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EFFECT OF PRELIMINARY INJECTION OF ALLOGENEIC CELLS ON TRANSPLANTATION IMMUNITY IN MICE RECEIVING CYCLOPHOSPHAMIDE

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Injection of 1×10^8 C57BL/6 mouse spleen cells into CBA mice 1 day before treatment with cyclophosphamide (CP) was shown to promote survival of 2×10^7 allogeneic or semiallogeneic cells injected later (3-6 h after CP). The criterion of survival was the ability of the donor cells to produce antibodies against sheep's red blood cells in recipients tolerant to this antigen. Injection of 1×10^8 allogeneic cells 2 days before CP treatment had no protective effect. After intravenous immunization with allogeneic cells, killer cells began to appear in the recipient's spleen as early as on the 2nd day, and their number reached a maximum on the 5th day. The results suggest that CP eliminates the recipient's lymphocytes responding to transplantation antigens, but the killer cells already formed are resistant to the action of CP.

KEY WORDS: transplantation immunity; tolerance; killer cells, cyclophosphamide.

The writers showed previously that by combined injections of nonlethal doses of cyclophosphamide (CP) and spleen cells of $(CBA\times C57BL/6)F_1$ hybrid mice, a long-lasting (up to 12 days) semiallogeneic chimerism of the lymphoid tissue can be induced in adult CBA mice [1, 2]. This chimerism is accompanied by tolerance to the donors' tissues, which differ from those of the recipient in their strong H-2 antigens. It has been shown that a transplanted allogeneic heart will survive for a long time, for 5 months or more, in such mice [4]. The

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TABLE 1. Effect of Allogeneic Cells Injected into CBA Mice 1 and 2 Days before Treatment with CP on Survival of Allogeneic and Semiallogeneic Cells Injected Subsequently

Group of animals	Treatment of recipient with $1 \cdot 10^8$ C57BL/6 spleen cells before injection		Injection of $2 \cdot 10^7$ spleen cells after CP *	Number of AFC per spleen in recipients					
				5th day		11th day		18th day	
	1 day	2 days		number of mice	number AFC	number of mice	number AFC	number of mice	number AFC
1-	—	—	+	24	2 173 (1 110—4 253)	43	265 (164—428)	7	1 194 (687—2 073)
2-	—	+	+	20	1 117 (407—3 058)	19	580 (289—1 165)	—	—
3-	+	—	+	24	12 303 (6 255—24 199)	38	3 236 (1 806—5 799)	12†	8 790 (3 158—24 468)
4-	+	—	—	5	99 (37—265)	5	212 (83—538)		
5‡	—	—	—	17	33 (22—47)	41	232 (171—316)	8	653 (183—2 336)

*When the number of AFC was determined on the 5th and 11th days, spleen cells of C57BL/6 mice were injected on the day of treatment with CP, when determined on the 18th day spleen cells of (CBA × C57/6)F_1 hybrid mice were injected on the day of treatment with CP.

†In some experiments 1×10^8 spleen cells from (CBA × C57BL/6)F_1 hybrid mice were injected 1 day before treatment with CP.

‡The mice of group 5 received only SRBC and CP.

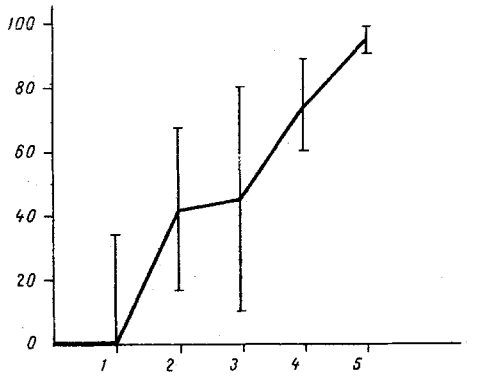

Fig. 1. Dynamics of formation of killer cells in spleens of CBA mice after intravenous injection of 1×10^8 spleen cells of C57BL mice or of (CBA × C57BL/6) F_1 hybrids. Abscissa, time after intravenous injection of C57BL/6 or (CBA × C57BL/6)F_1 cells into CBA mice (in days); ordinate, cytotoxic effect (in %), determined by the formula: $\frac{a-b}{a} \times 100\%$, where a is the number of cells in the tube after incubation with spleen cells of intact CBA mice; b the number of target cells in the tube after incubation with "immune" CBA spleen cells obtained 1-5 days after intravenous injection of allogeneic cells.

essential conditions for the creation of semiallogenic chimerism are a large dose of cells (not less than 1×10^8) a dose of CP not exceeding 200 mg/kg, and a short interval (3-6 h) between their injections [1]. However, it is impossible to use a large dose of lymphocytes in order to produce allogeneic chimeras because of the development of a graft versus host reaction (GVHR), as a result of which the recipient dies [7, 8]. Meanwhile, injection of a small dose of cells of the order of 2×10^7, as was shown previously [1], is inadequate for chimera production.

By using a method developed previously in the laboratory for obtaining tolerance with the aid of CP against a powerful antigen such as sheep's red blood cells (SRBC) [3], when CP injected 2 days after the antigen evidently eliminates the clone of specifically stimulated lymphocytes, in the present investigation various combinations of injection of 1×10^8 allogeneic spleen cells 1 and 2 days before CP, followed by injection of a relatively small number (2×10^7) of allogeneic or semiallogeneic cells were used. An attempt was thus made to test the possibility of specific elimination by CP of the clone of stimulated CBA-anti-C57BL/6 lymphocytes.

EXPERIMENTAL METHOD

Experiments were carried out on adult (18-25 g) mice of both sexes of strains CBA and C57BL/6, differing in the whole series of H-2 antigens, and on (CBA × C57BL/6)F_1 hybrids. The CBA mice (recipients) received an injection of 6.2×10^9 SRBC followed, 41-43 h later, by CP in a dose of 200 mg/kg, intraperitoneally in both cases. This treatment induced tolerance to SRBC in the recipients [3]. An injection of 2×10^7 spleen cells of the C57BL/6 or F_1 hybrid donors, immunized intravenously with 1×10^6 SRBC 1-4 weeks previously, was given to the recipient mice 3-6 h after CP. In addition, some recipients were given an intravenous injection of 0.8×10^8-1.0×10^8 C57BL/6 or (CBA × C57BL/6) F_1 spleen cells 48 or 24 h before the injection of CP. The number of antibody-forming cells (AFC) was determined by Jerne's method in the local hemolysis in gel test in the spleens of the experimental animals 5, 11, or 18 days after the last transplantation. Mice receiving 6.2×10^9 SRBC and CP but no cells served as the control. When tested on the 11th or 18th days, 4 days before Jerne's test the mice were given an intravenous injection of 5×10^8 SRBC. If survival of the cells was tested on the 5th day, SRBC were injected in a dose of 1×10^7 simultaneously with adoptive transplantation or none were injected whatever.* The criterion of survival of the donors' lymphocytes was the presence of an immune response to SRBC, for the recipients were tolerant to this antigen, whereas the donor cells were obtained from animals preimmunized against this antigen.

To study the dynamics of formation of killer cells, CBA mice were given an intravenous injection of 1×10^8 spleen cells from C57BL/6, DBA/2, and (CBA × C57BL/6)F_1 hydrid mice. The recipients were killed after various intervals and the cytotoxic activity of the spleen cells against macrophages of varied genetic origin was determined. The cytotoxic effect was determined 48 h after the addition of 1×10^7-1.5×10^7 cells of sensitized or intact spleens to the culture [5, 6].

EXPERIMENTAL RESULTS

The results of investigation of the spleen of CBA mice each receiving 2×10^7 allogeneic C57BL/6 cells sensitized to SRBC and a preliminary injection of 1×10^8 C57BL/6 cells 1 or 2 days before injection of CP, are given in Table 1. The recipients' spleens were tested on the 5th and 11th days after transplantation of sensitized C57BL/6 cells.

It will be clear from the results given in Table 1 that allogeneic cells, transplanted in a dose of 2×10^7, remained viable in the recipients' spleen for 5 days and died before the 11th day (Table 1, line 1). Injection of allogeneic cells 2 days before CP treatment worsened the conditions for survival of subsequently injected presensitized allogeneic cells a little (Table 1, line 2), although the difference was not statistically significant. Injection of the same dose of allogeneic cells 24 h before CP treatment, on the other hand, promoted the survival of the allogeneic cells; this protective effect, moreover, was observed not only on the 5th but also on the 11th day, when all the transplanted allogeneic cells in the control had died (Table 1, line 3). The difference between the number of AFC on the 5th and 11th days in the group receiving 1×10^8 allogeneic cells 1 day before CP treatment and the control at these same times is statistically significant. The same pattern also was observed on the 18th day of observation in experiments with transplantation of 2×10^7 semiallogeneic cells.

The reduction in the number of AFC when allogeneic cells were injected 2 days before CP treatment suggested the very early formation of killer cells resistant to subsequent CP treatment. Special experiments

*At the time of transplantation of the cells, the recipients' tissues still contained a large quantity of antigen (SRBC) [3].

accordingly were carried out in which the dynamics of killer formation was studied after intravenous injection of 1×10^8 allogeneic cells. As Fig. 1 shows, in agreement with the results obtained in vivo, injection of allogeneic cells 1 day previously did not lead to the formation of killer cells. The cytotoxic effect of the spleen cells began to appear, however, on the 2nd-3rd day after intravenous immunization with allogeneic cells, it reached a maximum on the 4th-5th day, and fell toward the 7th day. This effect was found to be immunologically specific, for spleen cells of CBA mice immunized with (CBA × C57BL/6)F_1 cells or C57BL/6 cells did not cause destruction of DBA/2 macrophages. Cytotoxic activity also was absent against CBA macrophages.

Injection of allogeneic or semiallogeneic cells 1 day before CP treatment thus improved the changes of survival of presensitized allogeneic lymphocytes transplanted after injection of CP. The mechanism of this protective effect is evidently connected with elimination by CP of the clone of lymphocytes specifically stimulated by C57BL/6 transplantation antigens. The absence of protection in the case when allogeneic cells were injected 2 days before CP treatment can be explained by the formation of killers more resistant to elimination by CP. This explanation agrees with the rapid formation of killers discovered under these conditions of immunization. The high resistance of the effector immune cells to the action of CP has also been observed with other models [3].

Early (on the 2nd day) killer formation has not been observed by other workers. In the present experiments it may perhaps have been due to the method of immunization used (intravenous injection of allogeneic cells), for it favors rapid contact between the recipient's lymphocytes and the antigen. Methods of immunization usually employed, such as skin grafting or transplantation of tumor cells, and so on, are evidently associated with slower penetration of antigens into the lymphoid organs, with the consequent later (7th-10th day) formation of killer cells [9, 10].

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