T-LYMPHOCYTE RESPONSE TO *H-2* MUTANTS

I. Proliferation is Dependent on Ly 1^+2^+ Cells*

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Lymphocyte differentiation (Ly)[1] antigens serve as useful markers for distinguishing thymus-derived (T) lymphocyte subpopulations. Initially, Boyse and co-workers (1, 2) discovered the Ly 1 and Ly 2, 3 alloantigen systems, expressed on partially overlapping subsets of T lymphocytes. More recently the number of known Ly antigen systems has expanded with antigens expressed on subpopulations of T lymphocytes (3, 4), B lymphocytes (5–7), and B and T lymphocytes (8). Boyse and Old proposed early on that Ly antigens are expressed differentially on lymphocyte subpopulations which have diverged in immunological function (9). Recently this proposal has been confirmed by Cantor and Boyse (10, 11) who distinguished lymphocytes responsive to major histocompatibility (H-2) complex antigens in mixed lymphocyte culture (MLC) and cell-mediated lympholysis (CML) on the basis of Ly 1, 2, and 3 phenotypes. Responder cells in MLC were Ly $1^+2^-3^-$ T lymphocytes whose proliferation was required for the generation of Ly $1^-2^+3^+$ effector cells detectable in CML. However, it is not clear whether the responsive lymphocyte subpopulations are specific for the assayed functions, proliferation versus cytolysis, or specific for the different target alloantigens, I region-associated (Ia) alloantigens in MLC (12) versus H-2K and H-2D alloantigens in CML (13).

To understand more fully the significance of the differential responses of Ly $1^+2^-3^-$ and Ly $1^-2^+3^+$ lymphocytes, it is particularly important to elucidate the basis for these differential responses. We wished to determine if the subpopulation of lymphocytes responsive to a class of determinants were dependent on the antigenic determinant, Ia or K/D, rather than on their immunologic function, proliferation or cytolysis. *H-2* mutants provide an opportunity to study both proliferation of responders and the generation of cytotoxic effectors to single antigenic determinants since mutations in *H-2K* and *H-2D* region genes have generated novel antigenic specificities detectable in both MLC and CML (14–16). In this communication we report observations made in Ly typing T lymphocytes proliferating in response to mutant H-2K and H-2D antigens in

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[1] *Abbreviations used in this paper:* C, complement, CML, cell-mediated lympholysis; FCS, fetal calf serum; H, histocompatibility; Ly, lymphocyte; MLC, mixed lymphocyte culture; NMS, normal mouse serum.

primary MLC. Proliferating T lymphocytes responsive to mutant H-2K^{ba} and H-2K^{bf} antigens were classified as Ly 1$^+$2$^+$ lymphocytes by selective depletion analysis. The implications of these results for our understanding of T-lymphocyte differentiation and maturation are discussed.

Materials and Methods

Mice. The mice employed in this study and their respective genotypes are presented in Table I. The mutant *H-2^{ba}* and *H-2^{bf}* haplotypes of the B6.C-*H-2^{ba}* By (B6-*H-2^{ba}*) and B6.C-*H-2^{bf}* By (B6-*H-2^{bf}*) mice were discovered by Bailey and Kohn through reciprocal circle grafting analyses designed to identify histocompatibility (*H*) gene mutations (17). Complementation tests demonstrated that both mutations occurred in the *K* end of the *H-2^b* haplotype (18, 19). Reciprocal, primary MLC of B6-*H-2^{ba}* and B6-*H-2^{bf}* lymphocytes and B6 lymphocytes result in responder proliferation (14, 19). The *H-2da* haplotype of B10.D2 (M504) (B10.D2-*H-2da*) was discovered by Egorov and collaborators to have diverged from the *H-2^d* haplotype of B10.D2/n by a mutation in the *D* region of *H-2^d* (20). B10.D2-*H-2da* and B10.D2/n lymphocytes are reciprocally stimulatory in primary MLC (15, 16). Mice of the B6-*Ly-1^a* strain were selected to be congenic with, but differ from, C57BL/6 (B6) mice at the *Ly 1* locus (21). B6-*Ly 1^a* mice are *Ly 1^a* whereas B6 mice are *Ly 1^b*. Both strains share the *Ly 2^b*, *3^b* genotype. According to genetic convention (22), *Ly 1^a* and *Ly 1^b* alleles determine the Ly 1.1 and Ly 1.2 alloantigens, respectively.

Antisera. Anti-Ly and anti-Thy 1 sera were raised by immunization of recipients with thymocytes obtained from congenic donors differing at the locus of interest. The cell dosage and schedule of immunization and bleeding have been described previously (23). Anti-Ly 1.1 serum was produced by immunization of (BALB/c × C57BL/10)F$_1$ recipients with B6-*Ly 1^a* thymocytes. Anti-Ly 2.2 serum was prepared by immunization of (C3H.OL × B6-Ly 2^a)F$_1$ hosts with C57BL/6 thymocytes. Individual sera were tested as suggested by Shen and co-workers (24); only active antisera were pooled. Anti-Thy 1.2 serum was produced by immunization of A.AKR(*Thy 1^a*,*H-2^{a1}*) mice with A.AL (*Thy 1^b*, *H-2^{a1}*) thymus cells. Specificity tests in complement-dependent cytotoxicity assays demonstrated that this anti-Thy 1.2 serum is specific for T cells from *Thy 1^b* donors (J. A. Frelinger, unpublished observations).

Complement-Dependent Cytotoxicity Testing. The alloantigenic specificity of all alloantisera were tested in a complement-dependent cytotoxicity assay described by David et al. (23). Selected young rabbit serum obtained from Pel-Freeze Farms, Inc., (Rogers, Ark.) served as the source of complement for tests with spleen lymphocyte targets.

Selective Lymphocyte Depletion. Lymphocytes bearing selectively expressed cell-surface alloantigens were eliminated from lymphocyte populations as described by Niederhuber et al. (25). Briefly, lymphocytes were suspended in alloantiserum diluted in RPMI-1640 (Microbiological Associates Walkersville, Md.) + heat-inactivated fetal calf serum (FCS) at a concentration of 5% at 10 × 10^6 cells/ml diluted antiserum. The antiserum-treated lymphocytes were incubated at 37°C for 30 min, centrifuged, and resuspended in rabbit complement appropriately diluted in RPMI-1640 at 10 × 10^6 cells/ml diluted complement. After incubation at 37°C for 40 min, the surviving lymphocytes were resuspended in RPMI-1640 + 5% FCS and washed three times in RPMI-1640.

Mixed Lymphocyte Culture. The MLC was performed as a modification of the procedure described by Peck and Bach (26) as we have described in a previous communication (27). Briefly, responder cells were T lymphocytes enriched from Tris-ammonium chloride-treated spleen cells by passage through nylon wool columns (27). After nylon wool passage, responder T cells were employed in MLC either without further treatment or after selective lymphocyte depletion with antiserum plus complement. Stimulator cells were Tris-ammonium chloride-treated spleen cells inactivated by mitomycin C (Sigma Chemical Co., St. Louis, Mo.) treatment. A total of one million responder and stimulator cells were mixed in either a 1:1 or 1:3 ratio in quadruplicate cultures in Click's medium (29) without nucleic acid precursors but supplemented with Hepes (30 mM) and normal mouse serum (1%). The cultures were incubated for 5 days at 37°C in humidified Lucite boxes with an atmosphere of 83% nitrogen, 10% carbon dioxide, and 7% oxygen. 24 h before harvest the cultures were pulsed with [^{3}H]thymidine (New England Nuclear, Boston, Mass. 2 Ci/mM) at 2 μCi/well. The cultures were harvested and [^{3}H]thymidine uptake determined as described previously (27). Specific [^{3}H]thymidine uptake was calculated as a Δ when the difference

TABLE I
H-2 Haplotypes and Ly 1,2 Genotypes of Employed Mouse Strains

Strain	*H-2* Haplotype	Origin of *H-2* regions				*Ly 1*	*Ly 2*
		K	*I*	*S*	*D*		
C57BL/6-*Ly 1*^{a}	*b*	*b*	*b*	*b*	*b*	*a*	*b*
C57BL/6-*H-2*^{ba}	*ba*	*ba*	*b*	*b*	*b*	*b*	*b*
C57BL/6-*H-2*^{bf}	*bf*	*bf*	*b*	*b*	*b*	*b*	*b*
B10.D2/n	*d*	*d*	*d*	*d*	*d*	*b*	*b*
B10.D2(M504) (B10.D2-*H-2*^{da})	*da*	*d*	*d*	*d*	*da*	*b*	*b*
B10	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B10.S	*s*	*s*	*s*	*s*	*s*	*b*	*b*
B10.A(1R)	*h1*	*k*	*k/d*	*d*	*b*	*b*	*b*
B10.A(2R)	*h2*	*k*	*k/d*	*d*	*b*	*b*	*b*
B10.C-*H-7*^{b}	*b*	*b*	*b*	*b*	*b*	*b*	*b*
C3H.OL	*o1*	*d*	*d*	*k*	*k*	*a*	*a*
C3H.Q	*q*	*q*	*q*	*q*	*q*	*a*	*a*

between mean uptake in allogenic and syngeneic cultures differed at the $\alpha = 0.001$ level according to Student's *t* test assuming equal variance (30).

Cell-Mediated Lympholysis. The CML assay was performed according to the technique described by Hirschberg et al. (31). All incubations and washes were performed in RPMI-1640 supplemented with heat-inactivated FCS (10%), gentamycin (0.5%) (Schering Diagnostics, Kenilworth, N. J.) and 2-mercaptoethanol (10^{-5} mM). Target cells were lymphoblasts which had been generated by incubation of splenic lymphocytes with concanavalin A (2 μg ConA/2 × 10^6 cells/ml) (Calbiochem, San Diego, Calif.) for 2–3 days. Viable lymphoblasts were separated by flotation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) followed by washes at 50 *g*. Lymphoblasts were labeled with ^{51}Cr (Amersham Corp., Arlington Heights, Ill.), at 200 μCi/2 × 10^6 cells for 60 min, washed four times, and suspended to 2 × 10^5 cells/ml. Effector cells were generated in MLC under conditions and cell concentrations identical to those employed in microculture as described above with the exception that 5 ml of both responder and stimulator cells were mixed in 25 cm^2 tissue culture flasks. Effector cells were harvested after 6–7 days of culture. Effectors were washed twice, counted, and resuspended. 100 μl of the target and effector suspensions were mixed in quadruplicate in U bottom wells of microtiter plates (Flow Laboratories, Inc., Rockville, Md.). The plates were centrifuged for 5 min at 50 *g*. The cultures were incubated for 4 h in an atmosphere of 83% N_2 10% CO_2, and 7% O_2 in humidified Lucite boxes at 37°C. After incubation the supernates were collected with the aid of a Skatron supernatant collection system (Flow Laboratories). Total releasable label was the ^{51}Cr released after two cycles of freeze-thawing. The specific ^{51}Cr release was determined by the following equation:

$$\text{percent specific release} = \text{experimental release} - \text{spontaneous release} \\ /\text{total releasable label-spontaneous release} \times 100.$$

Results

Ly Antisera Characterization. To insure the correct specificity of the anti-Ly 1.1 and anti-Ly 2.2 sera employed in this study, specificity tests were performed in complement-dependent cytotoxicity, MLC, and CML. The alloantigenic specificity of anti-Ly 1.1 and anti-Ly 2.2 sera were tested with B6-*Ly 1*^{a} and B6-*Ly 2*^{a} splenic lymphocytes in the complement-dependent cytotoxic test. The results of this assay are presented in Fig. 1. Both antisera were strongly positive on B6-*Ly 1*^{a} spleen cells and negative on B6-*Ly 2*^{a} lymphocytes, demonstrating that both sera were specific for their appropriate alloantigens.

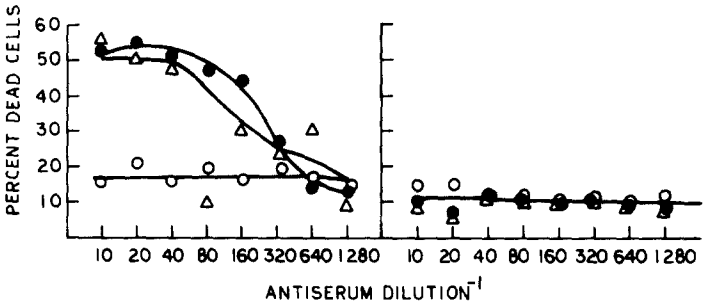

FIG. 1. Complement-dependent dye exclusion cytotoxic test of anti-Ly 1.1 and anti-Ly 2.2. Left panel sera tested on B6-*Ly 1*^{a} (Ly 1.1 and Ly 2.2) spleen cell targets, (○——○ NMS; △——△ anti-Ly 1.1; ●——● anti-Ly 2.2). Right panel, sera tested on B6-*Ly 2*^{a} (Ly 1.2 and Ly 2.1). Same symbols as left.

TABLE II

Specificity of Anti-Ly Sera for Selective Depletion of Responder T Cells in MLC

Responders	Ly 1, 2 Phenotype	Stimulator	Antiserum + C	Δ cpm*	Inhibition‡
					%
B6-Ly *1*^{a}	Ly 1.1, 2.2	B10.D2	NMS (1/40)	72,180	43
			α-Ly 1.1 (1/40)	45,768	
B10.S	Ly 1.2, 2/2	B10.D2	NMS (1/40)	114,011	
			α-Ly 1.1 (1-40)	102,512	10
C3H.Q	Ly 1.1, 2.1	C3H.OL	NMS (1/20)	211,298	
			α-Ly 1.1 (1/20)	25,470	88
B10.S	Ly 1.2, 2.2	B10.D2	NMS (1/20)	140,503	
			α-Ly 2.2 (1/20)	164,248	−17
B10	Ly 1.2, 2.2	B10.A (2R)	NMS (1/10)	129,383	
			α-Ly 2.2 (1/10)	112,055	13

* Δ = Mean [^{3}H]thymidine uptake for allogeneic combination − mean [^{3}H]thymidine uptake for syngeneic combination. Computed when the two means differ at $\alpha \leq 0.001$.

‡ Inhibition = (Δ after alloantiserum + C treatment/Δ after normal mouse serum + C treatment) × 100.

The slight degree of nonspecific lysis observed in the complement-dependent cytotoxic test does not necessarily indicate that a similar lack of specificity will be observed in depletion of T-lymphocyte function, due to the greater sensitivity of the analytical dye exclusion cytotoxic test. Both the alloantigenic and T-cell subpopulation specificity of the Ly antisera were tested by pretreatment of MLC responder T cells and CML effector T cells with antisera plus complement. Responder and stimulator cells mixed in MLC were incompatible at the *I* region, *K* region, and/or *D* region. Nylon wool T cells obtained from *Ly 1*^{a} and *Ly 1*^{b} donors were treated with anti-Ly 1.1 serum or normal mouse serum (NMS) + complement (C). The results are presented in Table II. Relative to the effects of NMS + C, anti-Ly 1.1 + C depleted the proliferative response of Ly 1.1-positive responder cells (B6-*Ly 1*^{a} and C3H.OL) with no effect on the

TABLE III

Depletion of H-2-Specific Cytotoxic Effector Cells with Anti-Ly 2.2 Serum

Effector cell donor	Ly 1, 2 Phenotype	Target	Antiserum + C	% ^{51}Cr Release*	Inhibition‡
					%
(B10.C-*H-7*^{b} × C3H.OL)F$_1$	Ly 1.1/1.2 Ly 2.1/2.2	C3H.Q	–	60.8 ± 3.8	
			NMS (1/20)	50.2 ± 4.2	
			α-Ly 1.1 (1/20)	41.2 ± 1.9	17.6
			α-Ly 2.2 (1/20)	0§	100.0
		B10.C-*H-7*^{b}	–	0	
			NMS (1/20)	0	
			α-Ly 1.1 (1/20)	0	
			α-Ly 2.2 (1/20)	0	

* Effector: target ratio of 20:1.

‡ Inhibition = (^{51}Cr release after antiserum + C/^{51}Cr release after NMS + C) × 100.

§ ^{51}Cr release reported as zero when value is less than or equal to zero.

TABLE IV

Ly 2 Typing of T Cells Proliferating in MLC to H-2D Alloantigen Determined by the H-2D^d· H-2D^{da} Allelic Combination

Responder	Ly 2 Phenotype	Stimulator	Target antigens	Antiserum + C	Δ cpm*	Inhibition‡
						%
B10.D2	Ly 2.2	B10.D2-*H-2*^{da}	H-2D^{da}	–	151,222	
				NMS	152,462	−1
				α-Thy 1.2	0	100
				α-Ly 2.2	12,337	92
B10.D2-*H-2*^{da}	Ly 2.2	B10.D2	H-2D^d	–	24,284	
				NMS	10,772	56
				α-Thy 1.2	0	100
				α-Ly 2.2	2,548	90

* As in Table II.

‡ As in Table II.

response of Ly 1.1-negative responders (B10.S). Therefore, the anti-Ly 1.1 serum was specific for the Ly 1.1 alloantigen on T cells proliferating in primary MLC. The T-cell subpopulation specificity of anti-Ly 2.2 serum was determined by treating nylon wool T cells obtained from *Ly 2*^{b} donors with anti-Ly 2.2 serum + C before culture with H-2-incompatible stimulators. The results of this assay are included in Table II. Anti-Ly 2.2 + C did not deplete B10.S and B10 T cells responsive in MLC to B10.D2 and B10.A(2R) stimulators. Anti-Ly 2.2 but not anti-Ly 1.1 serum plus complement depleted (C3H.OL × B10.C-H-7^b)F$_1$ effectors specific for C3H.Q targets (Table III). These observations are consistent with previous reports in demonstrating that T lymphocytes proliferating in response to H-2-incompatible stimulators in MLC are Ly 1$^+$2$^-$ whereas the cytotoxic effectors specific for H-2-alloantigens are Ly 1$^-$2$^+$ (10, 11).

Ly Typing of H-2 Mutant Antigen-Responsive T Lymphocytes. We Ly 2 typed the T cells proliferating in reciprocal primary MLCs of B10.D2/n and B10.D2-H-2da lymphocytes. The results of this experiment are presented in Table IV. Relative to the inhibition observed with NMS + C, anti-Ly 2.2 + C

TABLE V

Ly Typing of T Cells Proliferating in MLC to H-2K^{ba} and H-2K^{bf} Mutant Alloantigens

Responder	Ly 1, 2 Phenotype	Stimulator	Target antigen	Antiserum + C		Inhibition‡
					Δ cpm*	%
B6-Ly 1^a	Ly 1.1, 2.2	B6-*H-2*^{ba}	H-2K^{ba}	–	49,422	
				NMS	51,268	
				α-Thy 1.2	0	100
				α-Ly 1.1	0	100
				α-Ly 2.2	1,597	97
				α-Ly 1.1	699	99
				α-Ly 1.1 treated cells mixed with α-Ly 2.2-treated cells		
		B6-*H-2*^{bf}	H-2K^{b}*	–	13,325	
				NMS	5,866	
				α-Thy 1.2	0	100
				α-Ly 1.1	0	100
				α-Ly 2.2	0	100
				α-Ly 1.1-	0	100
				α-Ly 1.1 treated cells mixed with α-Ly 2.2-treated cells		
		B10.A(1R)	H-2K^{k}I$^{k/d}$	–	NT	
				NMS	124,857	
				α-Thy 1.2	2,065	98
				α-Ly 1.1	2,843	98
				α-Ly 2.2	97,335	22.1
				α-Ly 1.1-treated cells mixed with α-Ly 2.2-treated cells	50,030	60

* As in Table II.

‡ As in Table II.

and anti-Thy 1.2 + C eliminated the T cells responsive in reciprocal MLCs. This observation strongly indicated that T lymphocytes proliferating in response to the antigens defined by the H-2D^d: H-2D^{da} responder: stimulator combination are Ly 2$^+$. Although we did not have the anti-Ly 1.2 sera available to Ly 1 type these responders it is clear that these proliferating T cells expressed a different phenotype than Ly 1$^+$2$^-$ responders in *I*-region disparate MLC.

A more definitive Ly typing of H-2K mutant antigen-responsive T cells was accomplished through the use of anti-Ly 1.1 and anti-Ly 2.2 sera and B6-*Ly 1*^{a} T cells. B6-*Ly 1*^{a} T cells were pretreated with anti-Ly 1.1 or anti-Ly 2.1 + C and mixd in primary MLC with mitomycin C-treated spleen cells from B6-*H-2*^{ba}, B6-*H-2* ^{bf}, and B10.A(1R) mice. The results are presented in Table V. B6-*Ly 1*^{a} mice are congenic with the Boyse substrain of B6, differing at a segment of chromosome encompassing the *Ly 1* locus. The long separation of the B6/Boy

and B6/By (background strain of B6-*H-2*^{ba} and B6-*H-2*^{bf} congenic strains) suggests that there is a high probability that the two substrains differ by one or more *H* loci. However, primary cultures of B6-*Ly 1*^{a} responder cells and B6/By stimulator cells do not result in detectable proliferation. We are confident that the observed proliferation of B6-*Ly 1*^{a} responders mixed with B6-*H-2*^{ba} and B6-*H-2*^{bf} stimulators is specific for H-2K^{ba} and H-2K^{bf} alloantigens. Responders to both mutant stimulators and B10.A(2R) stimulators were depleted by anti-Thy 1.2 and anti-Ly 1.1 serum plus C. The crucial experiment was pretreatment with anti-Ly 2.2 + C. This treatment resulted in depletion of the T-cell response to both B6-*H-2*^{ba} and B6-*H-2*^{bf} stimulators. The same treated cells responded normally to B10.A(1R) stimulators. These results intimated that proliferation in MLC to H-2K-incompatible stimulators in contrast to *I* region-incompatible stimulators was mediated by Ly 1^{+}2^{+} T cells or required cooperation of Ly 1^{+}2^{-} and Ly 1^{-}2^{+} T cells. To discriminate between these two alternatives, we tested the ability of mixtures of T cells depleted of Ly 1^{+} cells and T cells depleted of Ly 2^{+} cells to respond to mutant stimulators. These results are included in Table V. Mixtures of Ly 1^{-} and Ly 2^{-} T cells were unresponsive to both *H-2K*^{b} mutant stimulators indicating that the lymphocytes responding to H-2K^{ba} and H-2K^{bf} antigens were Ly 1^{+}2^{+}.

Discussion

Lymphocyte differentiation antigens have long been believed to be expressed differentially on lymphocyte subpopulations which have diverged functionally during development and maturation. This supposition has been confirmed by the demonstration that T cells proliferating in primary MLC in response to *H-2K* plus *I* region gene coded alloantigens are Ly 1^{+}2^{-}3^{-} (10) whereas *H-2K* and *H-2D*-specific cytotoxic T cells (11) and specific suppressor T cells (32) are Ly 1^{-}2^{+}3^{+}. As a first step in elucidating the basis for the differential response of Ly 1^{+}2^{-}3^{-} and Ly 1^{-}2^{+}3^{+} cells to H-2 associated antigens, we have Ly-typed T cells responding in MLC to mutant H-2K and H-2D antigens, detectable in both MLC and CML. The results reported in this communication demonstrate that the B6-*Ly 1*^{a} T lymphocytes proliferating in response to B6-*H-2*^{ba} and B6-*H-2*^{bf} stimulators are Ly 1^{+}2^{+}. These observations are in contrast to those indicating that the B6-*Ly 1*^{a} responders to H-2K- and I-incompatible B10.A(1R) stimulators are Ly 1^{+}2^{-}. Based on numerous prior observations by others (12, 13) we presume that the proliferative response by B6-*Ly 1*^{a} T cells to B10.A(1R) stimulators was primarily specific for foreign I region determinants. Therefore, we have demonstrated that responder T cells specific for I-incompatible stimulators are Ly 1^{+}2^{-}3^{-}, whereas T cells required for proliferation in MLC to H-2K-incompatible stimulators are Ly 1^{+}2^{+}. While we have not yet established with certainty that the proliferating cells themselves are Ly 1^{+}2^{+}, preliminary data from experiments involving antiserum +C treatment 5 days after in vitro stimulation, but before [^{3}H]thymidine addition support the contention that the proliferating cells are Ly 1^{+}2^{+}. The obvious conclusion from these observations is that at least some of the T lymphocytes proliferating in response to foreign alloantigeneic determinants in primary MLC need not be limited to the Ly 1^{+}2^{-} subpopulation. It would appear that the subpopulation of responsive T cells is determined by

either the identity of the foreign alloantigenic determinants or the molecule carrying those determinants. To resolve this question we have initiated studies to Ly type T cells proliferating in MLC to alloantigens defined by combinations of responders and stimulators differing at only the *H-2K* or *H-2D* regions.

This is not the only system in which Ly 1^+2^+ cells have been implicated. Shiku et al. reported that cytotoxic effector cells for syngeneic tumors were Ly 1^+2^+ (33). These results have been recently expanded by Stutman et al. (34) who showed that the effectors were actually Ly 1^-2^+, but that an Ly 1^+2^+ amplifier was required for efficient expression of that cytotoxicity. Similarly Cantor and Boyse reported that an Ly 1^+2^+ population was required for the generation of killer cells specific for TNP modified syngeneic cells but that the killers themselves were Ly 1^-2^+ (35). Earlier Pang et al. showed that depletion by either anti-Ly 1^+ or anti-Ly 2^+ would inhibit a secondary, virus-specific cytotoxic response but they did not perform the critical reconstitution experiment necessary to determine if an Ly 1^+2^+ cell was required (36). In contrast to the data here for H-2 mutant products Pang et al. could demonstrate no effect of anti-Ly 2 on the secondary virus induced proliferative response. We should point out that the mutant stimulated MLC is the incorporation equivalent to that seen in most *I* region differences. Thus this makes the observation reported here that a Ly 1^+2^+ is required for proliferation stimulated *K* or *D* mutants even more striking.

The observations reported in this communication are important for the understanding of the development and maturation of T lymphocytes. Previous hypotheses of T cell differentiation have proposed that Ly 1^+2^+ cells serve as progenitors for more mature Ly 1^+2^- and Ly 1^-2^+ T cells (10). Our results in MLC reject this simple progression as an all inclusive explanation for T cell differentiation. T cells capable of responding immunologically to in vitro stimulation in MLC must exist within the Ly 1^+2^+ subpopulation of a normal animal. Before a clear impression of T lymphocyte development and maturation can be obtained, a more extensive characterization of the Ly-differentiation antigen phenotype of virgin and mature T cells is required.

Summary

We have determined the Ly phenotype of the T lymphocytes which proliferate in response to mutant H-2K and H-2D alloantigens in primary mixed lymphocyte culture. Responder T cells proliferating in reciprocal cultures of H-2^d(K^dD^d) and *H-*2^{da}(K^dD^{da}) lymphocytes were typed Ly 2^+ through selective depletion with specific alloantiserum plus complement. Further, B6-Ly 1^a lymphocytes proliferating in response to B6-*H-*2^{ba} and B6-*H-*2^{bf} stimulators were typed as Ly 1^+2^+ through similar analysis. These results are discussed with regard to their impact on views of lymphocyte differentiation and factors determining the identity of alloreactive lymphocytes.

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