THE PARENTAL RESISTANCE PHENOMENON AND ITS GENETIC REGULATION

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UDC 612.6.05:612.41

Lymphocytes of (CBA × M523)F_1 or (A × M523)F_1 mice, if transplanted into CBA or A recipients irradiated in a dose of 1000 rad, react to test antigens (sheep's red cells, Salmonella typhi Vi-antigen) by the formation of only 1/100-1/1000 of the number of antibody-forming cells formed by syngeneic recipients. An intermediate result was observed after transplantation of the same cells into irradiated M523 recipients. Conversely, lymphocytes of (A × CBA)F_1, (CBA × C57BL/6)F_1, or (A × A.CA)F_1 mice gave an equal immune response in syngeneic recipients and in CBA or A recipients. The ability of M523 lymphocytes or their hybrids to give an immune response to sheep's red cells did not differ from the immunoreactivity of lymphocytes of other lines either in situ or in a syngeneic adoptive system. Hematopoietic stem cells from (CBA × M523)F_1 mice formed only 40-50% of the number of colonies in the CBA spleen as in the spleen of syngeneic recipients. It is concluded that the M523 mutation interferes with the proliferation and differentiation of hematopoietic cells and lymphocytes in nonsyngeneic irradiated recipients.

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

If hematopoietic stem cells are injected into lethally irradiated F_1 hybrid mice, differing from the donors in their Hh-genes, hematopoiesis is known to develop more slowly than in a syngeneic system [7, 11-13]. This phenomenon, known as hybrid resistance, extends also to lymphocytes functioning in nonsyngeneic recipients [2, 6]. It has been suggested that Hh-genes are expressed only in homozygotes, i.e., that their phenotypic manifestation is corecessive in character [11, 13].

Data on the possibility of a similar phenomenon in the reverse system (F → P)* are few in number and contradictory in nature. In combinations of lines so far investigated, inhibition of hematopoiesis was only moderate in character [10, 12].

The present writers previously [5] found almost complete inability of lymphocytes of (CBA × CBA.M523)F_1 mice to give an immune response to a foreign antigen (sheep's red blood cells – SRBC) in lethally irradiated CBA mice.

The object of the present investigation was to study this phenomenon and its link with the phenomenon of hybrid resistance.

EXPERIMENTAL METHOD

Mice of lines CBA/CaLacSto (abbreviated to CBA), CBA.M523/Y (M523), C57BL/6 YSto (C57BL/6), A/SnY (A), and A.CA/K1Y (A.CA), and their F_1 hybrids, reared in the writers' own laboratories and also at the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used as experimental animals. The M523 ($H\text{-}2^{Ka}$) mutation appeared spontaneously in a population of CBA/CaLacSto mice [1] and was mapped in the H-2K locus [9]. The ages of the experimental mice ranged from 1.5 to 6 months.

*P stands for animals of the parental line.

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0007-4888/79/8703-0239$07.50

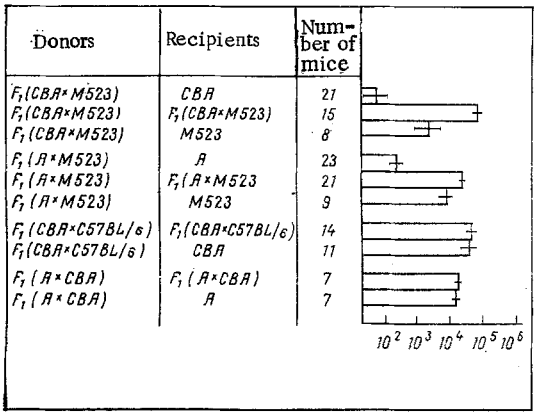

Fig. 1

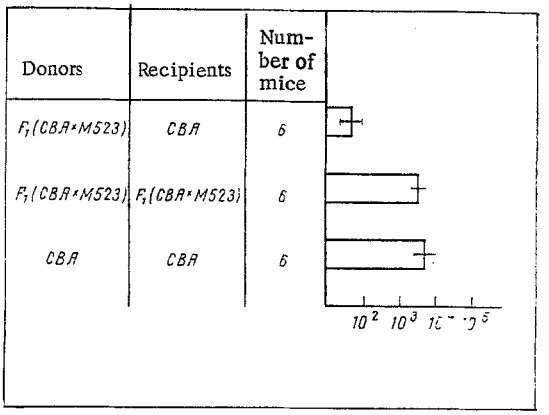

Fig. 2

Fig. 1. Immune response of F_1 mouse spleen cells to SRBC in syngeneic irradiated recipients and recipients of parental lines. Here and in Fig. 2 abscissa is the number of AFC in spleen.

Fig. 2. Defectiveness of immune response of (CBA × M523)F_1 spleen cells to S. typhi Vi-antigen in lethally irradiated CBA recipients.

SRBC and Salmonella typhi Vi-antigen, obtained from the Mechnikov Research Institute of Experimental Microbiology, Ministry of Health of the RSFSR, were used as antigens. A suspension of spleen or bone marrow cells was injected intravenously in a volume of 0.5-1 ml into recipients irradiated a few hours previously in a dose of 1000-1100 rad from a cobalt source. The donors and recipients were usually chosen to be of the same sex.

To obtain hematopoietic colonies, 5×10^4 bone marrow cells were injected into the recipients; the number of colonies was counted 8 days later. Vi-antigen was injected intravenously in a dose of 10 μg 0.5-1 h after transplantation of 1×10^8 donors' spleen cells.

SRBC were injected in a dose of 1×10^6 intravenously into the donors 6-30 days before the experiment and in a dose of 5×10^8 into the recipients 0.5-1 h after transplantation of 5×10^7 spleen cells.

On the 5th day after transplantation of the cells the number of antibody-forming cells (AFC) in the recipients' spleen was determined by the local active [8] or passive [3] hemolysis in gel test.

To study the comparative immunoreactivity of animals of different lines (without cell transplantation), 5×10^8 SRBC or 10 μg Vi-antigen was injected intravenously into them and the number of AFC was determined 4 days later.

EXPERIMENTAL RESULTS

In the experiments of series I the ability of spleen cells of F_1 hybrids of the various lines to give an immune response to SRBC was compared in lethally irradiated syngeneic and semisyngeneic (parental line) recipients. As Fig. 1 shows, (CBA × C57BL/6)F_1 and (A × CBA)F_1 lymphocytes gave an equal immune response in syngeneic and semisyngeneic recipients. The same result was obtained when the immune response of (A × A.CA)F_1 cells was tested in A or (A × A.CA)F_1 mice (not shown in Fig. 1). Conversely, spleen cells of (CBA × M523)F_1 and (A × M523)F_1 mice produced only 1/100-1/1000 the number of AFC in lethally irradiated CBA or A mice respectively as in syngeneic recipients. Transplantation of the same cells into lethally irradiated M523 mice, as Fig. 1 shows, gave an intermediate result.

In the experiments of series II, instead of SRBC a different test antigen was used, namely S. typhi Vi-antigen. As Fig. 2 shows, in this case also the (CBA × M523)F_1 spleen cells produced only 1/100 the number of AFC in the irradiated CBA mice as during syngeneic adoptive transplantation. Special experiments also showed that CBA, M523, and (CBA × M523)F_1 mice formed equal numbers of AFC in response to injection of SRBC. The pattern observed was thus unconnected with any special features of the test antigen or the genetic features of immunoreactivity of the mice and could be ascribed to the weakened ability of lymphocytes of hybrid mice to proliferate and differentiate in recipients of the parental line.

Does the pattern discovered extend also to F_1 cells other than splenic lymphocytes? The writers showed previously [5] that a mixture of (CBA × M523)F_1 thymus and bone-marrow lymphocytes also produces far fewer

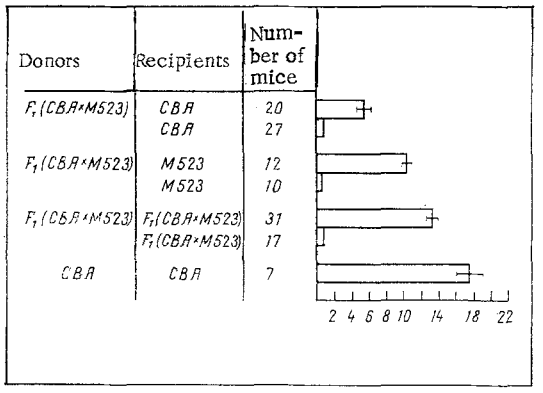

Fig. 3. Formation of hematopoietic colonies of (CBA × M523)F_1 bone marrow cells in spleen of recipients of different genotypes. Abscissa, number of colonies.

AFC in irradiated CBA than in syngeneic recipients. In the present investigation the classical method of counting colonies formed by hematopoietic stem cells in culture in vivo was used.

As Fig. 3 shows, after injection of equal numbers of (CBA × M523) F_1 bone marrow cells, only 40-50% of the number of hematopoietic colonies was formed in the spleen of the irradiated CBA recipients as in the spleen of syngeneic recipients. The number of colonies in the M523 spleen was intermediate. Not only immunocompetent lymphocytes, but also hematopoietic stem cells of (CBA × M523)F_1 mice thus proliferate and differentiate less readily in the CBA than in the syngeneic spleen.

It will be noted that the phenomenon of parental resistance described above, whatever the method of testing used, was more marked in the (CBA × M523)F_1 → CBA system than in the (CBA × M523)F_1 → M523 system. To determine whether this fact is connected with the so-called maternal inheritance [14], the efficiency of the immune response (to SRBC) of splenic lymphocytes of (CBA × M523)F_1 and (M523 × CBA)F_1 mice when transplanted into irradiated CBA mice was compared. The results proved to be identical, so that the hypothesis was not confirmed.

The weaker functioning of (CBA × M523)F_1 lymphocytes or stem cells in lethally irradiated CBA mice likewise cannot be ascribed to residual phenomena of ordinary transplantation immunity: Such an explanation is contradicted by the experimental conditions (irradiation of recipients in doses of 1000-1100 rad) and also by the fact that cells of other hybrids not containing the M523 mutation function normally under the same conditions despite differences from the recipients with respect to "strong" (H-2) transplantation antigens.

It can be concluded from these results that partial or total incompatibility caused by the M523 mutation leads to marked impairment of function of the lymphocytes or (to a lesser degree) hematopoietic stem cells of F_1 mice in lethally irradiated mice of the parental line. This phenomenon differs from ordinary transplantation immunity. Unlike allogeneic or hybrid resistance, determined by corecessive Hh genes [11, 13], this phenomenon is regulated by a dominant or codominant gene, which is evidently [9] located in the H-2K locus. The phenomenon now described differs from cases of weakened functioning of F_1 cells in irradiated recipients of the parental line described previously [4, 10, 12], first, in the very high level of resistance of the recipients and, second, by the fact that the effect is independent of differences in immunoreactivity of the donor and recipient to the test antigen. By contrast with the known phenomena of allogeneic ($P_1 \rightarrow P_2$)* resistance and hybrid $P_1 \rightarrow (P_1 \times P_2)F_1$ resistance, it is suggested that the phenomenon now described be called parental resistance.

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*P_1 and P_2 stand for different lines of animals.

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PHARMACOKINETIC ASPECTS OF THE IMMUNODEPRESSIVE ACTION OF CYCLOPHOSPHAMIDE

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UDC 612.017.1.014.46:615.277.3

The alkylating and immunodepressive activity of the serum of CBA, BALB/c, and DBA/2 mice after administration of cyclophosphamide was studied. Interlinear differences were found in these parameters, but no direct correlation could be shown to exist between them. The DBA/2 mice, which were most sensitive to the immunodepressive action of cyclophosphamide, had the highest serum immunodepressive activity.

KEY WORDS: cyclophosphamide; metabolism; immunodepression; genotype.

A previous investigation [5] showed that mice of different lines are unequally sensitive to the immunodepressive action of cyclophosphamide (CP). All stages of the pharmacokinetic process determining the fate of a drug in the body are considered to "take place by means of specific and nonspecific enzymes whose synthesis is unquestionably under genetic control" [2]. It can therefore be tentatively suggested that differences found in the immunodepressive activity of CP are connected with differences in its pharmacokinetics in mice of different genotypes. This suggestion is supported by earlier observations [5] showing differences in the rate of oxidative hydroxylation of CP in mice of different lines.

CP is an alkylating agent but, unlike many other immunodepressants of this group, in the intact state it has virtually no cytotoxic activity. It owes its biological effect to the formation of active metabolites, produced as a result of activation of the substance by an NADPH-dependent enzyme system of the endoplasmic reticulum of the liver [10]. It was accordingly decided to study the alkylating and immunodepressive activity of the blood serum, i.e., indices reflecting the formation of active CP metabolites in the body, in mice of different lines.

EXPERIMENTAL METHOD

Male CBA, BALB/c, and DBA/2 mice weighing 18-25 g (from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR) were used. The Soviet preparation cyclophosphan was used as CP. The alkylating activity of CP metabolites was determined in the blood serum of the mice at various times after intraperitoneal injection of CP. Each sample consisted of pooled sera from three mice. The NBP-test [9] by the method described previously [4], with slight modifications, was used for the determination. The immunodepressive activity of the serum was tested by a method developed by the writer for mice. CBA mice were sensitized intravenously with 10^6 sheep's red cells (SRBC) 7 days before the experiment. A cell suspension was prepared from the spleens of these mice in medium 199 with antibiotics (100 units penicillin and 100 units streptomycin to 1 ml), which was incubated for 1 h at 37°C in the presence of serum from mice receiving CP ("active"

Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 3, pp. 250-252, March, 1979. Original article submitted June 16, 1978.

 0007-4888/79/8703-0242$07.50