Brief Definitive Report

HISTOCOMPATIBILITY ANTIGENS CONTROLLED BY THE *I* REGION OF THE MURINE *H-2* COMPLEX

II. *K/D* Region Compatibility is not Required for *I*-Region Cell-Mediated Lymphocytotoxicity*

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The *I* region of the *H-2* complex was originally defined by its regulatory role in the immune response to synthetic polypeptides (1). Later, this region was demonstrated to control several other immunological traits: strong mixed lymphocyte reaction (2, 3), graft-vs.-host reaction (4), serologically detectable alloantigens with restricted tissue distribution (5, 6), and strong allograft reaction (7). The involvement of the *I* region in allograft reaction was first demonstrated in vivo by skin grafting of *I*-region congenic lines (7). More recently, we have demonstrated that the *I* region contains at least two histocompatibility (*H*) loci: a strong (*H-2A*) locus in the *IA* subregion, and a weak (*H-2C*) locus in the *IC* subregion (8). We have also demonstrated that in vivo primed and in vitro restimulated effector cells lyse labeled target cells in the cell-mediated lympholysis (CML) assay in strain combinations differing at the *H-2A* or *H-2C* loci (8, 9). Investigators in other laboratories have shown that antigens controlled by the *I* region can also serve as targets in a CML assay with effector cells produced in vitro (10, 11). In their capacity to reject skin grafts rapidly and to serve as targets in the CML assay, the H-2A antigens resemble those coded for by the *H-2K* and *H-2D* loci of the *H-2* complex and differ from all minor H antigens controlled by non-*H-2* loci. However, there still remains an important difference between the H-2K and H-2D antigens, on the one hand, and H-2A antigens on the other hand. Several investigators have demonstrated that specific in vitro lysis by effector T cells of virus-infected cells (12, 13), cells chemically modified by certain haptens (14, 15), or allogeneic cells differing at minor *H* loci (16, 17) occurs efficiently only when donors of both the T cells and the target cells share an allele at the *H-2K* or *H-2D* loci; sharing or nonsharing of alleles at the *H-2I* loci appears to be irrelevant for the occurrence of cell lysis in this system.

Because of this reported difference between H-2K/D and H-2A antigens, the question must be asked: do the H-2A antigens themselves require H-2K/D identity in order to function as targets in the CML assay? Experiments reported in this communication were designed to establish an answer to this question.

Materials and Methods

Mice. All mouse strains used were bred at the University of Texas Health Science Center at Dallas. Mice of both sexes and approximately 12-20-wk old were used.

Skin Grafting. Skin from the tail was placed on the dorsum of recipient mice according to the method described elsewhere (18).

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CML Assay. The assay used was described in detail elsewhere (8). The effector cells were obtained from spleens of mice presensitized by skin grafting. These spleen cells were restimulated in vitro by co-culturing them for 5 days with X-irradiated stimulating cells obtained from the same strain that donated the skin grafts. Target cells were blasts stimulated in vitro by concanavalin A (Con A). Each strain combination was tested in three effector:target cell ratios (approximately 50:1, 25:1, and 12:1). The percent release of ^{51}Cr from the labeled target cells was calculated according to the formula:

$$\frac{(\text{cpm supernate} - \text{background}) \times 2}{(\text{cpm supernate} + \text{pellet}) - 2 \times \text{background}} \times 100.$$

Results and Discussion

Two sets of experiments were performed to test whether H-2A antigens require H-2K/D identity to function as CML targets. In both experiments, congenic lines were used that differ in the central portion of the *H-2* complex (including the *I* region) but presumably are identical in the remainder of the genome. The two pairs of congenic lines were A.TH – A.TL and B10.T(6R) – B10.AQR. In the former combination, A.TL skin grafts were transplanted on A.TH mice, and at the time when the grafts were being rejected (between 10 and 14 days after transplantation), the recipients were sacrificed and their spleens were used as the source of effector cells in the CML assay. Upon in vitro restimulation with irradiated A.TL lymphocytes, the effector cells were tested, on the 5th day of culture, against ^{51}Cr-labeled Con A-blasts derived from A.TL lymphocytes. Specific lysis, comparable in its extent to that observed with strain combinations differing at the *H-2K* or *H-2D* loci, was observed (Table I). Genetic tests reported by us previously demonstrated that this lysis is mediated by *I*-region-controlled antigens (8). To determine whether or not *H-2K*- or *H-2D*- region compatibility was required for this lysis to occur, the A.TH effector cells sensitized to A.TL stimulating cells were tested against ^{51}Cr-labeled C3H/HeJ targets. In the A.TH anti-A.TL combination, sensitization occurs against I^k antigens, which are also carried by the C3H/HeJ strain. However, in the A.TL strain, I^k occurs in combination with $H\text{-}2K^s$ and $H\text{-}2D^d$ (the latter two alleles are also present in the A.TH strain), whereas in C3H/HeJ, it occurs in combination with $H\text{-}2K^k$ and $H\text{-}2D^k$. If, therefore, sharing of *K* and *D* alleles were not required for *I*-region CML to occur, the C3H/HeJ cells should also be lysed by the A.TH anti-A.TL effector cells. Table I shows that they indeed are. The effector cells are apparently sensitized to antigens controlled by the *IA* subregion (in which the *H-2A* locus resides), since B10.A(4R) cells, which share only the *IA* subregion with A.TL, were also lysed. As expected, the A.TH anti-A.TL effector cells also lysed A/J cells which share with A.TL not only the IA^k but also the $H\text{-}2D^d$ allele. The effector cells did not lyse B10.P target cells which differ from A.TL ($H\text{-}2^{t1}$) in the entire *H-2* haplotype (the B10.P strain is $H\text{-}2^p$).

In the second combination, B10.T(6R) mice were sensitized by grafting with B10.AQR skin, and their spleen cells were rechallenged in the CML assay with irradiated B10.AQR lymphocytes. The B10.T(6R) anti-B10.AQR effector cells lysed not only B10.AQR but also B10.BR and B10.BYR cells (Table II), again indicating that *K* or *D* region compatibility was not required for CML lysis mediated by *I*-region antigens. As expected, the effector cells also lysed B10.A

TABLE I
Cell-Mediated Lymphocytotoxicity of A.TH Effector Cells Obtained from Mice Presensitized to A.TL by Skin Grafting. Effector:Target Cell Ratio 50:1

Responder	Stimulator	Target	H-2 region composition of target*						Mean release of ^{51}Cr ± SE	Mean net release of ^{51}Cr
			K	*A*	*C*	*S*	*G*	*D*		
									%	%
A.TL	A.TH	A.TL	*s*	*k*	*k*	*k*	*k*	*d*	30.3 ± 2.4	–
A.TH	A.TL	A.TL	*s*	[*k*	*k*	*k*	*k*]	*d*	52.6 ± 3.8	22.3
C3H/HeJ	A.TL	C3H/HeJ	*k*	*k*	*k*	*k*	*k*	*k*	32.1 ± 1.8	–
A.TH	A.TL	C3H/HeJ	*k*	[*k*	*k*	*k*	*k*]	*k*	51.8 ± 3.5	19.7
B10.A(4R)	A.TL	B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	30.9 ± 1.9	–
A.TH	A.TL	B10.A(4R)	*k*	[*k*]	*b*	*b*	*b*	*b*	49.8 ± 2.5	18.9
A/J	A.TL	A/J	*k*	*k*	*d*	*d*	*d*	*d*	33.4 ± 2.1	–
A.TH	A.TL	A/J	*k*	[*k*]	*d*	*d*	*d*	*d*	54.1 ± 3.7	17.8
B10.P	A.TL	B10.P	*p*	*p*	*p*	*p*	*p*	*p*	38.4 ± 4.5	–
A.TH	A.TL	B10.P	*p*	*p*	*p*	*p*	*p*	*p*	40.1 ± 2.9	1.7

* Regions to which effector cells can react are boxed.

TABLE II
Cell-Mediated Lymphocytotoxicity of B10.T(6R) Effector Cells Obtained from Mice Presensitized to B10.AQR by Skin Grafting. Effector:Target Cell Ratio 50:1

Responder	Stimulator	Target	H-2 region composition of target*						Mean release of ^{51}Cr ± SE	Mean net release of ^{51}Cr
			K	*A*	*C*	*S*	*G*	*D*		
									%	%
B10.AQR	B10.T(6R)	B10.AQR	*q*	*k*	*d*	*d*	*d*	*d*	31.5 ± 3.2	–
B10.T(6R)	B10.AQR	B10.AQR	*q*	[*k*	*d*	*d*	*d*]	*d*	43.9 ± 3.8	12.4
B10.BR	B10.AQR	B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	36.7 ± 3.5	–
B10.T(6R)	B10.AQR	B10.BR	*k*	[*k*]	*k*	*k*	*k*	*k*	46.4 ± 3.4	9.7
B10.A	B10.AQR	B10.A	*k*	*k*	*d*	*d*	*d*	*d*	33.2 ± 2.7	–
B10.T(6R)	B10.AQR	B10.A	*k*	[*k*	*d*	*d*	*d*]	*d*	44.5 ± 3.5	11.3
B10.BYR	B10.AQR	B10.BYR	*q*	*k*	*d*	*d*	*d*	*b*	42.1 ± 3.9	–
B10.T(6R)	B10.AQR	B10.BYR	*q*	[*k*	*d*	*d*	*d*]	*b*	51.4 ± 4.1	9.3
B10.M	B10.AQR	B10.M	*f*	*f*	*f*	*f*	*f*	*f*	33.6 ± 2.5	–
B10.T(6R)	B10.AQR	B10.M	*f*	*f*	*f*	*f*	*f*	*f*	35.2 ± 3.1	1.6

* Regions to which effector cells can react are boxed.

cells, but did not lyse B10.M cells. Although the B10.T(6R) anti-B10.AQR cells were relatively inefficient effectors in most tests, the results shown in Table II were reproducible in repeated experiments. Table I and II show data from individual experiments; similar results were obtained in four repeats of each experiment.

The data shown in Tables I and II demonstrate that *K/D*-region compatibility is not needed for *I*-region CML to occur. In this respect, the H-2A antigens behave differently from the minor H antigens, a conclusion which is consistent with the rapid rejection of skin grafts across the H-2A barrier (8). Nevertheless, one functional difference between *H-2K/D* and *H-2A* loci still remains: so far, nobody has been able to demonstrate that H-2A antigens, like the H-2K or H-2D antigens, are able to help virus antigens, haptens, or minor H antigens

to mediate CML. However, it is worthwhile to recall that originally *I*-region antigens had been claimed not to be capable of mediating CML at all (19) and only a closer look and improved techniques demonstrated incorrectness of this claim. Perhaps, the CML-helper capacity of *I*-region antigens also deserves a reinvestigation.

Summary

In the cell-mediated lymphocytotoxicity assay, A.TH effector cells sensitized to A.TL lymphocytes lyse not only A.TL but also C3H/HeJ and B10.A(4R) targets. Similarly, B10.T(6R) anti-B10.AQR effector cells lyse B10.BR and B10.BYR target cells in addition to B10.AQR cells. These findings indicate that for CML to occur across the *IA* region barrier, compatibility at *K* or *D* regions is not required.

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