2 were grafted on the dorsum of mice of the coisogeneic line C57BL/10Sn. Skin grafts (25 × 12 mm) from mice of line CBA/He or rats of the August line were grafted onto Wistar rats. Noninbred rabbits were grafted with skin (35 × 20 mm) from rabbits of another color. After appropriate preparation, the skin grafts were fixed to all the animals by a continuous suture and held in position by a pressure bandage. Antibiotics were given.

Cultivation. Sterile, chemically pure cover slips (24 × 24 mm) were framed by immersing their edges for 2 mm in molten paraffin containing 5% wax. Framing in this manner prevented the medium from escaping. The cover slips were placed on glass slabs (12 × 40 cm), and 0.25-0.35 ml of medium No. 199 containing 20-30% inactivated (56°C, 30 min) serum was applied to each cover slip. Penicillin and streptomycin (100 units/ml of each) were first added to the medium. Pieces of spleen measuring up to 2 × 3 mm, washed with medium No. 199 to remove blood, were placed in the center of the cover slips. The pieces were usually taken from the subcapsular part of the spleen, opposite to the hilus. For this purpose, the

Central Research Laboratory, Vitebsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 6, pp. 119-122, June, 1971. Original article submitted March 3, 1970.

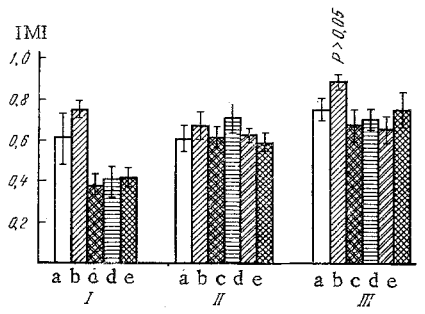

Fig. 2. Inhibition of migration of spleen cells of intact animals after addition of lymph gland cells of recipients and antigens of donors to them, compared with migration of cells of same spleen in pure medium (a), and in the presence of recipients' lymph gland cells without antigen (b), or the same cells with intact antigen (c), recipients' cells killed by freezing, with donor's antigen (d), lymph gland cells of intact animals with donor's antigen (e), and donor's antigen alone (f). I) Lymph glands of rats (5) on 8th day after xenogeneic grafting ($P < 0.05$-0.01); II) lymph glands of rats (5) on 11th day after allogeneic grafting ($P < 0.05$-0.01); III) lymph glands of mice (4) on 20th day after allogeneic grafting ($P < 0.05$-0.02).

spleen was cut into parts in two mutually perpendicular planes so that the capsule covered only the topside of the piece. Cover slips with the pieces applied to them were covered by special glass caps. Sometimes a group of these cover slips was covered by a Petri dish. In that case, to prevent drying, several drops of sterile water were applied to the inner surface of the dish. The edges of the caps and dishes were framed with molten paraffin. The chambers were incubated at 37°C for 48 h. However, as special experiments showed, the results can be read after 24 h.

Pieces of mouse spleen were cultivated in medium No. 199 containing 20% inactivated guinea pig serum, pieces of rabbit spleen were cultivated in medium No. 199 with 20% homologous or autologous serum, and pieces of rat spleen also in medium No. 199, but with not less than 30% of serum.

Analysis of Migration. The zones of migration and the area of the piece of spleen (Fig. 1) were measured with an ocular scale (20 divisions = 1 mm) under a stereoscopic microscope giving a magnification of 16-20 times. Some of the medium was first removed. The mean radius of migration was found from eight mutually perpendicular radii measured. Since the radius of migration largely depended on the size of the piece, the migration index (MI) was determined as the ratio between the mean radius of migration and the area of the piece of spleen:

$$MI = \frac{\text{mean radius of migration}}{\text{area of piece of spleen.}}$$

If the pieces were unequal in thickness (in rabbits), the thickness was measured by the micrometer screw of the microscope; in such cases the migration index was determined as the ratio between the mean radius of migration (in millimeters) and the volume of the piece (in cubic millimeters). The migration index was determined at the same time by a gravimetric method. The slab with the cover slips was placed in the position of the frame of a photographic enlarger, and the image of the piece and the zone of migration, magnified 10-15 times, was projected on a flat sheet of squared paper on which their outlines were traced. The migration index was found as the ratio between the weight of the image of the zone of migration and the weight of the image of the piece. In some cases the zones of migration were photographed in a similar manner, in which case their image was projected on photographic paper. The inhibition of migration index (IMI) was calculated by the formula:

$$IMI = \frac{\text{migration index in experiment (with antigen)}}{\text{migration index in control (without antigen)}}.$$

From each spleen of 3 to 5 recipients, 3-10 pieces were cultivated with antigen and the same number in the control without antigen. A second parallel control was set up (for antigen toxicity); pieces of spleen of intact animals were cultivated under the same conditions. In addition, the specificity of inhibition of migration was tested by adding intact antigens to other experimental chambers. In the case of rabbits sensitized with allogeneic grafts, antigens of intact rabbits were used for this purpose, antigens of C3H mice were used for the mice, and antigens of noninbred rats for the rats. Lymph gland cells in a number of $5\text{-}10 \cdot 10^4$ to each chamber were used as the antigens. Statistical analysis of the results was carried out by Student's method. After measurement of the migration zones the cells were fixed with methanol and stained with hematoxylin-eosin.

The experimental results showed that lymph gland cells (antigen) of donor animals (or lines of donors) inhibited migration of the cells of the pieces of spleen obtained from the recipients (Fig. 1).

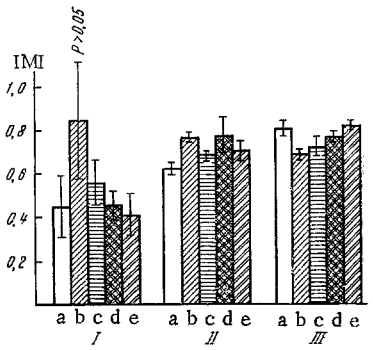

Fig. 3. Inhibition of migration of spleen cells from intact animals in the presence of supernatant from recipients' lymph gland cells incubated with donors' antigens, compared with migration of same spleen cells in medium (a), and in presence of supernatant from recipients' lymph gland cells without antigen (b); of same cells incubated with intact antigen (c), of same cells distintegrated and incubated with donors' antigens (d); of lymph gland cells from intact animals incubated with donors' antigens (e). I) Lymph glands of rabbits (3) on 16th day after allogeneic grafting ($P < 0.01$); II) lymph glands of mice (3) on 20th day after allogeneic grafting ($P < 0.05$-0.001); III) lymph glands of rats (3) on 8th day after allogeneic grafting ($P < 0.05$-0.2).

Most of the cells migrating on the surface of the glass from the pieces of spleen were macrophages of blast-like cells, and there were considerable numbers of lymphocytes, giant cells, and cells of unknown nature. Immature eosinophils were seen; other granulocytes were usually not present in the zones of migration. After addition of the antigen to the pieces of spleen of the sensitized animals, foci of karyorrhexis were found.

Study of Sensitization of Lymph Gland Cells. Explants of lymph glands were found to be unsuitable for the study of cell migration (because of the small amount of material, the difficulty of freeing the explants from the capsule, and the relatively weak migration of the cells). Sensitized lymphocytes, on contact with antigen, are known to secrete a factor inhibiting migration [2-4]. To study the sensitization of the lymph gland cells, two versions of the indirect method were therefore used.

1. Suspensions of viable lymph gland cells, or of lymph gland cells killed by repeated freezing and thawing (control), from recipients (0.5-$2 \cdot 10^6$), were added to chambers containing pieces of spleen of intact animals. Antigen, consisting of living cells ($0.5 \cdot 10^5$) from lymph gland cells from animals which were donors (or belonged to the donors' line) of the skin grafts was added (or not added – control) to the chambers. The same number of lymph gland cells from intact animals and the same antigen (Fig. 2) were added to other control chambers.

2. Suspensions of cells (20-$30 \cdot 10^6$/ml) obtained from the lymph glands of recipients or intact animals were incubated in tubes for 24-48 h at 37°C in medium No. 199 with serum. Antigen cells in a number of 0.5-$1 \cdot 10^5$ or intact antigen (only to experimental samples) in the same number, were added to some of the tubes containing recipients' lymph gland cells and to all tubes containing cells from intact animals. In addition, control tubes were set up, to which recipients' lymph gland cells disintegrated by freezing, and living antigen cells were added. The supernatant of the incubated mixtures was tested for its ability to inhibit migration of spleen cells from normal animals when added to the chambers in a volume of 0.05-0.1 ml. Some pieces of the test system were incubated without supernatant (Fig. 3).

In both the first and second versions of the experiment, lymph gland cells of animals sensitized by allogeneic or xenogeneic skin grafts, incubated with donor's antigens, or the supernatant obtained after such incubation was found to be capable of inhibiting migration of the spleen cells of intact animals (Figs. 2 and 3).

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