PARENTAL RESISTANCE OF IRRADIATED MICE TO (CBA × M523)F_1 LYMPHOCYTES. FATE OF TRANSPLANTED CELLS, DURATION OF RESISTANCE, AND ITS SPECIFICITY

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The immunologic resistance of (CBA × M523)F_1 mouse lymphocytes to a foreign antigen (sheep's red blood cells) in lethally irradiated CBA mice was investigated. If irradiation, transplantation of the cells, and the test injection of antigen were carried out on the same day, the activity of the graft was inhibited (compared with a syngeneic system); if the interval between these operations was increased to 3 days the activity of the donor's cells was restored. Retransplantation of recipients' spleen cells into irradiated CBA and F_1 mice showed the viability of the transplanted cells and absence of their readaptation to the nonsyngeneic microenvironment. The resistance of the recipients could be specifically overcome by previous injection of cells from F_1 mice in combination with or without cyclophosphamide. It can be concluded from the results that genetic parental resistance of CBA mice to cells of F_1 mice is due to the recipient's immunologically competent cells, which are inactivated 3 days after irradiation. They have no cytotoxic action on the donor's cells but temporarily restrict their activity.

KEY WORDS: parental resistance; allogeneic inhibition; hybrid resistance.

The writers described previously the sharply reduced ability of hematopoietic stem cells and lymphocytes of F_1 mice to proliferate and differentiate in lethally irradiated mice of the parental lines [3]. This effect was observed only in hybrids of mutant mice CBA.M523 with other lines [4]. The phenomenon described was called parental resistance.

The object of this investigation was to study the fate of transplanted cells of F_1 mice and also certain properties of the recipient's cells responsible for parental resistance.

EXPERIMENTAL METHOD

Mice of lines CBA/Ca Lac Sto (CBA), A/Sn Y (A), and CBA · M523/Y (M523), and their hybrids aged 1.5-2 months were used. Donors and recipients of the same sex were chosen.

The recipients were irradiated in a dose of 1000 rad from a cobalt source a few hours before transplantation of the cells, which were injected in a dose of $5 \cdot 10^7$ in 0.5 ml. In some specially indicated experiments, irradiation was given 3 days before transplantation of the cells.

Sheep's red blood cells (SRBC) were used as antigen and were injected intravenously into the donors in a dose of $1 \cdot 10^6$ cells 6-30 days before the experiment, and into the recipients in a dose of $5 \cdot 10^8$ cells. The SRBC were usually injected 30 min-1 h after transplantation of the cells; in some specially indicated experiments the antigen was injected 3 days after injection of the cells. The number of antibody-forming cells (AFCs) in the recipients' spleen was determined on the 5th day by the method of local hemolysis in gel.

In some experiments CBA or A recipients were subjected to preliminary tolerogenic treatment by the scheme described previously [2]. For this purpose, the A or CBA mice were given an intraperitoneal injection of cyclophosphamide (CP) in a dose of 200 mg/kg, and 3 h later they were given an intravenous injection of $1 \cdot 10^8$ spleen cells from (A × M523)F_1 or (CBA ×

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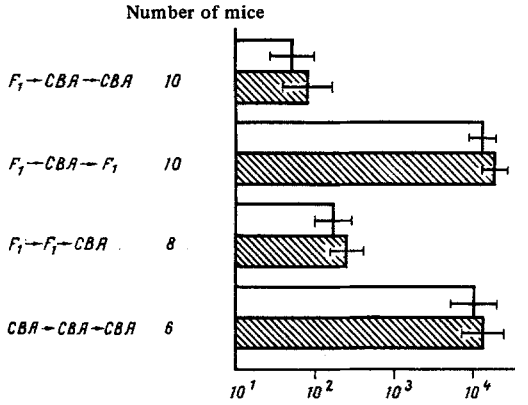

Fig. 1. Passage of lymphocytes of (CBA × M523)F_1 mice through semisyngeneic intermediate recipients. Abscissa, number of AFC against SRBC inspleen of secondary recipients 8 days after injection of $5 \cdot 10^7$ cells (unshaded columns). Shaded columns represent number of AFC in spleen of secondary recipients assuming injection of whole spleen of intermediate donor.

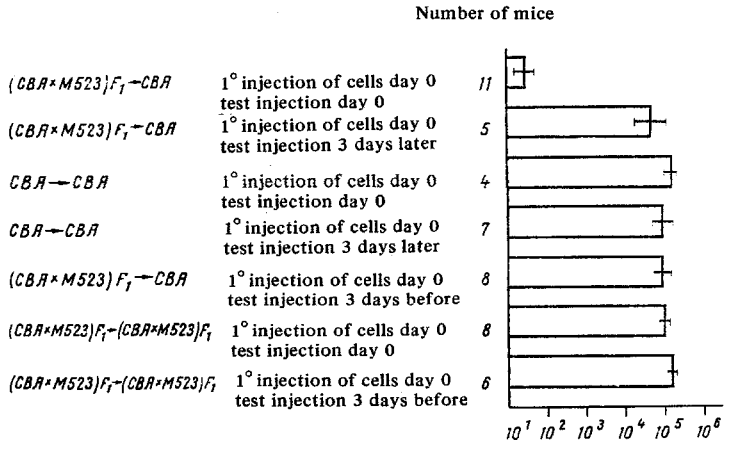

Fig. 2. Time characteristics of parental resistance. Abscissa, number of AFC against SRBC 5 days after test injection of antigen.

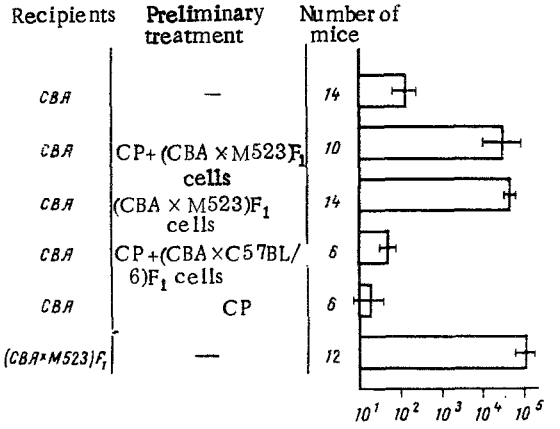

Fig. 3. Specific abolition of parental resistance. Abscissa, number of AFC against SRBC 5 days after injection of (CBA × M523)F_1 mouse cells and test injection of antigen.

M523)F_1 mice respectively. Mice receiving CP alone or CP in combination with (A × CBA)F_1 or (CBA × C57BL/6)F_1 mouse cells, and also intact animals served as the control. Some animals received (CBA × M523)F_1 mouse cells without CP. After 1-1.5 months all the animals were used as recipients in the scheme described above.

In another series of experiments the "double passage" method was used: spleen and thymus cells from (CBA × M523)F_1 mice were injected in a dose of $5 \cdot 10^7$ into lethally irradiated CBA and F_1 mice. Spleen cells from both groups of primary recipients were transplanted 8 days later into lethally irradiated CBA and (CBA × M523)F_1 mice in a dose of $5 \cdot 10^7$ cells together with $2 \cdot 10^6$ SRBC. Four days later the secondary recipients received a further injection (intraperitoneally) of $5 \cdot 10^8$ SRBC. On the 5th day the number of AFC in the spleen was determined by the local hemolysis in gel test.

EXPERIMENTAL RESULTS

One possible cause of defective function of (CBA × M523)F_1 mouse spleen cells in a lethally irradiated CBA recipient could be loss of the homing instinct by the donor's cells, as the result of which they did not enter the spleen. Accordingly, in the first series of experiments $25 \cdot 10^6$ (CBA × M523)F_1 mouse spleen cells were injected in a volume of 0.05 ml into the spleen of lethally irradiated CBA mice. In this case, however, the immune response to SRBC was reduced to less than one-hundreth of that in the syngeneic system.

In the next series of experiments the fate of transplanted cells of F_1 mice in the irradiated recipient was investigated. The "double passage" method was used for this purpose. As Fig. 1 shows, cells of (CBA × M523)F_1 mice, after a stay of 8 days in the spleen of lethally irradiated intermediate CBA recipients, were still able to give an immune response to SRBC on subsequent transplantation into F_1 recipients (the $F_1 \rightarrow$ CBA $\rightarrow F_1$ group), but did not adapt to CBA recipients (the $F_1 \rightarrow$ CBA $\rightarrow$ CBA group). A nonsyngeneic microenvironment in the spleen of the CBA mice thus merely limited proliferation and differentiation of the F_1 mouse cells but did not cause their death. Was this limiting effect permanent or temporary in character? To study this problem two possible methods can be used: a) The time between irradiation of the recipients and transplantation of the cells can be increased; b) the duration of stay of the cells in the recipient can be prolonged by delaying the test injection of antigen.

As Fig. 2 shows, both methods abolish parental resistance. The results show that 3 days after irradiation the spleen of CBA mice became just as suitable for normal functioning of F_1 mouse lymphocytes as the spleen of syngeneic recipients.

In the next series of experiments, the immunologic specificity of parental resistance was investigated. As Fig. 3 shows, preliminary induction of tolerance with (CBA × M523)F_1 mouse

cells in combination with CP led to loss of parental resistance in the CBA mice to (CBA × M523)F_1 mouse cells. Tolerogenic treatment with (CBA × C57BL/6)F_1 mouse spleen cells or injection of CP alone did not abolish the phenomenon. However, preliminary injection of (CBA × M523)F_1 mouse cells without CP also abolished resistance. Similar results were obtained with the combination of A and (A × M523)F_1 lines.

When the results are analyzed, the first problem to be studied is the connection between this phenomenon and the principles of, on the one hand, transplantation immunity and, on the other hand, hybrid or allogeneic resistance, that are already known.

During ordinary reactions of transplantation immunity, allogeneic cells are known to be eliminated. In allogeneic or hybrid resistance various workers, using the method of retransplantation of injected cells, observed either irreversible elimination of the cells [6] or merely temporary inhibition of their proliferation (lengthening of the lag phase) [5]. In parental resistance, as is clear from the results described above, limitation of activity of the donor's cells was only temporary (3 days) in character. Similar results (3-5 days) have been obtained for hybrid and xenogeneic resistance [5, 10]. The "readaptation" of injected cells to the new microenvironment, which Lengerova et al. [8] observed, was not found in the present investigation.

As the results show, parental resistance, like hybrid resistance [1, 6, 7, 9] and, unlike transplantation immunity, is specifically abolished by preliminary injection of cells of the donor's genotype.

The results suggest that genetic parental resistance of lethally irradiated CBA mice to lymphocytes of (CBA × M523)F_1 mice obeys basically the same rules as hybrid and allogeneic resistance. This resistance is due to immunologically competent cells of the recipient which are inactivated 3 days after irradiation. They have no cytotoxic action of the donor's cells but temporarily restrict their activity. It is possible that the ability of the transplanted material to differentiate is selectively impaired, whereas proliferative activity is less severely affected [3, 11].

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