

ABSENCE OF H-2 RESTRICTION IN PRIMARY AND SECONDARY MIXED-LYMPHOCYTE REACTIONS TO STRONG *Mls* DETERMINANTS*

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Most T-cell responses are restricted by products of the major histocompatibility complex (MHC),¹ i.e., T cells do not recognize antigen per se but an association of antigen with MHC determinants (1, 2). The bulk of evidence suggests that the capacity of T cells to react to antigen presented in association with a particular set of MHC determinants depends upon the T cells encountering these determinants during early differentiation, particularly in the thymus (2). Despite intensive speculation, the key question of precisely how T cells recognize an association of antigen plus MHC determinants remains unresolved. In approaching this question it is of obvious importance to know whether all T-cell responses are H-2 restricted. In this respect, it is generally agreed that responses to MHC determinants per se, and to certain cell-surface antigens encoded by genes closely linked to the MHC, do not exhibit MHC restriction (3-6). These findings appear to be the only unequivocal exceptions to the rule of MHC restriction. Hence, it is tempting to conclude that MHC restriction is an integral feature of T-cell responses to all antigens other than MHC or MHC-linked determinants.

This paper contradicts this generalization by showing that mixed lymphocyte reactions (MLR) to certain allelic products of the *Mls* locus fail to show H-2 restriction. The *Mls* locus is not linked to the *H-2* complex and has at least four alleles: *a*, *b*, *c*, and *d* (7-9). Of these, the products of the *Mls*^{*a,d*} alleles are strongly stimulatory and lead to high proliferative responses in primary MLR, both in vitro (7) and in vivo (10). The *Mls*^{*b,c*} determinants are weakly stimulatory and give appreciable responses only in secondary MLR. In a recent study by Peck et al. (11), it was concluded that secondary responses to *Mls*^{*c*} determinants are H-2 restricted. By contrast, we report here that responses to the strong *Mls*^{*a,d*} determinants do not exhibit H-2 restriction, either in primary or secondary MLR.

Materials and Methods

Mice. C3H/HeJ (*H-2*^{*k*}, *Mls*^{*c*}), AKR/J (*H-2*^{*k*}, *Mls*^{*a*}), CBA/J (*H-2*^{*k*}, *Mls*^{*d*}), BALB/c (*H-2*^{*d*}, *Mls*^{*b*}), DBA/2 (*H-2*^{*d*}, *Mls*^{*a*}), C57BL/6J (*H-2*^{*b*}, *Mls*^{*b*}), and SJL (*H-2*^{*s*}, *Mls*^{*c*}) were purchased from The Jackson Laboratory, Bar Harbor, Maine. AKR/Cum (*H-2*^{*k*}, *Mls*^{*a*})² were

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¹ *Abbreviations used in this paper:* LN, lymph node(s); MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction(s); TDL, thoracic duct lymphocytes.

² Although there is no genetic evidence that AKR/Cum mice possess the *Mls*^{*a*} allele, we have tentatively assigned this allele because (a) AKR/Cum mice were originally derived from the AKR/J (*Mls*^{*a*}) strain, and (b) AKR/Cum spleen cells do not stimulate (C3H/HeJ × DBA/2)F₁ (*Mls*^{*c*} × *Mls*^{*a*}) LN in primary MLR (K. Molnar-Kimber. Unpublished data.)

obtained from Cumberland View Farms, Clinton, Tenn. D1.C ($H-2^d$, $M1s^a$) and D1.LP ($H-2^b$, $M1s^a$) (originally obtained from The Jackson Laboratory), and CBA/CaHn ($H-2^k$, $M1s^b$) (originally obtained from the National Institutes of Health, Bethesda, Md.) as well as all F_1 hybrids were bred in our own colony.

Preparation of Cells. Suspensions of spleen, lymph nodes (LN), and thoracic duct lymphocytes (TDL) were prepared as described elsewhere (12) in cold medium lacking fetal calf serum. LN cell suspensions were prepared either from the mesenteric nodes or from pooled mesenteric, cervical, inguinal, and axillary nodes. Cell viability, as measured by trypan blue dye exclusion, was usually 80–95%.

Irradiation. All mice and cells were exposed to ^{137}Cs γ -irradiation at a dose rate of ≈ 100 rad/min.

Negative Selection In Vivo. LN cells were depleted of specific alloreactive lymphocytes by acute blood to lymph recirculation through irradiated allogeneic mice as described elsewhere (13). In brief, 2×10^8 LN cells were injected intravenously into lethally irradiated (850 rad) allogeneic recipients. The mice were cannulated 16 h later and TDL were collected from 20 to 40 h postinjection. With H-2-incompatible combinations, the use of appropriate alloantisera and complement (13) showed that 85–95% of the lymph-borne cells were T (Thy-1.2-positive) lymphocytes of donor origin.

Positive Selection In Vitro. 2×10^7 LN cells were cultured with 2×10^7 irradiated (1,000 rad) allogeneic stimulator cells in upright 50-ml flasks (No. 25100, Corning Glassworks, Science Products Div., Corning, N. Y.) in EHAA (Eagle's Hanks' Amino Acid) culture medium (Altec Associates, Hudson, Wis.) supplemented with 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 2 mM glutamine, 4×10^{-5} M 2-mercaptoethanol, 70.4 μg sodium bicarbonate/ml and 0.5% fresh mouse serum (obtained from the responder strain). The cultures were incubated at 37°C in a humidified atmosphere of 7.5% CO_2 in air. These bulk cultures were harvested on day 13 or 14. Approximately 10–15% of the responding population was recovered; the use of appropriate alloantisera and complement showed that 90% of the surviving cells were Thy-1.2 positive.

Positive Selection In Vivo. As described in detail elsewhere (10), $2\text{--}4 \times 10^6$ LN cells were injected intravenously into lethally irradiated (800 rad) allogeneic mice and then recovered from thoracic duct lymph of the recipients 4–5 d later; >90% of the lymph-borne cells were T-blast cells.

Primary MLR. LN cells or TDL at doses of 2×10^5 were cultured with 2.5×10^5 irradiated (1,000 rad) spleen cells as stimulator cells in a total vol of 0.2 ml RPMI-1640 culture medium (New England Nuclear, Boston, Mass.) supplemented with 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 2 mM glutamine, 4×10^{-5} M 2-mercaptoethanol, and 2.5% normal rat serum. Cultures were set up in triplicate in round-bottom microtiter plates (75-013-05; Linbro Chemical Co., Hamden, Conn.). The cultures were incubated at 37°C in a humidified atmosphere of 7.5% CO_2 in air. On the appropriate day, each well was pulsed with 0.25 μCi [^3H]Tdr (6.7 Ci/mmol) (New England Nuclear). The plates were harvested onto glass filters 8 h later with a Skatron multicell harvester and counted with 1 ml of Econofluor (New England Nuclear, Pilot Chemical Div., Watertown, Mass.) in an Intertechnique SL 30 liquid scintillation spectrometer.

Secondary MLR. Two-fold serial dilutions of 5×10^4 primed lymphocytes were cultured in triplicate with 2.5×10^5 stimulator cells in round-bottom microtiter plates in 0.2 ml supplemented RPMI or EHAA medium; as serum sources in the medium, normal mouse serum (0.5%) was added for cells selected in vitro and normal rat serum (2.5%) was used for the in vivo selected cells. Cultures were harvested as indicated in the figure legends. Peak responses usually occurred on days 2–3 for in vitro primed cells and on days 1–2 for in vivo primed cells.

Results

Kinetics of the Primary MLR to M1s Determinants. Fig. 1 compares the proliferative responses of a constant dose of normal responder cells (2.5×10^5) against M1s determinants (Fig. 1A and B) and H-2 determinants (Fig. 1C and D); cells were cultured in 2.5% rat serum (Materials and Methods).

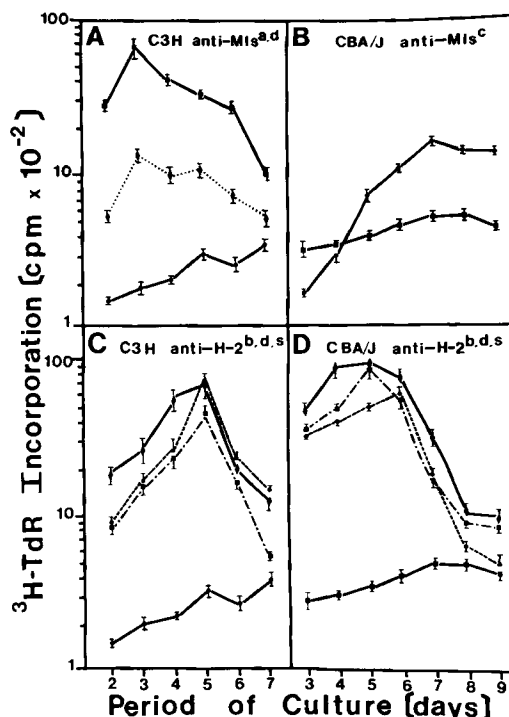


FIG. 1. Comparison of proliferative responses of normal TDL to MIs differences vs. H-2 differences. (A) Response of C3H/HeJ against H-2-compatible AKR/Cum (MIs^a) (◆---◆), CBA/J (MIs^d) (■—■), and syngeneic C3H/HeJ (●—●). (B) Response of CBA/J against H-2-compatible C3H/HeJ (MIs^c) (●—●) and syngeneic CBA/J (■—■). (C) Response of C3H/HeJ ($H-2^k$, MIs^c) against H-2-different BALB/c ($H-2^d$, MIs^b) (■---■), C57BL/6 ($H-2^b$, MIs^b) (◆—◆), SJL ($H-2^s$, MIs^c) (●---●), and syngeneic C3H/HeJ (●—●). (D) As in (C) except that the responder cells were CBA/J ($H-2^k$, MIs^d); (■—■), response to syngeneic CBA/J cells. Cell populations used as stimulators were all from F₁ hybrid mice, i.e., were hemizygous with the responder cells.

The response of C3H/HeJ ($H-2^k$, MIs^c) (k,c) LN cells against strong MIs^a (AKR/Cum)² and MIs^d (CBA/J) determinants is shown in Fig. 1A. With these two H-2-compatible combinations the responses were high (10- to 40-fold above background) and usually reached a peak on days 3-4; similar kinetics were observed with BALB/c (d,b) cells responding against DBA/2 (d,a) cells (data not shown). Responses to H-2 determinants were similar in magnitude to anti-MIs responses but reached a maximum at a later stage, i.e., on days 5-6. This is apparent in Fig. 1C, which shows responses of C3H/HeJ (k,c) cells against SJL (s,c), C57BL/6 (b,b) and BALB/c (d,b) stimulators. (The MIs^b allele of C57BL/6 and BALB/c is virtually nonstimulatory [see below].) Although the precise reason for the difference in the kinetics of the response to MIs vs. H-2 incompatibilities was not clear, studies on the effects of titrating the number of responding cells suggested that differences in precursor frequency for the two sets of determinants was probably a contributing factor. Thus, in the case of responses to MIs^d determinants (C3H/HeJ anti-CBA/J), reducing the number of responding cells usually shifted the peak response to a later stage (days 4-5 (data not shown)).

TABLE I

Specificity of H-2^k T Cells Negatively Selected Against H-2^d Determinants: Primary MLR Against M1s^a Determinants Presented on H-2^d Stimulator Cells

Responder cells*	Stimulator cells					
	C3H/HeJ (<i>k,c</i>)‡	CBA/J (<i>k,d</i>)	BALB/c (<i>d,b</i>)	D1.C (<i>d,a</i>)	DBA/2 (<i>d,a</i>)	SJL (<i>s,c</i>)
C3H/HeJ _{-C3H/HeJ} TDL	1.6§ (0.3)	29.8 (3.4)	116.2 (26.4)	249.8 (33.6)	180.6 (31.0)	78.6 (6.8)
C3H/HeJ _{-BALB/c} TDL	1.4 (0.5)	24.9 (8.4)	0.4 (0.1)	120.1 (18.6)	28.0 (3.3)	55.0 (16.2)
BALB/c Spleen	ND	ND	6.5 (0.7)	43.1 (4.0)	26.8 (2.4)	44.7 (2.5)

* Normal C3H/HeJ LN cells were transferred intravenously into irradiated C3H/HeJ and BALB/c mice and recovered from TDL of the recipients 1 d later (Materials and Methods).

‡ H-2 haplotype, M1s allele.

§ Incorporation of [³H]TdR (cpm × 10⁻²) in primary MLR measured on day 4. Mean of triplicate wells; standard deviation shown in parentheses.

|| Not determined.

Responses to weak M1s^c determinants (CBA/J [*k,d*] anti-C3H/HeJ [*k,c*]) are shown in Fig. 1 B. Responses were low (two- to fivefold above background) and reached peak responses only after day 6. The same responder cells reacted well against H-2 differences (Fig. 1 D). Responses to cells carrying the nonstimulatory M1s^b determinants, e.g., with DBA/2 (*d,a*) against BALB/c (*d,b*), were usually not detectable (data not shown).

To minimize back-stimulation, F₁ hybrid cells were used as stimulators in the above experiments. In practice, homozygous and F₁ hybrid cells gave overlapping results, and, for convenience, parental strain stimulators were used in all subsequent experiments. The data shown in the tables below were obtained on day 4 of culture; data from days 3 and 5 gave similar results. In each table the data are representative of three or more experiments.

Recognition of Strong M1s Determinants Presented on H-2-different Stimulator Cells in Primary MLR. To determine whether responses to M1s determinants were H-2 restricted, the first approach was to establish whether T cells could recognize M1s determinants presented on H-2-incompatible stimulators in primary MLR. For this approach it was necessary to use T cells that had been rendered unresponsive to the H-2 determinants on the stimulator cells. The negative selection procedure used here was to passage LN cells from blood to lymph through irradiated allogeneic mice of the required H-2 haplotype (Materials and Methods); LN cells passaged through irradiated syngeneic mice were used as a control.

The response of C3H/HeJ cells negatively selected to H-2^d determinants in irradiated BALB/c (*d,b*) mice (C3H/HeJ_{-BALB/c} TDL) is shown in Table I. These cells gave no response to BALB/c in MLR but, significantly, gave good responses towards D1.C (*d,a*) and DBA/2 (*d,a*), i.e., two strains of the same H-2 haplotype as BALB/c but with a different M1s allele foreign to the responding cells. These responses were less than those obtained with the syngeneic-passaged C3H/HeJ_{-C3H/HeJ} cells (presumably reflecting the removal of the H-2^d-reactive cells) but were clearly no lower than the responses given by BALB/c cells, i.e., cells that were H-2-compatible with the stimulator cells.

TABLE II

Specificity of $H-2^d$ T Cells Negatively Selected Against $H-2^k$ Determinants: Primary MLR Against $M1s^a$ and $M1s^d$ Determinants Presented on $H-2^k$ Stimulator Cells

Responder cells*		Stimulator cells					
		BALB/c (d,b)‡	D1.C (d,a)	CBA/CaHn (k,b)	AKR/Cum (k,a)	CBA/J (k,d)	SJL (s,c)
BALB/c-BALB/c	TDL	1.3§ (0.1)	180.7 (15.6)	96.1 (25.1)	56.0 (4.4)	114.8 (8.5)	70.2 (3.3)
BALB/c-CBA/CaHn	TDL	0.6 (0.1)	119.2 (20.0)	1.0 (0.1)	8.4 (3.7)	33.0 (4.1)	41.6 (3.4)
CBA/CaHn	LN	ND	ND	3.4 (0.6)	13.8 (1.5)	27.0 (1.5)	126.8 (13.7)

* Analogous to Table I, i.e., BALB/c LN cells were recirculated through irradiated BALB/c or CBA/CaHn mice.

‡ $H-2$ haplotype, $M1s$ allele.

§ See Table I.

|| See Table I.

TABLE III

Specificity of $H-2^k$ T Cells Negatively Selected Against $M1s^a$ Determinants in Irradiated ($H-2^d \times H-2^k$) F_1 Mice: Unresponsiveness to $M1s^a$ Determinants Presented Either on $H-2$ -Compatible or $H-2$ -Incompatible Stimulator Cells

Responder cells*		Stimulator cells						
		C3H/HeJ (k,c)‡	AKR/Cum (k,a)	CBA/J (k,d)	BALB/c (d,b)	D1.C (d,a)	DBA/2 (d,a)	SJL (s,c)
C3H/HeJ	LN	2.6§ (0.3)	18.7 (2.2)	33.7 (5.2)	37.8 (0.8)	51.1 (3.6)	25.7 (2.5)	27.7 (5.5)
C3H/HeJ-BALB/c	TDL	0.7 (0.2)	8.1 (0.4)	24.9 (3.3)	0.2 (0.2)	12.7 (3.4)	22.7 (4.8)	27.7 (5.1)
C3H/HeJ-(BALB/c \times AKR/J) F_1	TDL	1.0 (0.1)	0.7 (0.3)	0.5 (0.1)	0.3 (0.1)	1.1 (0.3)	0.6 (0.2)	26.8 (4.3)

* Analogous to Table I, i.e., C3H/HeJ LN cells were recirculated through irradiated BALB/c or (BALB/c \times AKR/J) F_1 mice.

‡ $H-2$ haplotype, $M1s$ allele.

§ See Table I.

Table II illustrates an analogous experiment in which BALB/c cells were negatively selected to $H-2^k$ determinants in irradiated CBA/CaHn (k,b) mice. These BALB/c-CBA/CaHn TDL were unresponsive to CBA/CaHn but gave definite responses to two $M1s$ -different strains of the $H-2^k$ haplotype, i.e., AKR/Cum ($M1s^a$) and CBA/J ($M1s^d$). The latter responses were comparable (Δ cpm) or higher (stimulation indices) than the responses given by $H-2^k$ cells (CBA/CaHn LN cells).

Negative Selection to Strong $M1s$ Determinants. The data in Table III demonstrate that recirculation of C3H/HeJ LN cells through $M1s$ -incompatible irradiated mice induced negative selection to the $M1s^a$ determinants of the host. In this experiment, to determine whether selection affected the response to both $H-2$ -compatible and $H-2$ -different $M1s^a$ -bearing stimulators in MLR, irradiated (BALB/c \times AKR/J) F_1 ($d,b \times k,a$) mice were used for selection, i.e., mice expressing both an $H-2$ ($H-2^d$) difference and an $M1s$ ($M1s^a$) difference with respect to the donor T cells. It can be seen that, in contrast to the control C3H/HeJ-BALB/c TDL, C3H/HeJ-(BALB/c \times AKR/J) F_1 TDL

TABLE IV

*Specificity of H-2^k T cells Negatively Selected Against M1s^a Determinants In Irradiated H-2^d Mice:
Failure to Respond to M1s^a Determinants Presented on H-2-Compatible Stimulator Cells*

Responder cells*		Stimulator cells							
		C3H/HeJ (<i>k,c</i>)‡	AKR/Cum (<i>k,a</i>)	ARK/J (<i>k,a</i>)	CBA/J (<i>k,d</i>)	BALB/c (<i>d,b</i>)	D1.C (<i>d,a</i>)	DBA/2 (<i>d,a</i>)	SJL (<i>s,c</i>)
C3H/HeJ _{-C3H/HeJ}	TDL	3.6§ (0.4)	57.8 (7.8)	89.6 (15.7)	53.1 (5.4)	383.4 (47.6)	295.8 (17.3)	149.3 (19.9)	283.3 (28.4)
C3H/HeJ _{-BALB/c}	TDL	1.5 (0.1)	15.8 (3.8)	17.2 (2.1)	10.6 (4.0)	1.1 (0.2)	49.0 (3.5)	14.8 (1.1)	65.2 (8.2)
C3H/HeJ _{-DBA/2}	TDL	1.0 (0.2)	2.7 (0.1)	2.8 (0.7)	1.6 (0.4)	1.3 (0.2)	0.9 (0.2)	1.2 (0.5)	69.4 (6.2)
BALB/c	LN	ND	ND	ND	ND	1.3 (0.6)	50.0 (5.8)	7.5 (1.7)	129.6 (17.3)

* Analogous to Table I, i.e., C3H/HeJ LN cells were recirculated through irradiated C3H/HeJ, BALB/c, and DBA/2 mice. LN cells were passed through nylon wool columns before transfer.

‡ H-2 haplotype, M1s allele.

§ See Table I.

|| See Table I.

were totally unresponsive to M1s^a-bearing stimulator cells irrespective of whether the stimulators were H-2 compatible (AKR/Cum) or H-2 incompatible (D1.C and DBA/2) with the responders. Good responses were observed against third-party H-2-different SJL stimulators. (It should be noted that unresponsiveness was also observed towards CBA/J stimulators, i.e., cells expressing M1s^d determinants. In this respect, for reasons mentioned in the Discussion, we believe that M1s^{a,d} determinants are either identical or highly cross-reactive.)

The simplest interpretation of the data in the preceding three tables is that T-cell responses to M1s determinants are H-2 unrestricted. However, it could also be argued that T cells that recognize M1s determinants on H-2-compatible and H-2-different stimulators are distinct subpopulations restricted to self H-2 and allo-H-2 determinants, respectively. According to this latter viewpoint, negative selection of T cells to M1s determinants presented in totally H-2-different irradiated mice (rather than the H-2-hemizygous hosts used in Table III) should retain the capacity to respond to these same M1s determinants presented on H-2-compatible cells. To test this prediction, C3H/HeJ (*k,c*) LN cells were negatively selected to M1s^a determinants in H-2-different irradiated DBA/2 (*d,a*) mice; to minimize the possibility that the few macrophages in the donor LN inoculum could process the host M1s determinants (i.e., present the determinants in association with donor H-2 determinants), the donor cells were nylon-wool-purified before injection (14).

As shown in Table IV, C3H/HeJ_{-DBA/2} T cells were totally unresponsive to H-2-different M1s^a-bearing cells (DBA/2 and D1.C, both H-2^d). In addition the selected T cells gave only minimal responses to cells from two H-2-compatible M1s^a-bearing strains, i.e., AKR/Cum and ARK/J (note that there was also a reduction in the response to H-2-compatible CBA/J [*M1s^d*]). These responses were reduced by ≈90% compared with the responses given by the control cells filtered through BALB/c mice, i.e., mice expressing the H-2^d determinants of DBA/2 but lacking M1s^a determinants; responses to third-party SJL cells were unchanged. Analogous experiments in which BALB/c cells were negatively selected to M1s^a determinants in H-2-different D1.LP

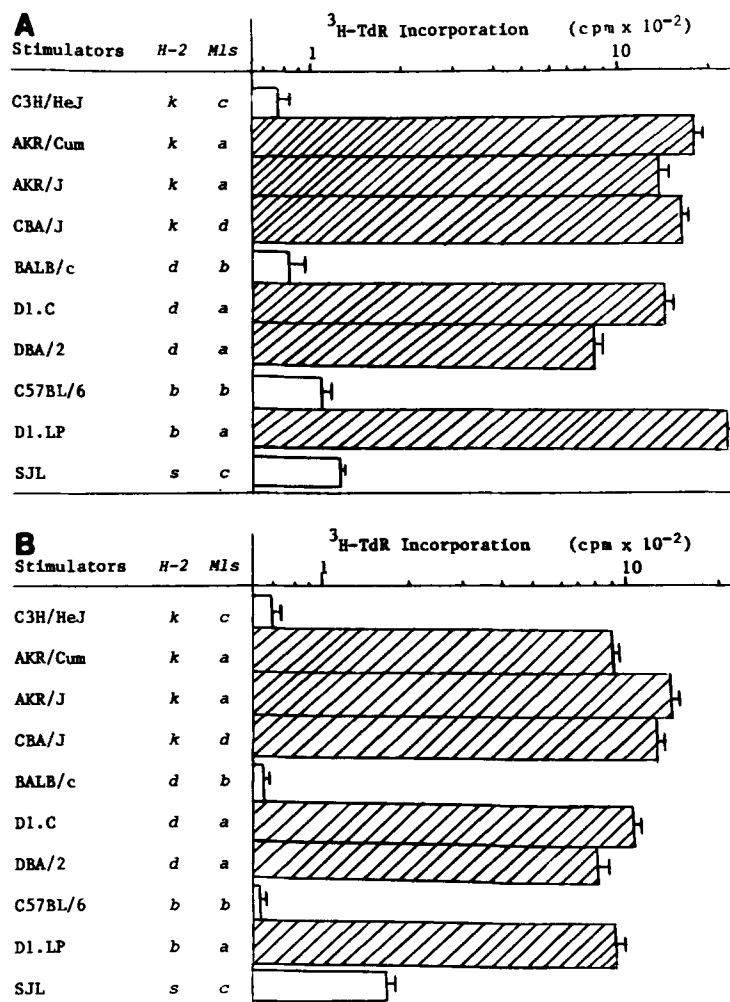


FIG. 2. Secondary MLR of C3H/HeJ LN cells primed to H-2-compatible AKR/Cum (MIs^a) in vitro (A) or in vivo (B). Response of 2.5×10^4 responder cells measured on day 2 and day 1, respectively.

(*b,a*) mice gave a similar 90–95% reduction in the response to MIs^a determinants on H-2-compatible DBA/2 and D1.C cells (data not shown). (Note that in Table IV the apparent difference in the response of C3H/HeJ_{BALB/c} and C3H/HeJ_{C3H/HeJ} T cells to AKR/Cum and AKR/J cells is largely a result of differences in the background counts found with syngeneic cells. No such differences were observed in other experiments, e.g., Table I.)

Collectively, the data on the results of negative selection shown in Tables I–IV thus strongly suggest that the response to MIs^a determinants is not H-2 restricted. To strengthen this conclusion the effects of positive selection were investigated.

Positive Selection to Strong MIs Determinants. Two approaches showed that T cells positively selected to MIs^a determinants in primary MLR failed to exhibit H-2 restriction upon restimulation in secondary MLR. Responses were assayed on days 1–3 of secondary culture.

Selection In Vitro. Normal unprimed C3H/HeJ (k,c) LN cells were stimulated in bulk cultures in vitro with H-2-compatible, M1s-different AKR/Cum (k,a) spleen cells. After 2 wk the surviving cells were restimulated in secondary MLR with a variety of cell types. As shown in Fig. 2A, high responses were obtained with stimulators bearing the priming M1s^a determinants (or M1s^d determinants); this applied irrespective of whether the stimulators were H-2 compatible (AKR/Cum and AKR/J) or H-2 incompatible (D1.C, DBA/2, and D1.LP). Responses to third-party H-2 differences (BALB/c, C57BL/6, and SJL) were low or absent.

Selection In Vivo. Essentially similar data were obtained when C3H/HeJ LN were positively selected to M1s^a determinants in irradiated AKR/Cum mice for 5 d, recovered from TDL of the recipients, and then restimulated in vitro (Fig. 2B). In this experiment the T cells used for positive selection were first negatively selected through irradiated (BALB/c × B6)F₁ mice. This was necessary because, in contrast to primary stimulation in vitro, C3H/HeJ anti-AKR/Cum T cells generated in vivo gave appreciable secondary responses to both BALB/c and C57BL/6 cells, i.e., H-2-different cells lacking the primary M1s^a determinants (data not shown).

Discussion

The procedure of depleting T cells of alloreactivity by acute negative selection in vivo is a useful technique for determining whether normal (nonchimeric) T cells can recognize antigen presented by H-2-different target cells. With this procedure, T cells in some situations show no reactivity to antigen presented on H-2-different cells (13, 15–17); such findings imply that the responding T cells are restricted to interact with antigen in association with self MHC determinants. In certain other situations, however, T cells do interact with H-2-different targets (18, 19). In interpreting these latter findings, it is imperative to determine whether the T cells are truly H-2 unrestricted, i.e., are specific for the antigen per se, or, alternatively, consist of a mixture of cells restricted to self H-2 or allo-H-2 determinants. To date, the available evidence supports the second possibility, i.e., T cells able to recognize antigen on H-2-different targets are held to be a separate subgroup restricted to allo-H-2 determinants (18, 19).

The latter viewpoint cannot account for our observation that T cells recognize M1s^a determinants across H-2 barriers. The key findings here were that (a) negative selection of T cells to M1s^a determinants in totally H-2-different irradiated mice removed 90–95% of reactivity to these determinants presented on H-2-compatible stimulators (Table IV), and (b) T cells primed to M1s^a determinants on H-2-compatible cells gave high secondary responses to these determinants on H-2-different cells (Fig. 2). Such findings lead to the conclusion that T cells are specific for M1s^a determinants per se rather than an association of M1s^a plus H-2 determinants.³ The one reservation to this conclusion is that negative selection to M1s^a determinants in H-2-different mice did leave a small (5–10% of normal) response to H-2-compatible M1s^a-bearing cells. This might signify that a minor component of the anti-M1s^a response does exhibit H-2 restriction. A more likely explanation, however, is that the response was directed not to M1s determinants but to the various other antigens

³ It should be mentioned that the data do not exclude the rather unlikely possibility that M1s determinants are recognized in association with public H-2 determinants common to H-2^k, H-2^d, and H-2^b haplotypes.

separating the two strains, e.g., minor histocompatibility determinants. Without the availability of M1s-congenic strains, it is difficult to assess these possibilities.

The data on the specificity of M1s^a-primed T cells (Fig. 2) contrast with the findings of Peck et al. (11) that secondary responses to M1s^c determinants do show H-2 restriction. Because both the amplitude and the kinetics of responses to M1s^a and M1s^c determinants are quite different (Fig. 1 and [10]), it is perhaps not surprising that there are also differences in terms of H-2 restriction. Indeed it is possible that M1s^a and M1s^c are not true alleles. In this respect, other data of Peck et al. ([20] and [A. Peck. Personal communication.]) suggest that the M1s locus may in fact be a complex of genes comprising a number of subloci. To date, we have performed only two experiments on the effects of priming T cells to M1s^c determinants and find that these responses are apparently unrestricted. These data, however, are only preliminary and need to be extended.

Establishing whether responses to M1s^d determinants are H-2 restricted is beset by the problem that the M1s^d allele is described in only two strains (CBA/J and RF/J), both of which have the same H-2 (*H-2^k*) haplotype. However, from a variety of evidence we are of the opinion that the products of the M1s^d and M1s^a alleles are either identical or highly cross-reactive (K. Molnar-Kimber and J. Sprent. Manuscript in preparation.); other workers have reached a similar conclusion (21). In particular, negative selection against M1s^a determinants removes $\geq 90\%$ of reactivity against M1s^d-bearing cells (Tables III and IV) and vice versa. Likewise, T cells primed against M1s^a determinants give strong secondary responses against M1s^d cells (Fig. 2). This marked cross-reactivity is also apparent across H-2 barriers, i.e., BALB/c (*d,b*) T cells primed to M1s^a on H-2-compatible (DBA/2 [*d,a*]) stimulators give high secondary responses to M1s^d on H-2-different CBA/J (*k,d*) cells (K. Molnar-Kimber and J. Sprent. Unpublished data.). Similarly, Peck et al. (20) have found that B10.BR (*k,b*) T cells primed against CBA/J cells gave high responses to DBA/2 stimulators. Hence, at least for the determinants which cross-react with M1s^a, M1s^d determinants appear to be recognized in an H-2-unrestricted fashion.

Our findings that responses to strong M1s determinants do not show H-2 restriction adds yet another complexity to the problem of the biological function of the M1s locus. The M1s locus is unique in four respects. First, homologues of this locus have not been found in species other than mice (22). Second, M1s determinants do not evoke antibody responses and hence may not be recognized by B cells (23, 24). Third, T-cell responses to M1s determinants are sterile in the sense that these determinants do not evoke cell-mediated lympholysis (25) or lethal graft-versus-host reactions (26, 27). Fourth, on the basis of the magnitude of primary MLR (Fig. 1 and [10]), the precursor frequency for strong M1s determinants is probably as high or higher than for MHC determinants. Consideration of these bizarre properties begs the question of how T cells recognize M1s determinants. Thus, from the point of view of the two receptor model, Are M1s determinants recognized by anti-MHC receptors or by receptors specific for conventional antigens? Perhaps the simplest explanation here is to presume that the M1s locus is a gene translocation of the MHC (8) and thence argue that M1s determinants are recognized by anti-MHC receptors; this could account for the lack of H-2 restriction of recognition of M1s determinants. The main problem with this interpretation is that M1s determinants do not have the typical properties of any known MHC determinants.

Another possibility is that M1s determinants are not recognized by anti-MHC receptors or by receptors for conventional antigens but by a third set of "mitogen" receptors analogous to receptors for concanavalin A or phytohemagglutinin. According to this viewpoint receptors for M1s and MHC determinants would not necessarily be mutually excluded. If so, cloned lines of M1s-reactive T cells in certain situations might have concomitant reactivity for particular H-2 determinants, and vice versa. This question is currently under investigation.

Summary

Negative and positive selection procedures were used to establish whether the strong proliferative response of T cells to M1s^a determinants is H-2 restricted. After negative selection to H-2 determinants *in vivo*, it was shown that T cells give high primary mixed lymphocyte reactions *in vitro* to M1s^a determinants presented on H-2-incompatible stimulator cells. Other studies demonstrated that (a) negative selection of T cells to M1s^a determinants on H-2-incompatible cells removed T cells with specificity for M1s^a-bearing H-2-compatible cells, and (b) T cells primed *in vitro* or *in vivo* to M1s^a determinants on H-2-compatible cells gave high secondary responses to M1s^a determinants presented either on H-2-compatible or H-2-incompatible stimulator cells. From these data we conclude that T cells recognize M1s^a determinants *per se* rather than an association of M1s^a plus self or allo-H-2 determinants.

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