AUTOAGGRESSIVE IMMUNOCOMPETENT CELLS IN MICE IN THE LATE STAGES AFTER IRRADIATION

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Lethally irradiated DBA/1 or (C57BL × DBA/1)F_1 mice were injected simultaneously with therapeutically effective doses of isologous bone marrow cells and syngeneic lymphocytes from intact mice (control) or from animals surviving different lengths of time after sublethal irradition. In the control the presence of lymphocytes in the mixed graft did not affect the survival rate of the recipients. Lymphocytes from mice surviving 6-12 months after irradiation in a dose of 600-700 R blocked the therapeutic effect of bone marrow (the killing effect). The intensity of the killing effect depended on the number of lymphocytes transplanted and the number of bone marrow cells in the graft. No killing effect was found if mice surviving 1 month after irradiation were used as donors of the lymphocytes. The results are regarded as evidence of autosensitization of the animal in the late stages after irradiation.

KEY WORDS: lymphocytes; late effects of irradiation; autoimmunity; transplantation of bone marrow.

After transplantation of the tissues of animals surviving after irradiation into syngeneic recipients rejection of skin grafts [11] or the development of a disease similar to secondary disease in the recipients [3, 4] are observed, i.e., the characteristic phenomena of tissue incompatibility. On the basis of these findings it has been suggested that the cells of the irradiated organism loose some of their ability to behave as syngeneic with respect to tissues of the same genotype. In the investigation described below the autoaggressive properties of lymphocytes of mice surviving a long time after irradiation were studied on the basis of their ability to block the therapeutic action of isologous bone marrow, transplanted into lethally irradiated recipients (the so-called killing effect [12], present in nonsyngeneic systems).

EXPERIMENTAL METHOD

Mice of strains DBA/1 (H-2^q) and BALB/c (H-2^d) from the Rappolovo nursery were used. The animals were irradiated on the RUM-17 apparatus at a dose rate of 60-100 R/min (0.5 mm Cu+1 mm Al, 200 kV, 15 mA). A transparent plastic container with 10 mice was placed 50 cm from the anode on a paraffin scatterer. Bone marrow for transplantation was obtained from intact animals. The cells were flushed out of the femora with balanced salt solution [7], containing antibiotics. The donors of lymphocytes were mice which had survived 1, 6, 8, or 12 months after whole-body sublethal irradiation, and also intact animals. The tissue of the superficial lymph nodes was minced and rubbed through a nylon sieve by means of a small bristle brush, the use of which enabled a small number of undamaged cells to be obtained. The cell suspensions were washed by centrifugation (200g, 10 min) and resuspended. The number of nucleated cells and the fraction of cells permeable for the dye (0.02% erythrosin), which were regarded as damaged, were counted. The damaged cells counted for 10-15% in the bone marrow suspension and 30-40% of the lymphocytes. Suspensions from the organs of the irradiated animals contained altogether fewer cells, but the fraction of damaged cells was the same as in the control. The resulting suspensions were mixed in the necessary proportions and injected intravenously into the mice 24 h after irradiation. The number of recipients in each group varied from 15 to 30. To prevent early death of the recipients they were given 0.4 mg kanamycin by intraperitoneal injection daily for the first 5 days of the experiments. The clinical state and survival rate of the recipients were studied during 2-3 months after transplantation.

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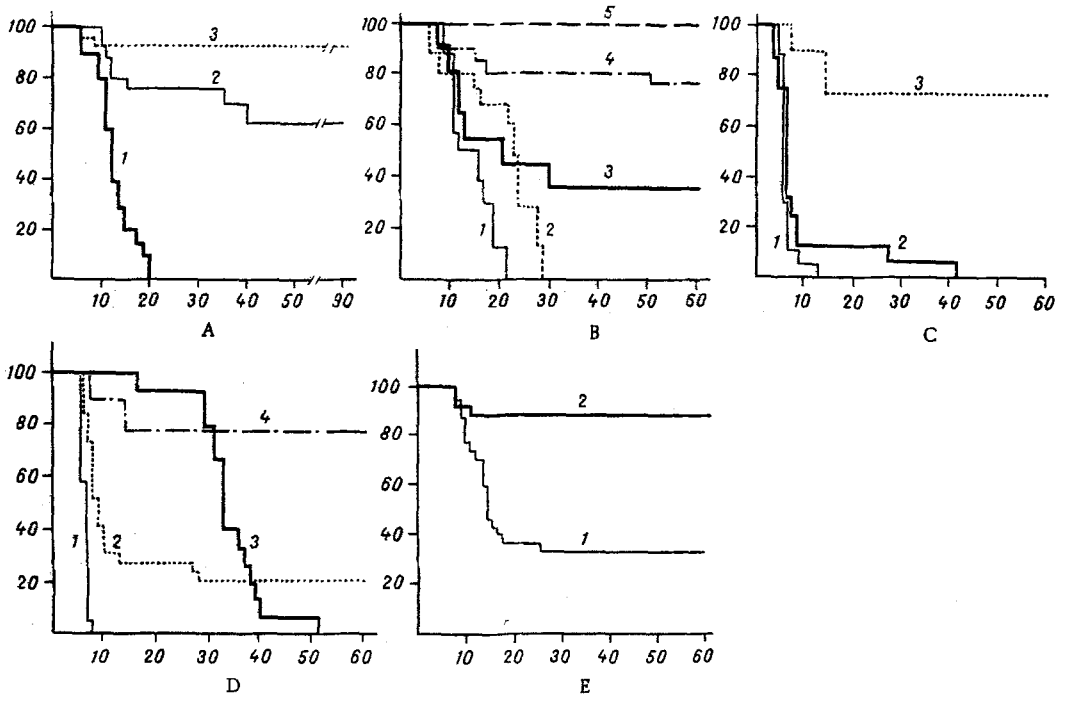

Fig. 1. Effect of lymph node cells (LNC) of irradiated and unirradiated mice on survival of lethally irradiated syngeneic recipients protected by isologous bone marrow cells (BMC). A, B, C, and D) Transplantation into DBA/1 mice; E) into F_1 mice. A) Allogeneic or syngeneic LNC of intact animals: 1) 10^6 BALB LNC + 0.5×10^6 BMC; 2) 10^6 BALB LNC + 10^6 BMC; 3) 10^6 DBA/1 LNC + 0.5×10^6 BMC. B, C, D) LNC of syngeneic mice surviving after irradiation with 650-700 R. B) Six months: 1) 10^6 LMC + 0.25×10^6 BMC; 2) 3×10^6 LNC + 10^6 BMC; 3) 10^6 LNC + 10^6 BMC; 4) 10^5 BMC; 1 month: 5) 3.5×10^6 LNC + 10^6 BMC; C) 6 months: 1) 6×10^6 LNC + 0.75×10^6 BMC; 2) 10^6 LNC + 0.75×10^6 BMC; 3) 3.5×10^6 intact LNC + 0.75×10^6 BMC; D) 12 months: 1) 6×10^6 LNC + 0.75×10^6 BMC; 2) 10^6 LNC + 0.75×10^6 BMC; 3) 10^6 LNC + 10^6 BMC; 4) 0.75×10^6 BMC. E: 1) 10^6 BMC + 10^6 LNC of syngeneic mice surviving 8 months after irradiation; 2) 10^6 BMC. Abscissa, days after irradiation; ordinate, survival rate of recipients (in % of initial number).

EXPERIMENTAL RESULTS

Data on the survival rate of the mice in the 10 experimental groups and in some of the control experiments are given in Fig. 1. The dose of irradiation causing death of 100% of the animals in the course of 30 days was 750 R for DBA/1 mice and 800 R for F_1 hybrids. An increase in the survival rate of the irradiated animals was observed after transplantation of 10^5 isologous bone marrow cells (Fig. 1B), but this dose of cells gave a therapeutic effect only in some experiments. Transplantation of $(0.5\text{-}1.0) \times 10^6$ bone marrow cells gave a reproducible therapeutic effect (Fig. 1D, E). The addition of twice or 4-5 times the number of lymph node cells of syngeneic unirradiated donors to a suspension of bone marrow cells (Fig. 1A, C) did not affect the viability of the recipients. If the lymph node cells were transplanted from donors incompatible for the H-2 complex (BALB/c mice) the outcome of the experiment was determined by the number of bone marrow cells in the mixed graft. If transplanted in a suboptimal number their therapeutic effect was completely blocked. If the dose of bone marrow was optimal, a considerable proportion of the recipients did not die in the course of 3 months (Fig. 1A). Consequently, transplantation of $(0.5\text{-}1.0) \times 10^6$ isologous bone marrow cells provided a background against which the killing effect could be induced only by lymphocytes with a sufficiently high level of aggressiveness.

Lymphocytes of DBA/1 mice surviving 6 months after irradiation in a dose of 650-700 R gave the strongest killing effect (Fig. 1B, C). The results indicate that the survival rate of the recipients was determined both by the number of transplanted lymphocytes and by the dose of bone marrow cells. The aggressiveness of the immunocompetent cells in a syngeneic system, just as an allogeneic system, can evidently be blocked to some extent by intact bone marrow cells.

After transplantation of lymph node cells from mice surviving 12 months after irradiation (650 R) the killing effect was somewhat weaker (Fig. 1D). In one experiment (Fig. 1D, 3) the clinical state of the recipients began to worsen only in the fourth week after transplantation. The mice started to loose weight rapidly, they developed dermatitis, their hair fell out, and some of them developed gastrointestinal disorders. On the whole, the picture corresponded to that of the isologous variant of secondary disease [1, 6].

Lymph node cells from DBA/1 mice irradiated with 650 R 1 month before transplantation did not cause a killing effect (Fig. 1B).

In the experiments on F_1 hybrids lymph node cells from animals surviving 8 months after irradiation in a dose of 600 R induced a considerable killing effect (Fig. 1E).

The results thus indicate that mouse lymphocytes in the late stages after irradiation acquire aggressiveness toward syngeneic recipients that is normally not characteristic of them. This property arises after the animals have recovered from radiation sickness and can be regarded as a late consequence of irradiation.

In previous experiments on this theme [3, 4] spleen cells were transplanted from irradiated animals. Under these circumstances stem cells and immunocompetent cells were transplanted from donor to recipient and the reduced viability of the recipients could be explained by imperfection of the former or aggressiveness of the latter. The experimental model used in the present investigation was free from this indeterminacy.

Aggressiveness of lymphocytes on transplantation can be explained either by incompatibility of donor and recipient or by the state of the donor's autosensitization. Experiments in which lymphocytes of BALB/c mice were used showed that in the model system described above the action of knowingly foreign immunocompetent cells was blocked by intact bone marrow cells. Nonsyngeneity of donors and recipients cannot therefore be regarded as the basis of aggressiveness of the lymphocytes of irradiated mice.

There are several considerations which suggest that the cause of the aggressiveness may be autosensitization of the donors. Autosensitization is nowadays regarded as a shift in immunologic homeostasis during which the regulatory suppressor mechanisms are weakened, and as a result there is activation of autoimmune processes [9]. It has been shown that irradiation may be a factor abolishing tolerance [8], that the medium of the irradiated organism favors the materialization of the autoimmune potential, and that the latter can be adoptively transferred to a syngeneic recipient [10]. Autoimmune shifts are characteristic of irradiated animals and have been described by many workers [2, 3, 5]. It can accordingly be postulated that the aggressiveness found in lymphocytes is essentially autoaggressiveness, reflecting the state of autosensitization of the body in the late stages after irradiation.

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