EFFECT OF ABOLITION OF HYBRID RESISTANCE ON SURVIVAL OF RADIATION CHIMERAS

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The effect of abolition of hybrid resistance by injection of lymphocytes on survival of lethally irradiated mice protected by transplantation of semiallogeneic bone marrow was studied. Injection of C57BL lymphocytes into (C57BL × CBA)F_1 recipients 1 day before irradiation and transplantation of bone marrow of the same genotype (C57BL) increased the survival rate of the chimeras compared with untreated recipients, whereas the same treatment, given 7 days before irradiation, reduced the survival rate of the chimeras. Injection of lymphocytes of the parental line had a negligible action on the radioresistance both of the hematopoietic stem cells (as shown by the endocolonization test) and on the body as a whole (as shown by the 30-day survival test) of the F_1 hybrid; it is accordingly concluded that differences in the effectiveness of the splenocytes when injected at different times before irradiation cannot be explained by changes in radioresistance.

KEY WORDS: hybrid resistance; radiation chimeras.

On transplantation of bone marrow of the parental line into an F_1 hybrid the injected hematopoietic cells often grow less successfully than syngeneic cells. This conclusion is based on the sharply reduced number of hematopoietic colonies in the spleen [8], evidence of poor growth of the hematopoietic stem cells; of the reduced iron consumption [6], evidence of depressed erythropoiesis; and the reduced consumption of IUdR* [4], evidence of delayed proliferation of hematopoietic cells of all branches. The phenomenon has been called repression of colony-forming units, allogeneic inhibition, resistance to bone marrow grafts, poor growth of hematopoietic cells, and so on. The most popular name at the present time is "hybrid resistance." The intensity of the phenomenon differs in mice of different strains. Usually, to judge from the number of colonies in the spleen, strong hybrid resistance leads to a reduction in the number of colonies by 3 to 10 times. It has been shown that hybrid resistance can be weakened by various forms of treatment, such as preliminary sublethal irradiation of the recipient [4, 6], injection of antilymphocytic serum [3, 9], injection of sera against weak and strong transplantation antigens [7], and so on. One of the most effective ways of overcoming hybrid resistance to bone marrow of C57BL mice is by injecting lymphocytes of the same genotype as the bone marrow into the (C57BL × CBA)F_1 recipient [2, 5]. Under optimal conditions this method enables hybrid resistance to be virtually completely abolished.

In this investigation the possibility of increasing the effectiveness of semiallogeneic bone marrow for the protection of irradiated animals was studied by injecting lymphocytes abolishing hybrid resistance into it.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice and (C57BL/6 × CBA)F_1 hybrids. The animals were irradiated with ^{137}Cs γ rays on the IPK apparatus with a dose rate of 21.6 rad/min. To obtain chimeras the recipients were irradiated in doses of between 1200 and 1500 rad, to study radioresistance in doses of between 550 and 1200 rad, and to study endocolonization, in doses of between 850 and 1200 rad. The bone marrow was

*5-iodo-2′-deoxyuridine.

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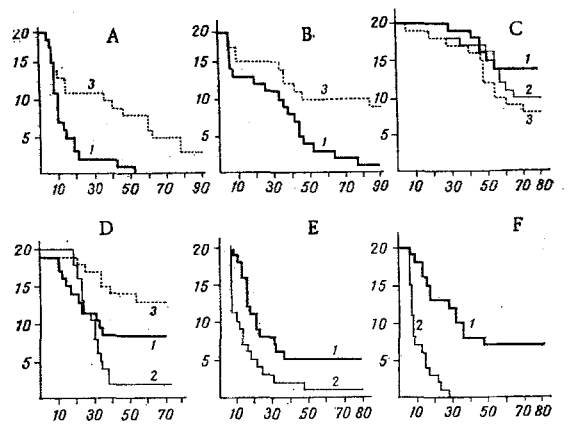

Fig. 1

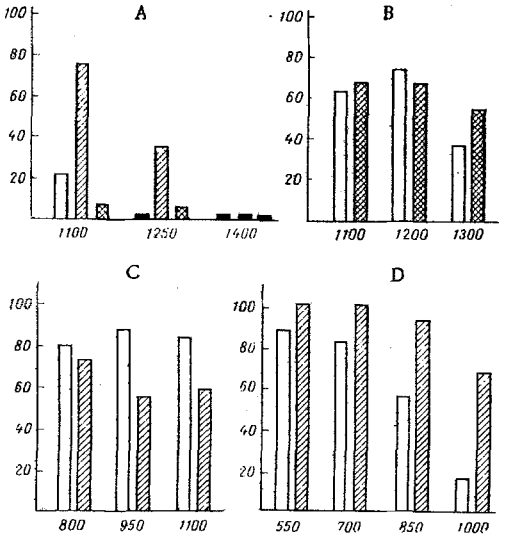

Fig. 2

Fig. 1. Effect of preliminary treatment with lymphocytes on survival of radiation chimeras: 1) control; 2) treatment 7 days before irradiation; 3) treatment 1 day before irradiation. Dose of bone marrow cells: C) 10^5; A, D, E) 10^6; B, F) 10^7. Abscissa, days after irradiation and transplantation of bone marrow; ordinate, number of mice.

Fig. 2. Effect of preliminary treatment with lymphocytes on survival rate of irradiated mice. Unshaded columns) Control; obliquely shaded columns) treatment 7 days before irradiation; cross-hatched columns) treatment 1 day before irradiation. Abscissa, dose of irradiation, rad; ordinate, 30-day survival rate, %.

obtained by flushing the femur with medium No. 199. The marrow was injected intravenously in the desired dose in the course of 2 h after irradiation of the recipients. To abolish hybrid resistance in the future recipients, 1 or 7 days before irradiation they were given an intravenous injection of spleen cells in a dose of one spleen to six mice (mean $2 \cdot 10^7$ cells per mouse). To prevent thrombosis, 50 units heparin was injected intraperitoneally into the mice 10-30 min before injection of the splenocytes. Endocolonies were studied in spleens, fixed in Bouin's fluid, taken from irradiated mice killed 8 days after irradiation. Radioresistance was assessed on the basis of the 30-day survival rate of mice irradiated with different doses. Survival of the chimeras was recorded in the course of 2-4 months. In the experiments with chimeras and to study radioresistance the groups consisted of 17 to 30 mice, and in the endocolonization experiments they consisted of 10 mice.

EXPERIMENTAL RESULTS

In experiments with chimeras bone marrow from C57BL mice was injected into irradiated F_1 mice. In each experiment bone marrow was injected into either intact or "derepressed" mice (in which hybrid resistance had been abolished by preliminary injection of splenic lymphocytes), and in some experiments syngeneic bone marrow also was used. In each experiment up to four doses of bone marrow, from 10^5 to 10^7 cells, was injected so that the number of groups in the individual experiments was between six and twelve. Altogether seven experiments were carried out. Because of the impossibility of showing all the results obtained, typical results of a few experiments are illustrated in Fig. 1.

It will be clear from Fig. 1D, E, and F, that injection of derepressed cells 7 days before irradiation not only did not increase the survival rate of the chimeras, but actually reduced it compared with the control recipients. Meanwhile survival of the chimeras was due to repopulation by the injected hematopoietic cells. This repopulation was substantially improved by preliminary treatment of the recipients with splenocytes. Despite this fact, the survival rate of the chimeras decreased. To analyze these paradoxical results it was necessary to rule out the possibility of development of a "graft versus host reaction" (GVHR), reducing radioresistance. In fact, under these conditions the recipient received large numbers of immunologically competent cells of the C57BL genotype, capable of reacting against transplantation antigens of the second parental line (CBA). Although no GVHR is usually exhibited by unirradiated animals because of rejection of the injected allogeneic

TABLE 1. Effect of Treatment with Lymphocytes on Endogenous Colony-Forming Units in Irradiated Mice

Expt.	Dose of irradiation, rad	Time of treatment	Number of colonies per spleen (M ± m)	P
1	850	—	10,4±4,0	—
		3a 7 days previously	12,4±4,3	>0,05
	950	—	3,0±0,6	—
		3a 7 days previously	6,7±1,7	>0,05
	1050	—	2,0±0,3	—
		3a 7 days previously	0,6±0,4	<0,02
	900	—	3,6±1,6	—
		3a 7 days previously	13,7±6,4	<0,001
		3a 1 day previously	12,4±7,1	<0,001
2	1050	—	2,2±1,0	—
		3a 7 days previously	3,6±1,1	>0,05
		3a 1 day previously	3,3±2,0	>0,05
	1200	—	0,6±0,3	—
		3a 7 days previously	0,7±0,5	>0,05
		3a 1 day previously	0,3±0,2	>0,05

cells, in this system such rejection was impossible because of the genetic inability of the hybrid to react against the parental antigens, and a GVHR developed although in a milder form. Accordingly the effect of injection of splenocytes 24 h before irradiation on the survival rate of the chimeras was studied. This time was long enough to abolish hybrid resistance but not long enough to immunize the injected cells or for them to manifest their activity in the GVHR. The results showed that, after such treatment, the survival rate of the chimeras was substantially greater than in the control series and, more especially, than the recipients treated 7 days beforehand (Fig. 1A, B, D). Splenocytes of the C57BL parental line did not affect proliferation of the cells of its own genotype in the (C57BL × CBA)F_1 hybrid, i.e., of cells syngeneic with the recipient [1]. As would therefore be expected, preliminary treatment of the F_1 hybrids with lymphocytes of the C57BL parental line had virtually no effect on the survival rate of the syngeneic chimeras (Fig. 1C).

The results thus agreed with the hypothesis put forward, but it was also tested more directly. The GVHR is known to damage stem cells severely. Accordingly, the effect of injection of splenocytes 7 days or 1 day before irradiation on endogenous stem cells was studied. As the results (Table 1) showed, after relatively lower doses (up to 900 rad) injection of lymphocytes both 7 days and 1 day previously increased the number of endogenous colonies, a characteristic feature of many other stressors also. Meanwhile, after higher doses of irradiation (1050-1200 rad), injection of splenocytes at both times had virtually no effect on endocolonization and, consequently, did not reveal an active GVHR in this test. Accordingly a more integrative index was studied, namely the 30-day survival rate of mice treated with splenocytes and intact mice after irradiation in different doses (Fig. 2).

As Fig. 2A and B shows, this index also failed to confirm the hypothesis. When splenocytes were injected 1 day before irradiation the radioresistance of the mice was lowered or unchanged (Fig. 2A, B), when they were injected 7 days before irradiation it was reduced in one experiment (Fig. 2C) and increased in two others (Fig. 2A, D). Hence it follows that differences in the effectiveness of splenocytes when injected at different times before irradiation cannot be explained at least by changes in the radioresistance of the animals.

Consequently, no reason could be discovered for the opposite effects of splenocytes on the survival rate of chimeras when injected 1 day and 7 days before irradiation. However, the results do indicate that injection of lymphocytes 1 day before irradiation of the recipient results not only in improved growth of the bone marrow in a situation when it is inhibited, but also an increase in its protective activity for irradiated animals. It may be that the effectiveness of the scheme of preliminary treatment of the recipient of allogeneic bone marrow used in clinical practice, including injection of peripheral blood leukocytes of the future donor 24 h before the beginning of immunodepressive preparation [1], can be attributed not only to a hypothetical depression of the GVHR as a result of such treatment, but also to improved chances of taking of the allogeneic bone marrow in cases when phenomena of the hybrid resistance type are well marked in man also.

LITERATURE CITED

1. I. L. Chertkov, Clin. Med., No. 5, 28 (1974).
2. L. N. Lemenova and I. L. Chertkov, Probl. Gematol., No. 5, 9 (1976).
3. I. L. Chertkov, L. N. Lemenova, and O. A. Mendelevich, Probl. Gematol., No. 1, 3 (1972).
4. G. Cudkowicz and M. Bennett, J. Exp. Med., 134, 83 (1971).
5. J. W. Goodman and S. C. Shinpock, Proc. Soc. Exp. Biol. (N.Y.), 129, 417 (1968).
6. J. W. Goodman and H. B. Wheeler, Transplantation, 6, 173 (1968).
7. C. J. Gregory, E. A. McCulloch, and J. E. Till, Transplantation, 13, 138 (1972).
8. E. A. McCulloch and J. E. Till, J. Cell Physiol., 61, 301 (1963).
9. J. E. Till, S. Wilson, and E. A. McCulloch, Science, 169, 1327 (1970).

CLASSES OF CELL SURFACE IMMUNOGLOBULINS DETECTED ON RAT LYMPHOCYTES BY ENZYMIC RADIOIODINATION

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It was shown by the methods of enzymic radioiodination, immunoprecipitation, and electrophoresis in sodium dodecylsulfate–polyacrylamide gels that splenic lymphocytes of normal rats carry on their surface immunoglobulins of two main classes: monomeric IgM (IgM_S) and $Ig(H_2L_2)$, the heavy chains of which are a little smaller than μ chains and differ from them in their antigenic properties. The class of cell surface Ig thus revealed is evidently equivalent to human IgD and to the IgD-like protein of the cytoplasmic membrane of mouse lymphocytes. The presence of small quantities of IgG on the surface of lymphocytes can be explained both by its cytophilic properties and by the immunological state of the experimental animals.

KEY WORDS: enzymic radioiodination; classes of lymphocyte surface Ig.

The view that the immunoglobulins (Ig) of the cell membrane are antigen-identifying receptors of B lymphocytes is generally accepted [13]. However, it is not yet known how the surface Ig are bound to the cell membrane, whether the method of binding is common to all or special to each type of surface Ig, and whether differences are found in the structure of the surface and secreted Ig. As a first step toward the solution of these problems the various types of lymphocyte surface Ig must be isolated and described.

The object of this investigation was to analyze the surface immune proteins detectable by "lactoperoxidase radioiodination" of the splenic lymphocytes of normal rats.

EXPERIMENTAL METHOD

August rats aged 2-3 months were used. A suspension of splenic lymphocytes containing more than 90% of viable cells (test with 0.2% trypan blue) was obtained by density fractionation in a bovine serum albumin concentration gradient [10] and washed repeatedly in isotonic physiological buffered (pH 7.2) saline (PBS). Samples containing 10^7 cells in 20 μl PBS were treated with 10 μl of a solution of lactoperoxidase (0.25 mg/ml), 5 μl $Na^{125}I$ (100-200 μCi, specific activity 100-150 mCi/ml), and 5 μl $Na^{127}I$ (0.15 mM). Enzymic iodination

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