PRIVATE SPECIFICITIES OF *H-2K* AND *H-2D* LOCI AS POSSIBLE SELECTIVE TARGETS FOR EFFECTOR LYMPHOCYTES IN CELL-MEDIATED IMMUNITY*

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T cells responsible for allograft immunity are known to perform at least two functions: primary recognition of products of the major histocompatibility complex (MHC)[1] and an effector function which is completed with destruction of grafted tissue. Both functions have expression in vitro, the former in the proliferative response of normal lymphocytes in mixed culture (1), the latter in the cytotoxic effect (CE) of immune lymphocytes upon allogeneic target cells (TC) (2). TC destruction in the latter case is also preceded by recognition of an immunizing complex by lymphocyte receptors, as shown by the selective removal of killer lymphocytes from the population as a result of their specific adherence to the corresponding TC (3).

Recognizing T (RT) cells and effector T (ET) cells were shown to react to products of different MHC regions: RT recognize the products of the mixed lymphocyte culture (MLC) locus which is mapped mainly in the immune response (Ir) region of MHC (4), whereas ET cells react to *H-2K* and *H-2D* products, the induction of ET cells being preceded by MLC reaction (5) which, in its turn, requires differences in MLC locus (6). In case of differences in MLC locus but identity of *H-2* loci in mice (6) or HL-A loci in human (7–9), CE does not develop, even though MLC reaction is taking its normal course.

These findings were interpreted to mean that the reaction to transplantation antigens involves cooperation of two T-cell populations, each recognizing products of different MHC regions (10, 11). This model, however, is not in agreement with observations made on mutant strains of mice. A single difference in *H-2D* locus between the original B10.D2 strain and B10.504 mutant,

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[1] *Abbreviations used in this paper:* AI, absorption index; CE, cytotoxic effect; CML, cell-mediated lympholysis; ET cells, effector T cells; 199 LB, medium 199 with lactalbumin and bovine serum; LD, lymphocyte defined; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; MST, medium survival time; R_{im}, release with immune lymphocytes; R_{max}, maximum release; R_{norm}, release with normal lymphocytes; R_{sp}, spontaneous release; RT cells, recognizing T cells; SD, serologically defined; TC, target cells.

due apparently to a point mutation of this locus (12, 13), proved to be sufficient for induction of both ET cells (14) and RT cells (13). Serologically undetectable differences in the "K-end" of MHC between C57BL/6 strain and H(z1) mutant appeared to be responsible for both MLC reaction and cell-mediated lympholysis (CML), as well as for skin graft rejection (15, 16). Genes governing MLC reaction were suggested to be distributed through MHC and not restricted to Ir (17, 18). Thus, the arrangement of MLC locus genetic determinants, whose products are recognized by RT cells, remains obscure; at the same time, most data favor a decisive role of *H-2K* and *H-2D* loci products in both induction (6) and realization of CML (19). This does not mean, however, that the serologically defined (SD) entities of these loci are themselves recognizable by ET cell receptors.

Each of *H-2* loci (K or D) is known to govern only a single private *H-2* specificity which is strictly peculiar to the particular allele of the given *H-2* locus. Conversely, public *H-2* specificities may be coded by either *H-2* locus and are detectable in different *H-2* haplotypes (18, 20). Although both private and public *H-2* specificities are capable of inducing antibody formation, their role in cell-mediated immunity may prove to be nonequivalent. The present study was designed to test whether private or public *H-2* specificities, or both of them, constitute the recognizable unit for ET cell receptors during absorption of cytotoxic lymphocytes on TC and subsequent CE in vitro and also during accelerated skin graft rejection in vivo.

It has been demonstrated that ET do not recognize public *H-2* specificities but react selectively either to private *H-2* specificities or to serologically silent products of *H-2K* and *H-2D* loci closely linked to private *H-2* specificities. Preliminary results of the present study have been published elsewhere (21).

Materials and Methods

Animals. Mice of congeneic strains C57BL/10 ScSn ($H\text{-}2^b$) (abbreviated B10) and C57BL/10-$H\text{-}2^d$ (abbreviated B10.D2) were bred at the Gamaleya Institute, Moscow. Congeneic strains of mice with recombinant *H-2* haplotypes B10. A($H\text{-}2^a$), B10.D2 (R107) abbreviated R107 ($H\text{-}2^{d\text{-}Eg}$), B10.D2 (R101) abbreviated R101 ($H\text{-}2^{g\text{-}Eg}$), B10.A(2R) abbreviated 2R ($H\text{-}2^{h\text{-}2Sg}$), B10.A(5R) abbreviated 5R($H\text{-}2^{\text{-}2Sg}$), as well as B10.M($H\text{-}2^f$) and DBA/1 ($H\text{-}2^q$) were bred at the Laboratory of Tissue Compatibility Genetics of the General Genetics Institute, USSR Academy of Sciences. *H-2* haplotype of the above strains are presented in Table I according to Dèmant (18) and Vedernikov and Egorov (22). Mice aged 10–16 wk were used in the experiments.

Tumor. MX11 sarcoma in B10 mice induced with methylcholanthrene, converted into the ascite form and maintained by every week passages.

Immunization. B10.D2 mice were immunized with a single dose of twice washed sarcoma cells administered at five subcutaneous sites and intraperitoneally, 40 to 50 $\times$ 10^6 viable cells per mouse (23).

Experiments on Cytotoxici Effect of Immune Lymphocytes. The techniques for preparing immune lymphocyte suspensions and target cell cultures, those for assessment of cell viability and cytotoxic activity, as well as quantitation of the latter, have been described in detail previously (3, 14, 23). In the present study they were slightly modified, as follows.

Regional lymph nodes were taken 8 days after immunization. Dissociated cell suspensions were prepared by forcing the lymphoid tissue through a 100 mesh stainless steel seave with subsequent removal of cell clumps by sedimentation at unit gravity. Peritoneal macrophages, used as target cells,

were harvested in 48–72 h after intraperitoneal injection of an irritant (solution of 3% peptone and 0.15% glycogen in saline), counted in a hemocytometer and seeded into Leighton tubes in portions containing 2.5×10^5 cells per 1 ml of 199 medium supplemented with 20% bovine serum, 10% lactalbumin hydrolysate solution and antibiotics (199 BL Medium). Target cells were grown for 2 days as nonconfluent uniform monolayers in the atmosphere of 5% CO_2 or in the presence of 0.005 M Hepes. Immune and normal lymph node cells from mice of the same strain were washed twice with Hanks' solution, resuspended in 199 medium containing 5% fetal calf serum (FCS) and 0.005 M Hepes, and counted using a mixture of 0.1% solutions of aqueous eosine and trypan blue. 2×10^6, 4×10^6 or 8×10^6 lymphocytes were added in 1 ml vol to previously washed Leighton cultures of target cells to be investigated. Four replicate cultures were used for each lymphocyte dose. After incubation overnight, the culture medium with free lymphocytes was replaced by 199 medium containing 5% bovine serum; the next day, no less than 300 surviving macrophages were counted in each tube, using magnification ×200 and eosine-trypan blue mixture, and the mean numbers of target cells per field of vision were multiplied by 10^3 (the number of fields of vision per flat tube surface).

CE was calculated as (a – b) 100/a, where *a* and *b* represent mean numbers of surviving TC following incubation with normal and immune lymphocytes, respectively. Differences in results were assessed for significance by Student's *t* test.

The Cytotoxic Assay Based on ^{51}Cr Release. The cytotoxic assay based on ^{51}Cr release according to Häyry et al. (24) was used in some early experiments of this study with a number of modifications. 48-h Leighton cultures of TC were washed and incubated for 2 h with 1 ml 199 medium containing 10% FCS ans 100 μCi/ml ^{51}Cr (spec act 50 mCi/mg). Then followed four washings with 3-ml vol of 199 BL medium, and normal and immune lymphocytes were added to macrophages as above. To assess spontaneous release (R_{sp}) of the label, TC were incubated in 199 medium with 5% FCS without the addition of lymphocytes. Maximum release (R_{max}) was assessed following treatment of TC with 2% sodium dodecylsulphate (SDS) diluted in 0.05 M borate with 0.002 M EDTA. After incubation for 20 h at 37°C, the tubes were gently shaken, the culture medium harvested, and each culture washed twice with 0.5 ml of 199 BL medium. Radioactivity in pooled culture medium and washings was measured in a Nuclear Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). The culture fluid was not centrifuged, since preliminary experiments showed that some particular component of the label released from destroyed macrophages can thus escape determination. Under the above conditions, R_{max} amounted to 1.5×10^3–4×10^3 CPM, R_{sp} constituted 28 to 33% of R_{max}, while R_{norm} (release of the label following incubation with normal lymphocytes) exceeded R_{sp} by 10 to 20% making up 35 to 45% with respect to R_{max}. We succeeded in decreasing the magnitude of R_{norm} to the level of R_{sp} by cultivating TC on glass surfaces covered by poly-L-lysine (25). Still lower values of R_{norm} were obtained if normal lymphocytes were absorbed by syngeneic target cells, as described below.

In later experiments of this study the technique of CE determination using ^{51}Cr release was modified as follows (26). After harvesting into a siliconized tube from the peritoneal cavity, macrophages were centrifuged for 5 min at 900 rpm, suspended in 199 medium with 10% FCS and 75 μCi/ml ^{51}Cr, and incubated at the concentration of 1×10^7–2×10^7 cell/ml for 1 hr at 37°C in a water bath with occasional agitation. After the incubation, cells were washed three times with 199 BL, counted again, and delivered to Leighton tubes previously covered with poly-L-lysine by 4×10^5 cells in 1 ml of 199 BL medium containing 0.005 M Hepes. After 2 days of incubation at 37°C, the cultures were washed three times with 199 BL, and addition of lymphocytes and subsequent operations were made as described above. With this procedure, R_{norm} did not exceed 15–20% of R_{max}. The cytotoxic effect of immune lymphocytes was calculated as specific release = $(R_{im} - R_{norm})/R_{max} - R_{norm}) \times 100$.

Absorption of Immune Lymphocytes. Absorption was carried out using previously described methods (3, 19) with some modifications. Almost confluent monolayers were prepared by seeding 5×10^6 or 15×10^6 peritoneal macrophages per 60-mm Carrel flask or 250-ml plastic bottle (no. 3024, Falcon Plastics, Div of BioQuest, Oxnard, Calif.), respectively, 2 days before experiment. After washing out of nonadherent cells, the immune lymphocytes suspended in 199 medium with 10% bovine serum were added to the Carrel or plastic bottle cultures ($30–50 \times 10^6$ cells in 2.5 ml vol in the former case, and $90–150 \times 10^6$ cells in 7-ml vol, in the latter).

After incubation for 3 h at 30°C, the vessels were rocked on a shaker (New Brunswick Scientific Co.

Inc., New Brunswick, N. J.) at 100 rpm for 5 min, and the nonadherent lymphocytes were harvested using additional rinsing of the vessels with a small portion of the medium and manual shaking. The above procedure of absorption was repeated on a new monolayer of macrophages and all nonadherent lymphocytes were pooled, spun down, resuspended in 199 medium containing 5% FCS, and counted. Absorption of normal lymphocytes on syngeneic B10.D2 target cells was performed in the same way. Equal doses of viable nonadherent and untreated immune lymphocytes were then compared in parallel for cytotoxic activity. The absorption index (AI) was calculated as (a - b) 100/a, where *a* and *b* represent the cytotoxic effects of intact and nonadherent immune lymphocytes, respectively.

Skin Grafting. Tail skin grafts from four donors to the back of one recipient animal were made by a modification (27) of the Billingham and Medawar method (28). Plaster bandages were removed 6 days later, and grafts were scored as rejected when no viable epithelium remained on inspection of the grafts.

Experimental Design. As indicated in Table I, B10.D2 anti-B10 lymphocytes are potentially directed against seven *H-2* specificities of which two are private, coded by *H-2K*^{b} (specificity *33*) and *H-2D*^{b} (specificity 2), and five public ones (*H-2.5*, *39*, *53*, *54*, and *56*). These lymphocytes were brought in contact with different TC possessing the following specificities from the enumerated above: *H-2.5* (B10.A strain), *H-2.39* and *53* (B10.M strain), *H-2.54* and *56* (DBA/1 strain), *H-2.2* (R101 strain), *H-2.5*, *33*, and *39* (possibly also *H-2.53*, *54*, and *56*) (R107 and 5R strains), *H-2.2* and 5 (2R strain), as well as with a mixture of R107 and R101 target cells, possessing both private specificities, *H-2.2* and *H-2.33*.

Reactions with TC of the donor (B10) and recipient (B10.D2) were used as positive and negative controls, respectively. In in vivo experiments, B10.D2 mice were immunized by a single intraperitoneal injection of 5×10^6 B10 spleen cells and used, 7 days later, as recipients for skin grafting from four mouse strains: B10, B10.A, B107, and R101.

Results

With all the doses used (8, 4, and 2 millions), B10.D2 anti-B10 lymph node cells destroyed TC of the donor (B10) but did not affect those of the recipient (B10.D2) or third-party strains B10.A, B10.M, or DBA/1 (Table I). The degree of CE on B10 TC was proportional to the lymphocyte dose. Absence of the reaction with third-party TC indicates that public specificities *H-2.5; H-2.39, 53; H-2.54, 56* are not involved in the reaction, since, with the above three strains, the lymphocytes could theoretically respond to only those public specificities. These results are in agreement with our previous observations (3).

However, partial CE was produced in the above experiments by the same lymphocytes on TC from recombinant strains R107, R101, and 2R. If, based on Table I data, CE on B10 TC is assumed to be 100%, the corresponding effects on R107 and R101 TC amount to about 75% and 25%, respectively, and are, thus, in the proportion of 3:1. The results were similar whether the cytotoxicity tests employed viable cell count (Exp. 1–4) or ^{51}Cr release from destroyed TC (Exp. 5–7). So far as *H-2.5*, *39*, *53*, *54*, and *56* specificities do not seem to participate in the reaction, it should be assumed that 75% of the activity of B10.D2 anti-B10 lymphocytes is directed against the private specificity *H-2.33* and 25% against the private specificity *H-2.2*. This assumption is supported by the observation that the effect of the same lymphocytes on a 3:1 mixture of R107 and R101 TC approached 100% (or even exceeded that level). In some experiments, TC of 2R strain were destroyed somewhat more readily than those of R101, due, mainly, to a higher activity of the lowest lymphocyte dose (Table I).

The lack of CE in the immune lymphocyte reaction with third-party TC, which

TABLE I

Cytotoxic Effect (%) of Anti-B10 B10.D2 Lymphocytes on Target Cells Possessing Private or Public Specificities of H-2K and H-2D Loci

		Source, *H-2* genotype, and *H-2* specificities* of target cells								
Exp. no.‡	No. of lympho-cytes ($\times 10^6$)	B10(K^bD^b) 2; 33; 5; 39 53; 54; 56	B10.D2(K^dD^d) None	B10.A(K^kD^d) 5	B10.M(K^fD^f) 39; 53	DBA/1(K^qD^q) 54; 56	R107(K^bD^d) 33; 5; 39; 53; 54; 56	R101(K^dD^b) 2	2R(K^kD^b) 2; 5	R107 + R101 33; 5; 39; 53; 54; 56 +2
1	8	91.0		−31.5			64.2	23.5		95.0
	4	68.9		−17.5			27.5	2.1		
2	8	83.8	−1.5	0.8			75.3	34.5	55.7	79.1
	4	77.6					62.3	5.7	27.6	77.7
3	8	94.0	4.7	−11.1			77.3	46.9	46.0	90.6
	4	64.0					38.7	12.8	28.1	66.0
	2	42.4					19.7	0		41.1
4	8	76.2			2.7		60.0	25.8	34.0	
	4	56.4								
5	8	100	10.5	−4.4	−5.6		94.0		35.4	
6	8	100					55.6	22.5	31.7	
7	8					6.1	82.6	24.5	24.0	
	4	45.7								
Mean ±SE§	8	90.8 ± 3.8 (100)	4.6 ± 3.5 (5.9)	−12.8 ± 5.4 (0)	−1.5 ± 4.2 (0)	6.1 (6.7)	72.7 ± 5.1 (80.0)	29.6 ± 3.9 (32.6)	37.8 ± 4.6 (41.6)	88.2 ± 4.7 (97.1)
	4	62.5 ± 5.4 (100)					42.8 ± 10.3 (68.5)	6.9 ± 2.5 (11.0)	27.9 (44.5)	71.9 (>100)

* *H-2* specificities potentially capable of reacting with lymphocytes: 2 and 33 are private specificities of *H-2D*^{b} and *H-2K*^{b} alleles, respectively. 5, 39, 53, 54 and 56 are public specificities.

‡ Cytotoxic effect was assessed by target cell count in Exp. 1, 2, 3, 4 or by ^{51}Cr release in Exp. 5, 6, 7.

§ In parenthesis: relative cytotoxic activity of lymphocytes (%).

share only public *H-2* specificities with the donor could be connected with inability of the lymphocytes to attach to these TC, because their antigen-binding receptors do not recognize public *H-2* specificities. To test this possibility, B10.D2 anti-B10 lymphocytes were subjected to absorption by macrophages of different origin with subsequent testing of the nonadherent lymphocytes for CE on B10 TC.

It follows from Table II, that in two independent experiments (employing

TABLE II

Absorption of Anti-B10 B10.D2 Lymphocytes by Target Cells Possessing Private or Public Specificities of H-2K and H-2D Loci

Source of target cells for absorption*	*H-2* specificities‡	No. of lymphocytes ($\times 10^6$)		Cytotoxic effect on B10 target cells	Absorption index	Relative absorbing activity of target cells
		For absorption§	For test‖			
				%	%	%
None	—	—	8	83.8		
			4	64.0		
B10	2; 33; 5; 39; 53; 54; 56	50	8	22.6	73.0	100 (P¶ < 0.001)
		30	4	6.5	90.0	100 (P¶ < 0.001)
B10.D2	None	50	8	75.5	9.9	13.6
		30	4	64.2	0	0
B10.A	5	50	8	77.0	8.1	10.9 (P¶ > 0.1)
		30	4	61.8	3.4	3.8 (P¶ > 0.1)
B10.M	39; 53	30	4	64.8	0	0 (P¶ > 0.1)
R107	33; 5; 39; 53; 54; 56	50	8	56.4	32.7	44.8 (P¶ < 0.01)
		30	4	30.2	52.9	58.8 (P¶ < 0.001)
R101	2	50	8	84.8	0	0 (P¶ > 0.05)
		30	4	40.2	37.2	41.5 (P¶ < 0.01)
R107 + R101 3:1	2 + 33; 5; 39; 53; 54; 56	50	8	37.3	55.5	76.2 (P¶ < 0.001)
		30	4	−3.0	100	100 (P¶ < 0.001)
2R	2; 5	50	8	84.1	0	0 (P¶ > 0.05)
		30	4	40.4	36.9	41.0 (P¶ < 0.01)

* 5×10^6 peritoneal cells were seeded per Carrel flask 8 cm in diameter and cultivated for 2 days to obtain an almost confluent monolayer.

‡ *H-2* specificities of absorbing cells potentially capable of reacting with lymphocytes (see Table I).

§ Two times for 3 h at 30°C.

‖ Nonadherent lymphocytes were harvested, washed one time, counted, and tested on B10 target cells.

¶ Significance of the difference between the cytotoxic effects of lymphocytes absorbed with the given stated specific target and with B10.D2 target incapable of specific absorption.

different lymphocyte doses) CE of the lymphocytes, reaching 83.8% and 64%, was inhibited by 73% and 90%, respectively, following absorption with B10 macrophages, but not reduced significantly after absorption with B10.D2 TC. Macrophages of B10.A and B10.M strains, also, either failed to affect the activity of the nonadherent lymphocytes or reduced it nonspecifically by no more than 8.1% (differences in the absorbing activities between B10.D2, B10.A, and B10.M are not significant: $P > 0.1$). Similar results were obtained following absorption with DBA/1 TC (not shown in Table II). By contrast, absorption of the lymphocytes with R107, R101, and 2R TC reduced CE by 52%, 37.2% and 36.9%, respectively ($P < 0.01$ in all the cases), which constitutes 60%, 40%, and 40% of the AI of B10 TC. The latter result, shown in detail in Fig. 1, was obtained using comparatively

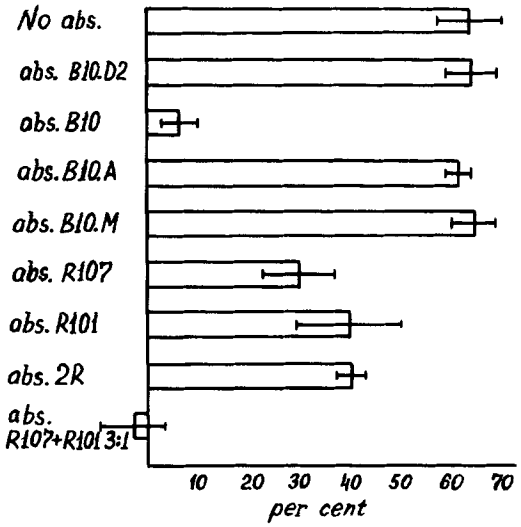

FIG. 1. Cytotoxic activity of anti-B10 B10.D2 lymph node cells absorbed by target cells possessing private or public specificities of *H-2K*^{b} or *H-2D*^{b} alleles. 3×10^7 lymphocytes were incubated two times for 3 h at 30°C on a monolayer of macrophages seeded two days previously in the amount of 5×10^6 per Carrel flask. Strains of mice used as a source of absorbing macrophages are shown on the ordinate. The nonadherent lymphocytes were harvested, washed one time, counted, and the cytotoxic effect of 4×10^6 lymphocytes was tested on B10 target cells. Each column represents the arithmetic mean (±SD) of cytotoxic effects found in four replicate tubes.

low doses of the lymphocytes for absorption (30×10^6 cells per Carrel flask) and for CE assay (4×10^6 cells per tube) which insures practically complete exhaustion of CE towards B10 TC.

Absorption with a mixture of R107 and R101 TC in this case resulted in complete inhibition of the lymphocyte activity. However, if the dose of absorbed and tested lymphocytes was in excess, not providing for complete exhaustion of CE towards B10 TC, the absorbing activity of R107 macrophages became lower, that of R101 and 2R strains was not evident at all, while the mixture of R107 and R101 TC resulted in incomplete reduction of the lymphocyte activity (Exp. 1, Table II).

These findings permitted a suggestion that the receptors of effector lympho-

cytes, determining their specific attachment to TC, do not recognize public *H-2* specificities and react only with private ones, there being two lymphocyte populations each recognizing only one of the private specificities, either *H-2K* or *H-2D*. Immunization with both *H-2.33* (K^b) and *H-2.2* (D^b) specificities could induce the two respective lymphocyte populations in unequal proportion: their greater part could be directed against the private specificity *H-2K*, while the smaller part against the private specificity *H-2D*. If the suggestion is correct, selective removal from the immune lymphocyte suspension of cells directed towards only one of the private specificities should not affect CE upon macrophages possessing the alternate private specificity.

It follows from Table III that experimental findings agreed with behavior expected from the above suggestion. Cytotoxicity of B10.D2 anti-B10 lymphocytes towards R107 or 5R TC, ranging in different experiments from 36.8% to 73.1%, was suppressed by 63–100% following absorption with R107 TC but did not diminish after absorption with R101 and 2R TC. Some insignificant reduction of CE in a part of experiments is nonspecific, since AI of the same magnitude are observed following incubation of the immune lymphocytes with syngeneic B10.D2 macrophages incapable of specific absorption. In the above experiments CE of the same lymphocytes against R101 and 2R macrophages, ranging from 17% to 34%, was maintained following absorption with R107 TC (exp. 1, 2, 4) or with 5R TC (Exp. 3) possessing similar *H-2* haplotypes (K^bD^d). At the same time, absorption with R101 macrophages removed lymphocytes cytotoxic for both R101 and 2R TC and, furthermore, absorption with 2R macrophages cancelled CE not only against 2R but against R101 TC, as well. In both cases, cross-absorption amounted to 79–100% (Table III). Just like in the previous series of experiments, the results did not depend on the method of testing whether by TC count or specific ^{51}Cr release.

Thus, absorption of d anti-b lymphocytes with macrophages, possessing *H-2D*^{b} private specificity, *H-2.2*, in combination with the *H-2K* private specificity of non-*H-2*^{b} origin (not *H-2.33*) cancels CE of the lymphocytes upon any TC possessing *H-2.2* specificity but does not affect CE upon TC bearing the other private specificity *H-2.33*. At the same time, sharing of public specificities by absorbing macrophages with *H-2*^{b} does not affect CE of the lymphocytes.

The ability of ET lymphocytes to react only with private *H-2* specificities is supported by results of in vivo experiments on skin transplantation to B10.D2 mice previously immunized with a small dose of B10 mouse spleen. Following such immunization not only donor (B10) grafts were rejected at an accelerated fashion, but also those of R107 and R101 recombinant strains, bearing single private specificities *H-2.33* (K^b) and *H-2.2* (D^b), respectively. On the contrary, there was no significant acceleration in rejection of B10.A skin graft, possessing only public *H-2.5* specificity out of donor *H-2* specifities foreign to the host (Table IV).

Discussion

The main two findings of the present work are the following: (*a*) ET lymphocytes do not recognize public *H-2* specificities. (*b*) ET lymphocytes recognize private *H-2* specificities or structures closely related to them.

TABLE III

*Cross-Absorption of Anti-B10 B10.D2 Lymphocytes with Target Cells Possessing H-2.33(K^b) or H-2.2(D^b) Private Specificities**

Source of target cells for test	Exp. no.‡	Source and *H-2* private specificity of absorbing target cells				
		None	R107 or 5R§ *H-2.33*	R101 *H-2.2*	2R *H-2.2*	B10.D2 None
		Cytotoxic effect (%)				
R107 or 5R§	1	60.0	−4.6‖ (>100)¶	60.5 (0)¶	60.3 (0)¶	NT
	2	55.6	−11.0‖ (>100)	50.9 (8.5)	48.7 (12.3)	50.3 (9.5)¶
	3	73.1	26.8‖ (63.3)	60.0 (17.9)	61.2 (16.3)	60.1 (17.8)
	4	36.8	2.7‖ (92.7)	30.3 (17.6)	19.2 (47.8)	27.3 (25.8)
R101	1	25.8	30.0 (0)	−2.2‖ (>100)	−1.6‖ (>100)	NT
	2	22.5	25.0 (0)	NT	3.7‖ (83.6)	NT
	3	24.5	14.2 (42.0)	−2.5‖ (>100)	−9.5‖ (>100)	25.0 (0)
	4	19.0	18.4 (3.2)	0‖ (100)	1.1‖ (94.2)	16.3 (14.2)
2R	1	34.0	29.6 (12.4)	−7.4‖ (>100)	−1.6‖ (>100)	NT
	2	31.7	23.8 (24.9)	6.6‖ (79.2)	NT	NT
	4	17.0	7.2 (57.7)	3.1‖ (81.8)	−1.5‖ (>100)	8.7 (48.8)

* 8×10^7 lymphocytes were incubated for 3 h at 30°C successively in two 250-ml plastic bottles, containing monolayer of peritoneal macrophages; the nonadherent cells were harvested, one time washed, counted, and the cytotoxic effect of 8×10^6 lymphocytes was tested.

‡ Cytotoxic effect was assessed by target cell count in Exp. 1 or by ^{51}Cr release in Exp. 2, 3, 4.

§ R107 target cells are used in Exp. 1, 2 and 4, 5R target cells are used in Exp. 3. NT, not tested.

‖ Difference between the cytotoxic effects of lymphocytes absorbed with the stated specific target and with B10.D2 target (or nonabsorbed lymphocytes) is highly significant ($P < 0.01$). In other cases the same difference is not significant ($P > 0.05$).

¶ In parenthesis: absorption index.

Previously, we obtained evidence that ET lymphocytes were incapable either of specific absorption on third-party TC or of destroying such cells, if the latter possessed only part of the immunizing *H-2* antigenic complex (3, 14). Moreover, no CE was produced on third-party TC even after mixing three lymphocyte populations, directed towards different *H-2* specificities, or after mixing TC of different origin, each possessing only some specificities of the immunizing *H-2* complex (14, 19). *H-2K* and *H-2D* specific complexes, localized on the same immunizing cell of A strain, were recognized by receptors of two different populations of C57BL anti-A lymphocytes, which was proved by selective removal of each of the populations by absorption of the lymphocytes with corresponding TC (19). These findings have been corroborated (29–31) and an explanation has been proposed (32) that, in the course of the immune response to *H-2K* or *H-2D* antigenic membrane complexes, the polyspecific precursors of ET cells give rise to the progeny-bearing receptors with complex polyvalent structure which is a mirror reflection of the reciprocal arrangement of *H-2K* or *H-2D* antigenic determinants on the surface of the immunizing cell.

In most of the experiments mentioned, third-party TC shared with the donor

TABLE IV

*Rejection of Skin Grafts of Mice with Different Genotypes by B10.D2 Recipients Preimmunized with B10 Spleen Cells**

Graft donor	Normal or immune recipients	No. of recipients	MST ± SE (days)	*P*
B10	Normal	9	9.7 ± 0.33	<0.001
	Immune	10	6.0 ± 0	
B10.A	Normal	8	9.6 ± 0.42	>0.05
	Immune	8	8.5 ± 0.5	
R107	Normal	8	9.3 ± 0.6	<0.001
	Immune	10	6.7 ±0.3	
R101	Normal	8	12.4 ± 0.56	<0.001
	Immune	10	7.9 ± 0.66	

* 5×10^6 spleen cells were injected intraperitoneally 7 days before skin grafting.

public *H-2* specificities only. In this connection, an alternative explanation of the results is possible, namely: that receptors of ET lymphocytes are not capable of recognizing public *H-2* specificities at all and selectively react to only private *H-2* specificities. In such a case, partial destruction of strain A TC by B10 anti-B10.D2 and C3H anti-B10.D2 lymphocytes (3, 14) could be due to the fact that the donor (B10.D2) TC and those of the third-party (A) strain possess a common private specificity *H-2.4* (D^d) which is lacking in the recipient.

Detailed investigation of this issue in the present study, carried out with recombinant strains of mice, has supported the above suggestion. B10.D2 anti-B10 lymphocytes, which are potentially directed against seven *H-2* specificities—five public ones (*H-2.5, 39, 53, 54, 56*) and two private ones (*H-2.2* and *H-2.33*)—did not react with macrophages of B10.A, B10.M, and DBA/1 strains possessing only public specificities *H-2.5, H-2.39, 53* and *H-254, 56* respectively, but reacted with R101 and R107 TC with their respective private specificities *H-2.2* (D^b) and *H-2.33* (K^b). Occurrence or absence of the reaction was expressed not only in direct TC destruction but also in the ability of TC to absorb the appropriate ET lymphocytes and to remove them from the suspension. It is evident that receptors of ET lymphocytes react only with private *H-2* specificities.

Data of the present work show that immunization with both private specificities (*H-2.2* and *H-2.33*) carried by the same immunizing cell results in induction of the two respective ET populations in unequal proportion: a greater part of the lymphocytes (60 to 80%) is directed towards *H-2K* private specificity, while the smaller one towards *H-2D* private specificity. That these two ET populations do not overlap is demonstrated by experiments on cross-absorption of the lymphocytes on TC of one of the recombinant strains (R107, R101, 2R, and 5R) with sub-

sequent testing of nonadherent lymphocytes upon each of the recombinant strains. Following complete removal of ET cells, which react with TC of R107 and 5R strains (reaction due to *H-2.33* specificity), CE is maintained towards R101 and 2R TC (reaction due to *H-2.2* specificity), and, conversely, removal of lymphocytes reactive with *H-2.2* specificity does not affect CE against R107 TC, bearing *H-2.33* specificity. In addition, if the lymphocytes are absorbed on the macrophages of one of the strains bearing *H-2.2* specificity (R101 or 2R), this results in removal of lymphocytes killing not only the particular strain used for absorption but also the other strain sharing that private specificity. Finally, a mixture of TC, each bearing one of the two immunizing private specificities, absorbs 100% of immune lymphocytes and is completely destroyed by them.

The ability of ET lymphocytes, immune to *H-2K* private specificity, to destroy greater numbers of TC, compared with lymphocytes immune to *H-2D* private specificity, is compatible with our previous data obtained in another system of strains (19) and seems to be due to more numerous ET cells induced as a result of incompatibility in *H-2K*, compared with that in *H-2D*. A number of other observations may be connected with this feature: *H-2K* incompatibility is known to be a more effective stimulator of blastogenesis in vitro (33) and in vivo (34), of humoral lymphotoxin elaboration in MLC (35), of graft-vs.-host reaction (36), and of plaque-forming cell development to SRBC (37), as well as it results in shorter survival time of skin allografts (38), than the incompatbility in *H-2D*. Greater immunogenicity of *H-2K* locus products, than that of *H-2D* locus products, is, possibly, related to the neighborhood of *H-2K* locus to *Ir*-region in IX linkage group (20). If a product of MLC gene of *Ir*-region is located on the cellular membrane close to those of *H-2K* gene, it could enhance indirectly the induction and/or proliferation of ET cells. An alternative view that a part of ET lymphocytes is directed, like RT lymphocytes, against the very products of MLC gene, mapped in *Ir*-region, does not agree with experimental findings (see introduction).

The ability of ET lymphocytes to react only with private *H-2K* and *H-2D* specificities and not with public *H-2* specificities is not merely a laboratory phenomenon: similar regularities are found in vivo in rejection of skin allograft under conditions of an overlap in private or public *H-2* specificities; it is in the first case, only, that the graft is rejected at an accelerated fashion in preimmunized animals (Table IV).

This result is in accordance with data obtained by one of us earlier (39), but at variance with those reported by Klein and Murphy (40). The discrepancy is, probably, due to the fact that mice in the latter study were immunized with a comparatively large antigenic dose, administered as a cell-free extract, and to assessment of the rejection rate by different techniques. A large antigen dose could induce an early elaboration of antibodies which act synergically with immune lymphocytes in the second-set rejection either of a third-party graft (39) or under the conditions of reducing lymphocyte activity (41).

The inability of ET lymphocytes to react with public *H-2* specificities suggests that there may exist structures recognizable by receptors of only B lymphocytes and not by those of T cells. It has been shown earlier that haptens do not activate

T-helper cells (42) and do not react with effectors of cell-mediated immunity (43, 44). It is established by the present work, based on absorption criterion, that a large group of natural cellular membrane determinants—public *H-2* specificities —are not recognized by receptors of ET lymphocytes, in spite of the fact that they are known to be capable of both inducing humoral antibodies and reacting with them readily.

It is not known what restricts the variety of T-cell receptors, compared with those of B cells, and what determines the privilege of being recognized by T cells. However, it is tempting to think that T-cell receptors recognize selectively some specific class of conformational determinants, whereas B-cell receptors can react with configurations of any type, including those structures which are recognized by T-cell receptors.

It is not excluded that a definite steric association of public specificities results in appearance of a conformational determinant which, in fact, constitutes a private specificity, in other words, that private specificities are determined by the primary structure of public specificities. Results of investigations with the use of mutant *H-2* haplotypes are indicative of such a possibility: a mutation in just one or two of the public *H-2* specificities is invariably accompanied by appearance of a new private *H-2* specificity (12).

Data obtained in this study provide evidence in favour of the contention that *H-2* antigens are natural hapten-carrier complexes (10, 11), public specificites being the equivalent of hapten in this complex, while the role of carrier determinants is, possibly, played either by private specificities themselves or some other serologically silent products of *H-2K* and *H-2D* loci, closely linked to the private specificities (45). Therefore, the carrier and hapten determinants could either overlap partially (in the former case) or be sterically spaced (in the latter case) just like it is observed in some other systems (46–48). It does not appear possible at present to chose between the two alternatives.

The suggestion about selective recognition of private SD specificities by ET lymphocyte receptors should be reconciled with the results of reciprocal immunization between B10-derived mutants (Hz1, M505) and the original B10 strain, which indicated development of CML in spite of absence of any SD differences (15, 16, 45). The apparent inconsistency may be explained by a requirement in accessory gene expression to produce antibody against some private specificities of B10 background (49). The latter feature appears to be unique for $H\text{-}2^b$ haplotype, since SD differences were invariably observed between original strains possessing other haplotypes ($H\text{-}2^d$ or $H\text{-}2^f$) and their respective mutants (12, 45). Thus, failure to reveal SD differences does not necessarily mean absence of such differences in private specificities.

If, on the other hand, some serologically silent portion of the molecule, coded by *H-2K* or *H-2D* locus, constitutes the target for ET lymphocytes, a question arises about the relation of this portion to the lymphocyte-defined (LD) determinants coded by MLC loci which map in *Ir* (4) and D (13, 50) regions of MHC.

It can be supposed that these determinants are identical and contribute to the composition of two different types of molecules coded by MHC. One of the

molecule types, carrying SD *H-2* specificities and capable of inhibiting the cytotoxic activity of ET lymphocytes (51) is a product of two genes, of *H-2K* or *H-2D*, on the one hand, and of MLC gene in the K or D regions, on the other hand. Therefore, this type of molecule possessed the property of a hapten-carrier complex. The other type of molecules, coded exclusively by MLC gene, does not bear SD *H-2* specificities and performs only carrier functions. For some reason, for instance, due to a difference in the determinant density on the cellular surface, molecules possessing SD *H-2* specificities present more adequate targets for ET cells, but less readily activate RT lymphocytes, whereas molecules devoid of the serological specificities are more active stimulators of RT lymphocytes but less suitable targets for ET lymphocytes (52).

It follows from the proposed model that RT and ET cell receptors react with identical or very similar products of MHC locus, as distinct from B-cell receptors which react with SD *H-2K* and *H-2D* specificities. Experiments utilizing the above models as a working hypothesis are being conducted at present.

Summary

Receptors of effector T lymphocytes of congeneic strains of mice do not recognize public *H-2* specificities and react to private *H-2* specificities only. This has been established with the use of three tests: direct cytotoxicity assay of immune lymphocytes upon target cells, specific absorption of the lymphocytes on the target cells, and rejection of skin grafts at an accelerated fashion. Immunization with two private *H-2* specificities in the system C57BL/10ScSn → B10.D2 induces formation of two corresponding populations of effector lymphocytes in unequal proportion: a greater part of them is directed against the private specificity *H-2.33* (K^b), while the smaller part is towards *H-2.2* (D^b) private specificity. These two populations of effector lymphocytes do not overlap, as demonstrated by experiments on their cross-absorption on B10.D2 (R107), B10.D2 (R101), B10.A(2R), and B10.A(5R) target cells, as well as on mixtures of R107 and R101 targets. Following removal of lymphocytes reacting with one of the private *H-2* specificities, lymphocytes specific to the other specificity are fully maintained. A mixture of target cells, each bearing one of the two immunizing private specificities, absorbs 100% of the immune lymphocytes and is totally destroyed by them.

It is suggested that *H-2* antigens are natural complexes of hapten-carrier type, in which the role of hapten is played by public *H-2* specifities and that of the carrier determinant by either private *H-2* specificities or structures closely linked to them. Various models of steric arrangement of MHC determinants recognized by receptors of effector T lymphocytes are discussed.

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