IMMUNOLOGICAL SPECIFICITY OF RAPID REJECTION OF A SKIN GRAFT THROUGH ANTIGENIC OVERLAP IN THE H-2 SYSTEM: ROLE OF IMMUNE LYMPHOCYTES AND ANTIBODIES

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In the early stages after immunization of mice by a single injection of allogeneic cells, a skin graft obtained from a mouse of a third strain exhibiting partial antigenic overlap with the donor does not undergo rapid rejection despite a maximal secondary response to a graft from the corresponding donor. The development of a secondary response to a third-party graft in the late stages after single or during repeated immunization is not due to a change in the properties of the lymphocytes, but to the synergic action of humoral antibodies. Immunization with single antigenic specificity or with a combination of antigens determined by two alleles of the H-2 locus induces rapid rejection of third-party skin grafts. Skin-grafting experiments confirmed the principles observed previously in the study of the specificity of lymphocytes on model systems in vitro.

Tissue destruction during allografting is due to direct contact between immune lymphocytes and the corresponding antigens of the target cells. Investigations of the immunological specificity of transplantation immunity using model systems in vitro have shown that, unlike humoral isoantibodies, immune lymphocytes are polyspecific [1-3], and they provided an explanation of the mechanism of development of the complex receptors of the T-lymphocytes [4]. However, the possibility cannot be ruled out that the polyspecificity of lymphocytes obtained 8 days after a single immunization is a laboratory phenomenon which does not reflect the natural principles of transplantation immunity.

To test this hypothesis the specificity of the secondary response to a skin graft in vivo and the role of lymphocytes and antibodies in this process were studied.

EXPERIMENTAL METHOD

Inbred mice aged 9-12 weeks belonging to congeneic lines C57BL/10ScSn ($H\text{-}2^b$), B10. D2 ($H\text{-}2^d$), B10. A ($H\text{-}2^a$), and B10. 504 ($H\text{-}2^{da}$), and also to lines C3H/Sn ($H\text{-}2^k$) and I/St ($H\text{-}2^1$) were used. Lines B10. D2 and B10. 504 differ only in a single antigenic specificity in the H-2 locus [5].

The tumor used was sarcoma MCh11, induced by methylcholanthrene and passed through C57BL/10 mice. A suspension of tumor cells was obtained by trypsinization.

The methods of obtaining cell suspensions from the spleen and lymph glands of the mice and of determining their viability were described previously [1, 2].

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TABLE 1. Rejection of Skin Graft of Strain B10.A by B10.D2 Mice Immunized with C57BL/10 Tissues (M ± m)

Interval between immunization and skin grafting (in days)	Type of C57BL/10 immunizing tissue	No. of B10.D2 mice	Period or survival of skin graft (in days)			
			C57BL/10	P	B10.A	P
—		24	10,7±0,2	—	8,6±0,3	—
3	Sarcoma	19	6,2±0,1	<0,001	8,4±0,4	>0,1
4	Skin	6	6,7±0,3	<0,001	8,3±0,6	>0,1
7	Spleen	15	6,1±0,1	<0,001	8,9±0,4	>0,1
9	Sarcoma	15	6,2±0,1	<0,001	7,4±0,5	<0,05
28	Skin	8	7,6±0,1	<0,001	7,6±0,2	<0,02
7	Sarcoma + spleen + sarcoma	9	6,2±0,2	<0,001	7,3±0,3	<0,01

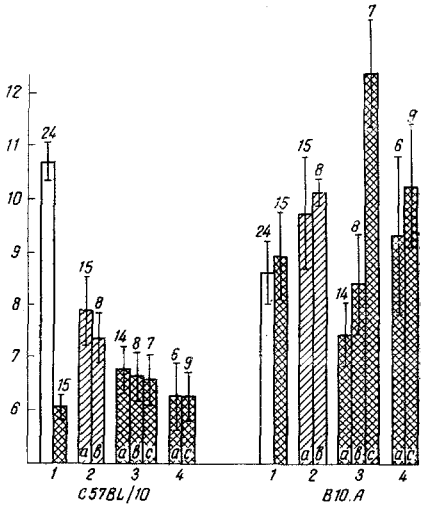

Fig. 1. Transfer of rapidly rejected graft of C57BL/10 and B10.A skin to B10.D2 mice with hyperimmune lymphocytes and with B10.D2 anti-C57BL/10 serum. Unshaded and obliquely shaded columns represent normal B10.D2 mice; cross-hatched columns represent immunization with C57BL/10 spleen ($4 \cdot 10^6$ cells intraperitoneally 7 days before transplantation). Intravenous injection of $1.5 \cdot 10^8$ immune lymphocytes (2) once (a) or twice (b). Intraperitoneal injection of antiserum (3) or normal B10.D2 serum (4) on days 0, 2, 4, and 6 in doses of 0.2 ml (a) or 0.1 ml (b), and on days 0, 1, 2, and 3 in a dose of 0.2 ml (c). Ordinate, mean duration of survival of skin graft (in days). Numbers above columns show number of recipient mice.

Transplantation of skin from the donor's tail to the recipient's back was carried out by the method of Billingham and Medawar [9] in the modification described in [6]. Grafts measuring 20-30 mm^2 in area were fixed by adhesive tape which was removed 6 days after grafting, when the state of the graft was recorded daily. The graft was regarded as rejected if all or nearly all of its surface was necrotic. The donors of the grafts were always females, but the recipients were either males or females. In each experiment skin from mice of 2-4 strains was grafted onto the same recipients. The statistical significance was determined by Student's t-test.

A single immunization was carried out with MCh11 cells in a dose of $3 \cdot 10^6$, injected subcutaneously on the left and right sides, or spleen cells injected intraperitoneally in a dose of $4 \cdot 10^6$ or by the skin graft. During triple subcutaneous immunization the B10.D2 mice received $6 \cdot 10^6$ MCh11 cells, $4 \cdot 10^7$ C57BL/10 spleen cells, and $3 \cdot 10^6$ MCh11 cells in succession at 5 points at intervals of 1-2 weeks. Blood serum and lymph gland cells were obtained 7 days after the last immunization. Antibodies against antigens of the H-2 locus were determined by dextran hemagglutination [10].

EXPERIMENTAL RESULTS

In the experiments of series I, B10.D2 mice were immunized with C57BL/10 tissues (incompatibility with respect to four H-2 antigens: 2, 5, 22, and 33), and tested simultaneously with two skin grafts: from the donor (C57BL/10) and from a third strain (B10.A). This last strain has one antigen (5) of the H-2 locus of the four immunizing antigens used in common with the donor.

Table 1 shows that in the early period after skin grafting (3, 4, or 7 days), after a single immunization with sarcoma or skin or with a small dose of spleen cells the donor's skin graft was rejected by a secondary type of mechanism, while the third-party skin underwent primary rejection. With an increase in the interval between immunization and test grafting to 9-28 days, rejection of the third-party skin graft was accelerated. This acceleration became highly significant if the grafting was carried out after triple immunization. The donor's skin graft underwent a secondary type of rejection in all cases.

TABLE 2. Rejection of Skin Graft of Third-Party Mice after Immunization with a Single Antigen of the H-2 Locus or with Antigens of Two Alleles of the H-2 Locus*

Donor	Recipient	No. of recipient mice	Duration of survival of skin graft of strains (in days)			
			B10. D2 (1/1)†	*P*	B10. A (1/1)†	*P*
—	B10. 504	9	12,1±0,7	—	8,8±0,2	—
B10. D2	B10. 504	6	10,3±0,2	<0,05	6,8±0,4	<0,001
			B10. A (8/8)†	C3H (5/8)†	B10. D2 (5/8)†	I/St (1/8)†
—	C57BL/10	8	9,3±0,4	8,8±0,3	10,3±0,7	9,0±0,4
B10. A	C57BL/10	10	6,1±0,1	7,4±0,4	9,0±1,0	10,1±0,6
			P<0,001	*P*<0,002	*P*>0,05	*P*>0,05

*Single intraperitoneal immunization with $4 \cdot 10^6$ spleen cells 7 days before skin grafting.

†Theoretical index of immunity shown in parentheses: ratio between number of reacting antigens of the graft and number of immunizing antigens of the donor.

Active immune lymphocytes, which attack the donor's skin graft, are evidently harmless for the skin graft from a third strain possessing only one of the four immunizing antigens. To determine the causes of acceleration of rejection of the third-party skin graft, at later stages or after triple immunization, B10.D2 lymphocytes obtained from lymph glands after triple immunization with C57BL/10 cells were injected intravenously into fresh syngeneic mice in a dose of $1.5 \cdot 10^8$ cells once or twice. Before and after the injection the recipients were grafted with the skin of the donor (C57BL/10) and of a third strain (B10.A). As Fig. 1 shows, in all cases the immune lymphocytes induced rapid rejection of the donor's skin, whereas rejection of the third-party skin not only was not accelerated, but was actually delayed.

In another series of experiments the immune serum obtained from those B10.D2 mice from which the immune lymphocytes had been taken was injected intraperitoneally into syngeneic mice 4 times in volumes of 0.1 or 0.2 ml on days 0, 2, 4, and 6 relative to the test transplantation. The titer of hemagglutinins against C57BL/10 erythrocytes in the serum was 9-10 $\log_2$. The recipients were first immunized with C57BL/10 spleen (this type of immunization does not accelerate rejection of B10.A skin; see Table 1). It will be clear from Fig. 1 that under these conditions injection of 0.2 ml of the immune serum caused rapid rejection of the B10.A skin. The use of smaller doses of serum (0.1 ml), or its injection too soon after grafting (days 0, 1, 2, and 3) did not lead to accelerated rejection of the B10.A skin. Normal serum, injected in doses of 0.2 ml four times at different periods after grafting likewise was ineffective.

By contrast with the system described above, in the case of incompatibility with respect to a single antigenic specificity (B10.D2 → B10.504), immunization with spleen cells led to rapid rejection of the skin both of the donor (B10.D2) and of the third strain (B10.A) having the same antigen (Table 2). In the case of immunization with a set of eight antigens determined by two H-2 alleles (d and k) (the B10.A → C57BL/10 system), skin both from the B10.A donor and from strains C3H and (not significantly) B10.D2, carrying antigens of the $H\text{-}2^k$ and $H\text{-}2^d$ alleles respectively, was rejected. Conversely, a graft of I/St skin, with only one of the eight immunizing antigens (10), underwent the primary type of rejection (Table 2).

The principles discovered previously in a study of the specificity of immune lymphocytes on model systems in vitro were thus confirmed by skin grafting at different times after single immunization: 1) a graft containing some of the immunizing antigens does not undergo rapid rejection in the presence of a maximal response to a graft from the corresponding donor; 2) during immunization with a single antigen, skin both of the donor and of a third strain containing this antigen undergoes secondary rejection; 3) immunization with a combination of antigens determined by two alleles of the H-2 locus (d and k) induces rapid rejection of grafts carrying each of these alleles separately; 4) rejection of a C3H ($H\text{-}2^k$) graft is accelerated more than rejection of a B10.D2 ($H\text{-}2^d$) graft.

The results described by statement 1 do not agree with observations published previously [8]. The reason is evidently because in that investigation the strains of mice used were not congeneic or not strictly congeneic with respect to the H-2 locus, as the result of which acceleration of rejection of the third-party skin could have been due to immunization against antigens of different H-loci.

The development of a secondary reaction to the third-party graft in the late stages after single or during repeated immunization is due, as the results of this investigation show, to the action of humoral antibodies and not to a change in the properties of the lymphocytes (accumulation of lymphocytes monospecific against the antigen of the third strain). In fact, hyperimmune lymphocytes induced secondary rejection of the donor's skin in syngeneic recipients, but primary rejection of the third-party skin, despite the fact that this skin underwent secondary rejection by the donor of the lymphocytes. On the other hand, antiserum of hyperimmune mice, containing a high titer of antibodies, induced rapid rejection of a third-party graft by recipients immunized against antigens of the corresponding donor. This action of the antiserum is immunologically specific, for a graft of an unrelated line (BALB/c), having none of the antigens of the immunizing complex, underwent primary rejection by these same recipients. Presumably the isoantibodies can reject the skin if the immune lymphocytes themselves are unable to destroy the graft. Similar results have recently been demonstrated under conditions in which the activity of the lymphocytes was weakened by cortisone and antilymphocytic serum [11]. This synergism may be the main function of humoral antibodies in the destruction of tissues during transplantation immunity. The mechanisms of this synergism may be activation of immune lymphocytes by the antigen–antibody complex on the surface of target cells [12], an increase in the vulnerability of the target cells when treated with antibodies, or complex interaction between several components: immune lymphocytes, soluble alloantigen, antibodies, and complement [7].

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