THYMUS-DEPENDENCE OF GENETIC RESISTANCE TO (CBA × M523)F_1 LYMPHOCYTES

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The writers previously described a phenomenon of parental resistance, consisting of sharply reduced ability of lymphocytes of (CBA × M523)F_1 mice to proliferate and differentiate in lethally irradiated recipients of the parental CBA line [2, 3]. The transplanted cells in this case were not rejected but simply temporarily (not more than 3 days) inhibited by the irradiated recipient [1, 4]. This phenomenon was shown to be immunological in nature, for parental resistance can be abolished by preliminary immunologically specific action on the recipients, namely by injection of splenocytes of (CBA × M523)F_1 mice [1, 4].

The object of this investigation was to study the nature of cells responsible for the manifestation of parental resistance.

EXPERIMENTAL METHOD

CBA/Cazacsto mice and (CBA × M523)F_1 hybrids were used (M523 is a gene mutation of the CBA line, mapped in the H-2K locus) [7]. The age of the experimental animals was 1.5-2 months. The donors and recipients were usually chosen to be of the same sex. The basic scheme of the experiment was as follows. CBA mice and their (CBA × M523)F_1 controls were irradiated from a cobalt source (1000 rads) and were given an intravenous injection of 5×10^7 spleen cells from (CBA × M523)F_1 mice immunized 6-30 days before the experiment with sheep's red blood cells (SRBC) in a dose of 2×10^6. Simultaneously with the cells, the recipients were injected intravenously with 5×10^8 SRBC. Five days later the number of antibody-forming cells (AFC) against SRBC in the recipients' spleen was determined by Jerne's method. The presence of genetic resistance was judged from the reduced number of AFC in a nonsyngeneic system ($F_1 \rightarrow$ CBA) compared with syngeneic controls ($F_1 \rightarrow F_1$, CBA → CBA).

The recipients were subjected to various preliminary procedures. In some experiments the future recipients were exposed to sublethal irradiation in a dose of 500 rads and were given an injection of syngeneic bone marrow cells, intact splenocytes, or splenocytes treated with anti-Thy-1-serum with complement 10 days before the main experiments [lethal irradiation and injection of (CBA × M523)F_1 splenocytes with SRBC]. The anti-Thy-1-serum was obtained by immunizing AKR mice with thymocytes from C3H mice. Serum for treating the spleen cells was used in a dilution of 1:20, and complement in a dilution of 1:10. The suspension of spleen cells with serum and rabbit complement was incubated for 45 min at 37°C, and then washed twice in the cold by centrifugation.

In the other experiments B and TB mice were used as recipients. For this purpose adult CBA mice were thymectomized [9]. The mice were irradiated 2 weeks later in a dose of 950 rads and protected with 10^7 syngeneic embryonic liver (EL) cells in (B mice), with EL cells and thymocytes (9×10^7) (TB mice), or with 5×10^7 splenocytes. These animals were investigated 1-1.5 months later for the presence of parental resistance by the method described above, involving reirradiation and injection of (CBA × M523)F_1 mouse spleen cells and SRBC.

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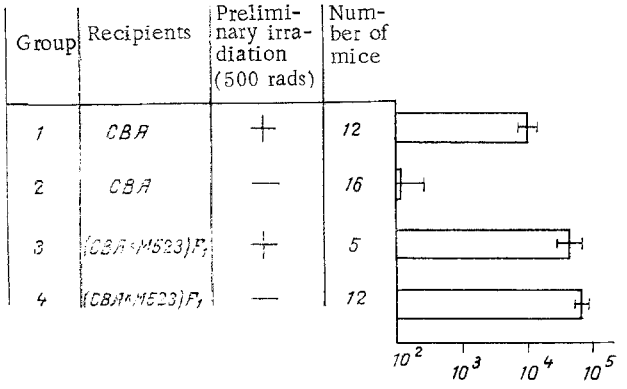

Fig. 1

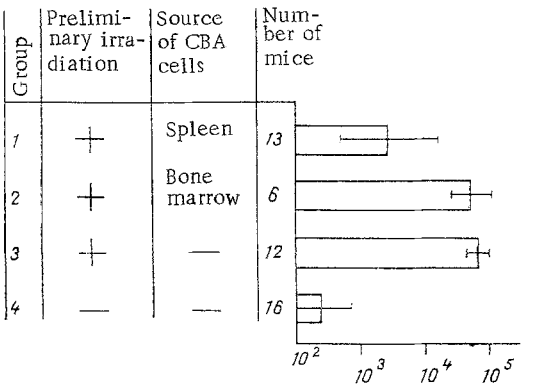

Fig. 2

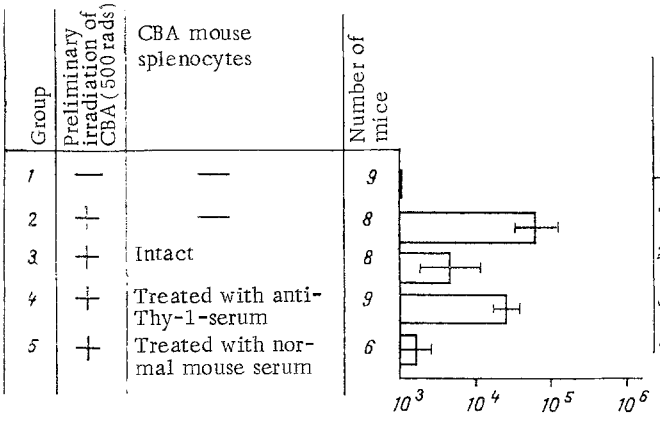

Fig. 3

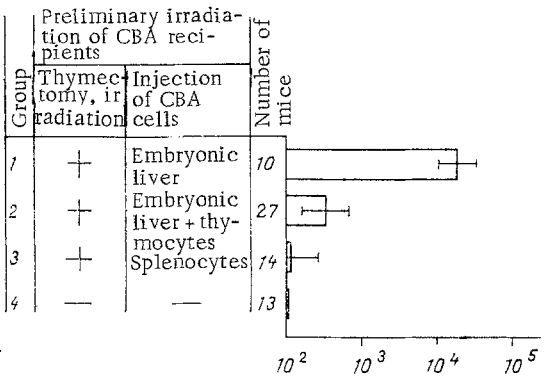

Fig. 3

Fig. 1. Effect of preliminary sublethal irradiation of recipients on survival of (CBA × M523)F_1 mouse lymphocytes. Abscissa, number of AFC in spleen 5 days after injection of (CBA × M523)F_1 splenocytes and SRBC into lethally irradiated recipients. Some recipients were irradiated in a dose of 500 rads 10 days before the experiment.

Fig. 2. Restoration of parental resistance in sublethally irradiated CBA recipients by various syngeneic cells. Abscissa, number of AFC in spleen of CBA recipients 5 days after lethal irradiation and injection of (CBA × M523)F_1 mouse lymphocytes with SRBC. Some recipients, irradiated in a dose of 500 rads 10 days before the experiment, were given an intravenous injection of syngeneic splenocytes or bone marrow cells.

Fig. 3. Effect of anti-Thy-1-serum on ability of CBA mouse spleen cells to restore parental resistance. Abscissa, level of immune response to SRBC on (CBA × M523)F_1 mouse splenocytes in spleen of CBA recipients 5 days after lethal irradiation. Recipients were irradiated 10 days beforehand in a dose of 500 rads and were given an intravenous injection of intact splenocytes or of splenocytes treated with anti-Thy-1-serum with complement.

Fig. 4. Ability of spleen, thymus, and embryonic liver cells of CBA mice to restore parental resistance. Abscissa, number of AFC in spleen 5 days after injection of (CBA × M523)F_1 splenocytes into lethally irradiated CBA recipients, subjected to various forms of preliminary treatment.

EXPERIMENTAL RESULTS

As Fig. 1 shows, preliminary (10 days beforehand) sublethal irradiation of the recipients abolished parental resistance. The immune response was restored in the animals of this group to its original level in syngeneic controls (group 1 compared with groups 2 and 4). Preliminary sublethal irradiation did not affect the level of the immune response in a syngeneic system (group 3).

Sensitivity to preliminary (10 days beforehand) sublethal irradiation and resistance to lethal irradiation given actually on the same day as the allogeneic cells were transplanted emphasize the similarity between this phenomenon and allogeneic and hybrid resistance [5, 6] and distinguish it from ordinary transplantation immunity.

In the next series of experiments an attempt was made to discover which cells can restore parental resistance when temporarily lost as a result of sublethal irradiation (Fig. 2). Injection of CBA splenocytes immediately after sublethal irradiation restored the recipients' resistance to (CBA × M523)F_1 mouse splenocytes (group 1). CBA mouse bone marrow cells were ineffective (group 2).

To determine more precisely the cells responsible for the manifestation of parental resistance, a suspension of CBA mouse spleen cells, which restores resistance to sublethally irradiated CBA recipients, was incubated before injection with anti-Thy-1-serum in the presence of rabbit complement (Fig. 3). Treatment of CBA splenocytes in this way abolished their ability to restore parental resistance of recipients to (CBA × M523)F_1 mouse cells (group 4). Incubation of CBA splenocytes with normal mouse serum did not affect their ability to restore resistance (group 5).

In the next experiments, B and TB mice were used as recipients. The results of determination of the number of AFC in such animals after reirradiation and transfer of (CBA × M523)F_1 mouse cells are illustrated in Fig. 4.

It will be clear from Fig. 4 that parental resistance in B-CBA mice was absent or was weak. The immune response in this group was 2 or 3 orders of magnitude higher than in the control animals, i.e., in CBA mice irradiated once and receiving donors' cells of the (CBA × M523)F_1 line (group 1 compared with group 4). However, when TB-CBA mice were used as recipients strong resistance was observed, as shown by the sharply depressed immune response of (CBA × M523)F_1 mouse lymphocytes transplanted into these animals (group 2). The same effect was observed when thymectomized, irradiated CBA mice were restored with syngeneic splenocytes (group 3).

Consequently, the cells responsible for manifestation of parental resistance are present in the spleen and thymus of CBA mice but not in bone marrow or embryonic liver. Direct experiments using anti-Thy-1-serum showed that these cells carry Thy-1 antigen on their surface and that they are thus T cells. They differ, however, from the T cells that are responsible for transplantation immunity and they have some common properties with the effectors of hybrid resistance: resistance to irradiation, later maturation during ontogeny, inactivation by preliminary injection of the cells of donor origin, and sensitivity to preliminary sublethal irradiation and to cyclophosphamide [1, 4-6, 8].

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