DIAGNOSIS OF THE CRISIS OF GRAFT REJECTION BY THE INHIBITION OF CELL MIGRATION TEST IN VITRO

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Intensive sensitization of lymphocytes of the blood, lymph glands, and spleen of mice and of recipient rabbits to antigens of the donors of allogeneic and xenogeneic skin graft was found a few days before their rejection by means of direct and indirect (using allogeneic and xenogeneic fragments of a test spleen) methods inhibiting migration of spleen cells. In the early periods (2nd-3rd days) after transplantation and during the period of marked destruction of the grafts their sensitization to antigens was weaker, and this was often expressed as stimulation of cell migration. Detection of lymphocytes sensitized to donors' antigens by the migration test may provide a warning of the impending crisis of graft rejection.

A problem of great importance in connection with progress in organ transplantation is the timely diagnosis of the crisis of rejection of incompatible grafts. Humoral antibodies of different types (hemagglutinins, cytotoxins, etc.) are usually found [1, 2, 5] either after death of the graft or, at best, at its beginning. This late appearance of immunoglobulins in the recipient's serum can be explained by their low concentration through adsorption in the earlier periods on the antigens of the grafts [2, 3]. However, even the detection of humoral antibodies against donors' antigens is not an absolute criterion of threatened rejection of the graft, for the main role in destruction of the grafts belongs to sensitized lymphocytes, responsible for the reaction of hypersensitivity of delayed type [7, 9. 15, 16]. The appearance of such cells in the blood or lymphoid organs can thus be used as a criterion of the onset of sensitization to the graft and, consequently, as a warning of its rejection. One way of detecting lymphocytes sensitized against various antigens (including transplantation antigens) is by the test based on inhibition of the migration of cells (macrophages and leukocytes) in vitro [4, 6, 8, 10-14].

The possiblity of using this test in the diagnosis of the graft rejection crisis was studied.

EXPERIMENTAL METHOD

Skin allografts were transplanted onto 16 rabbits (donor and recipient were of the same breed), on 80 mice of line B10/D2 from C57BL/10Sn mice, and on 30 Wistar rats from August rats. Xenogeneic skin grafts were transplanted onto 30 B10·D2 mice from August rats. In addition, autologous or syngeneic grafts were transplanted onto animals of the same lines [4]. At various times after grafting the presence of sensitized lymphocytes was tested in the recipient by direct and indirect methods of inhibition of migration of spleen cells in vitro by the technique described earlier [4]. When the direct method of inhibition of migration was used, donors' antigen cells were added (final concentration $0.5 \cdot 10^6$–$1 \cdot 10^6$/ml) were added to the fragments of recipients' spleen cultivated in vitro on coverslips in medium No. 199 with 15% inactivated guinea-pig serum. If the spleen cells were sensitized against this antigen, inhibition or stimulation

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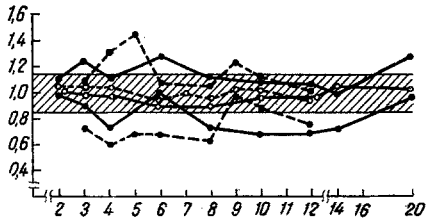

Fig. 1

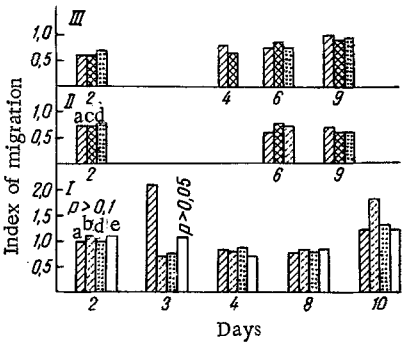

Fig. 2

Fig. 1. Effect of donors' antigens on migration of recipients' spleen cells compared with migration of the same cells without antigens. Continuous line with four circles shows action of antigens of donor rats on migration of spleen cells of recipient mice; continuous line with empty circles represents action of the same antigens on migration of spleen cells of intact mice; broken line with filled circles represents effect of antigens of donor mice (C57Bl/10Sn) on migration of spleen cells of recipient mice (B10 • D2); broken line with empty circles represents effect of the same antigens on migration of spleen cells of intact mice. Each point on curves represents data for three-six animals giving stimulation greater than 1.0 or inhibition less than 1.0 of migration.

Fig. 2. Inhibition or stimulation of migration of spleen cells of intact allogeneic animals under the influence of supernatant of lymph gland or blood leukocytes of recipients incubated with donors' antigens, relative to migration of cells of the same spleen: a) in presence of supernatant of recipients' leukocytes without antigen; b) in medium; c) in presence of supernatant of recipients' leukocytes incubated with intact antigen; d) in presence of supernatant of leukocytes of intact animals incubated with donors' antigens; e) in presence of supernatant of recipients' leukocytes disintegrated by freezing and incubated with donors' antigens; I) supernatant of lymph gland cells of mice receiving allografts; II) supernatant of lymph gland cells; III) blood leukocytes of recipient rabbits. In every case, unless specially mentioned, $P < 0.05$–0.02.

of their migration from the fragments was observed. In the direct method the supernatant obtained after preliminary incubation of blood leukocytes or lymph gland cells with donors' cells for 24 h at 37°C was tested for its ability to inhibit the migration of spleen cells of intact animals. For this purpose pieces of spleen of animals of the same species or line as the recipients and also of other lines or species were used. Appropriate controls [4] were set up (Figs. 1 and 2).

EXPERIMENTAL RESULTS

When the direct method of inhibition of migration was used, on the 3rd day after transplantion donors' antigens were shown to inhibit (index of inhibition of migration < 1.0) or to stimulate (index of stimulation of migration > 1.0) the migration of spleen cells of mice receiving allogeneic or xenogeneic skin grafts (Fig. 1). Stimulation and inhibition of migration reflect different degrees of sensitization to antigens [19, 20]. If strong sensitization to the donors' antigens was present in some mice, inhibition of migration was observed, while weak sensitization of other animals under the same experimental conditions led to stimulation of the migration of spleen cells. The strongest inhibition of migration was observed in the period preceding rejection of the grafts (Fig. 1). The degree of sensitization could depend not only on the number of sensitized cells in the lymphocyte population but also, evidently, on their sensitivity to the antigen.

It was shown by the indirect method that the supernatant obtained after incubation of viable blood leukocytes or lymph gland cells from the recipients with the donors' antigens inhibited migration of the

spleen cells of the intact animals (Fig. 2). The supernatant of sensitized cells, incubated with antigens and disintegrated by freezing and thawing, did not affect migration or had a weaker effect. By means of this method sensitized cells were found in the blood and lymph glands of some rabbits 2 days after transplantation, and in the lymph glands of mice 3 days after transplantation (Fig. 2). The migration-inhibiting factor secreted by sensitized lymphocytes into the supernatant under the influence of the specific antigen, it will be noted, was neither strain-specific nor species-specific. For instance, the supernatant obtained after incubation of lymph gland cells of B10·D2 recipient mice with antigens of C57B1/10Sn donors was able to inhibit the migration of spleen cells of mice belonging to lines C3H/He and A. On the other hand, the supernatant obtained after incubation of leukocytes of rabbits receiving allografts with the donors' antigens was able to inhibit migration of the spleen cells of intact mice by comparison with the controls. This last observation agrees with those obtained by other workers [7]. Sometimes the supernatant of recipients' lymph gland cells incubated with intact antigens obtained from other rabbits had some effect on migration of the test spleen cells. This effect was evidently due to the presence of common isoantigens in the intact animals and in the donors. In fact, migration of spleen cells of B10·D2 mice receiving skin grafts from C57B1/10Sn mice were inhibited by antigens of lymph gland cells of CC57W mice, containing the same allospecificities of the H-2 locus as the donors, but not by antigens of lymph glands of BALB/c mice, deficient in these allospecificities.

In the period of marked destruction of allogeneic (9th-10th days) and xenogeneic (6th day) grafts, the results of both the direct and the indirect migration methods showed that sensitization could be diminished (Figs. 1 and 2), presumably because of the participation (and, perhaps, the death) of the sensitized cells in the destruction of the grafts. This evidently could not have been the reason why the supernatant of the blood leukocytes obtained from recipient rabbits on the 9th day after grafting, and incubated with donors antigens, had no effect on migration of the allogeneic splenic test cells. However, definite inhibition of migration of the spleen cells of intact rabbits was observed in the supernatant of lymph gland cells taken at the same time and incubated with donors' antigens. In the period immediately after rejection of the grafts, sensitization of the cells was considerable, but later it weakened appreciably.

The results of both the direct and indirect tests of inhibition of migration of spleen cells in vitro thus showed that lymphocytes sensitized to donors' antigens appear in the blood, lymph glands, and spleen of the recipient before rejection of the grafts. Sensitization reaches its maximum a few days before the beginning of graft rejection, and has begun to weaken at the time of marked destruction of the graft. Since the participation of sensitized cells in the process of graft destruction is generally accepted, the appearance of these cells can be used as a criterion of the onset of sensitization and the impending crisis of rejection. The use of the macrophage migration-inhibition test in capillary tubes [8, 11, 13] or of the indirect-spleen-cell migration-inhibition test [4] with fragments of xenogeneic test spleen enables these methods to be used in clinical practice.

LITERATURE CITED

1. V. I. Govallo, Immunity in the Transplantation of Organs and Tissues (Humoral Indices, Methods of Overcoming It). Doctoral Dissertation, Moscow (1965).
2. E. A. Zotikov, The Isoserology of Homotransplantation [in Russian], Moscow (1969).
3. D. K. Novikov, G. P. Adamenko, V. I. Novikova, et al., in: Details of Manifestations of Tissue Incompatibility during Transplantation of Organs [in Russian], Moscow (1971), p. 141.
4. D. K. Novikov, G. P. Adamenko, and V. I. Novikova, Byull. Éksperim. Biol. i Med., No. 6, 119 (1971).
5. R. V. Petrov and Yu. M. Zaretskaya, Radioaction Immunology and Transplantation [in Russian], Moscow (1970).
6. S. Al-Askari and H. S. Lawrence, Transplant. Proc., 1, 400 (1969).
7. R. E. Billingham, Anat. Rec., 165, 121 (1969).
8. B. R. Bloom and B. Bennet, Science, 153, 80 (1966).
9. L. Brent, Progr. Allergy, 5, 271 (1958).
10. J. R. David et al., Ann. New York Acad. Sci., 120, 393 (1964).
11. R. E. Falk et al., J. Immunol., 104, 1287 (1970).
12. V. Haskova, et al., Folia Biol. (Prague), 13, 293 (1967).
13. E. R. Heise et al., J. Immunol., 101, 1004 (1968).
14. L. Korcakova, Folia Biol. (Prague), 14, 226 (1968).
15. P. B. Medawar, in: H. Lawrence (editor), Cellular and Humoral Aspects of the Hypersensitive State, New York (1959), p. 504.

16. N. A. Michison, Proc. Roy. Soc. B, 142, 72 (1954).
17. E. Scopinska et al., Arch. Immunol. Ther. Exp., 19, 377 (1971).
18. J. Svejcar and J. Johanovsky, Z. Immun. Forsch., 128, 1 (1965).
19. M. Sborg, Acta Med. Scand., 184, 135 (1968).