FINE SPECIFICITY OF ALLOIMMUNE CYTOTOXIC T LYMPHOCYTES DIRECTED AGAINST H-2K

A Study with K^b Mutants*

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Products of the K and D regions of the murine H-2 major histocompatibility complex serve as strong transplantation antigens (1–4) and are vital elements in associative recognition by T cells of viral, chemically modified self and minor histocompatibility antigens (5–10). The analysis of the role of the K, D, and L loci in these immune responses is facilitated by the availability of K-, D-, and L-locus histocompatibility mutants. The largest series of mutants is that of the H-$2K^b$ locus of the C57BL/6 mouse strain. So far, 16 H-$2K^b$-locus mutants have been detected by reciprocal skin graft rejection ([11–19]; R. W. Melvold. Unpublished observations.). Biochemically, the K^b mutations appear to be characterized by at least a single amino acid (AA)[1] substitution in the K^b glycoprotein ([20, 21]; B. M. Ewenstein, H. Uehara, T. Nisizawa, S. G. Nathenson, R. W. Melvold, and H. I. Kohn. Structural studies on the MHC mutants. IV. Characterization of the H-2K glycoprotein from two mutants, bm3 (M505, H-2^{bd}) and bm11 (H-2^{bk}). Manuscript in preparation; K. Yamaga, T. Nisizawa, D. McGovern, R. W. Melvold, H. I. Kohn, and S. G. Nathenson. Unpublished observations). The mutations elicit all of the T cell reactions associated with graft rejection, including positive mixed lymphocyte culture (MLC) and cell-mediated lympholysis (CML) reactions in vitro (22–25), as well as graft-versus-host reactivity in vivo (26, 27). Studies with K^b mutants have identified the H-2K glycoprotein as the active element in K-region-associated T cell recognition of virus-infected cells (28–30). Although early studies indicated that the mutations are associated with little or no changes in serologically detectable alloantigens, such changes have now been reported for several members of the K^b series (18, 31, 32). However, in reciprocal

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[1] *Abbreviations used in this paper:* AA, amino acid; CML, cell-mediated lympholysis; CTL, cytotoxic T lymphocyte(s); FCS, fetal calf serum; MLC, mixed lymphocyte culture(s); TNP, trinitrophenyl.

immunizations with the strain of origin, no alloantibody was detected (12, 33, 34), and this contrasts strongly to the ease with which T cell reactions are elicited.

We now report studies of the specificity of the cytotoxic T lymphocytes (CTL) generated in MLC in all possible combinations between seven K^b-locus mutants and the wild type H-2^b, as measured by CML. Three new K^b mutants (H-2^{bm9}, H-2^{bm10}, and H-2^{bm11}) showed positive MLC and CML reactions with the strain of origin. Two mutants (H-2^{bm6} and H-2^{bm9}) were found to be identical in CML. The analysis of CML cross-reactivity against third-party targets of the K^b series indicated extensive complexity of K^b CML target-antigenic determinants. Apparently, single AA substitution in the K^b molecule leads to the loss of multiple CML target determinants and gain of multiple new CML target determinants. In this study, 64 different CML target specificities were identified, 19 of which have been described before (25, 35, 36). The CML antigens of the bm3 mutation, which has two AA substitutions in its K^b glycoprotein, were closely related but not identical to those of the bm11 mutation, which shares its AA substitution with one of the two AA substitutions of bm3 (see above: B. M. Ewenstein et al. Manuscript in preparation).

Materials and Methods

Animals. All mice were bred at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. C57BL/6Kh (B6, H-2^b) mice as well as the mutants B6-H-2^{bm6}, B6-H-2^{bm8}, B6.C-H-2^{bm9}, B6.C-H-2^{bm10}, and B6.C-H-2^{bm11} were raised from breeding stocks, supplied by Dr. H. I. Kohn and Dr. R. W. Melvold, Shields Warren Radiation Laboratory, Boston, Mass. B6.C-H-^{bm1} mice were bred from stocks supplied by Dr. Donald Bailey, The Jackson Laboratory, Bar Harbor, Maine. Breeding stock of the B6-H-2^{bm3} line was obtained from Dr. J. Klein, then at the Department of Microbiology, University of Texas, Dallas, Tex. A list of the H-2-mutant lines used and references to their first description are given in Table I.

Preparation of Cell Suspensions. Cell suspensions were prepared from lymphoid organs as described previously (25) with minor modifications. Spleen or lymph node cells were mixed and pressed through stainless metal sieves into RPMI-1640 medium with 25 mM Hepes buffer and 2 mM L-glutamine, containing 10% fetal calf serum (FCS, heat-inactivated for 30 min at 56°C; Grand Island Biological Co., Grand Island, N. Y.). This cell suspension was washed twice in the same medium.

Generation of Killer Cells in MLC. Spleen cells were resuspended in culture medium consisting of RPMI-1640 with 25 mM Hepes buffer and 2 mM L-glutamine plus 100 IU penicillin and 100 μg streptomycin/ml, supplemented with 10% heat-inactivated (30 min, 56°C) human serum and containing 2-mercaptoethanol to a final concentration of 2×10^{-5} M. This cell suspension was used as the responding population in MLC. Viable responder cells (100×10^6) were mixed with 100×10^6 stimulator cells (irradiated with 2,500 rad from a ^{137}Cs source) in a 250-ml culture flask (Costar, Data Packaging, Cambridge, Mass.; catalog No. 3075) that contained 80 ml of culture medium. The flasks were tilted at a 45° angle to allow the cells to settle in one corner, and incubated at 37°C, after which the cells were harvested.

CML. After a total of 5 d of culture as described above, the cells were used as effector cells in CML assays. The culture supernate was removed, the cells were washed twice, and were resuspended in medium consisting of RPMI-1640 with 25 mM Hepes, supplemented with 10% heat-inactivated (30 min, 56°C) FCS.

Target cells for CML were obtained by culturing lymph node cells for 48 h in the presence of concanavalin A (2.5 μg/ml of culture medium). After one wash, 10×10^6 target cells, suspended in 1 ml of wash medium, were incubated for 30 min at 37°C with 150 μCi $Na_2[^{51}Cr]O_4$ (sp act: 50–400 mCi/mg ^{51}Cr, The Radiochemical Centre, Amersham, England). Then the cells were washed three times and resuspended in medium with 10% heat-inactivated FCS.

The CML assays were performed in microtiter plates with round-bottomed wells (Cooke

TABLE I

H-2K^b Mutant Lines Used in This Study

Line	H-2 haplotype	Former name(s)	First description
B6.C-H-2^{bm1}	bm1	H-2^{ba}, Hz1	Bailey and Kohn (11) Bailey et al. (12)
B6-H-2^{bm3}	bm3	H-2^{bd}, M505	Egorov and Blandova (13)
B6-H-2^{bm6}	bm6	H-2^{bg2}	Kohn and Melvold (14)
B6-H-2^{bm8}	bm8	H-2^{bh}	Kohn and Melvold (14)
B6.C-H-2^{bm9}	bm9	H-2^{bi}	Melvold and Kohn (15)
B6.C-H-2^{bm10}	bm10	H-2^{bj}	Melvold and Kohn (15)
B6.C-H-2^{bm11}	bm11	H-2^{bk}	Melvold and Kohn (15)

Additional details about these mutants have been published (19).

Engineering Co., Alexandria, Va.). Target cells (1×10^5) were mixed with varying numbers of effector cells in a total vol of 0.2 ml. The plates were incubated for 3 h at 37°C in humidified air with 5% CO_2. Subsequently, the plates were centrifuged at 700 *g* for 10 min. Next, 100 μl of the supernate was removed and transferred to a 70- × 11-mm plastic tube. The radioactivity in this tube was determined in a gamma scintillation counter, and the specific ^{51}Cr release (r) was calculated using the formula:

$$r = \frac{e - b}{s - b} \times 100\%,$$

where e is the ^{51}Cr release (counts per minute) in the experimental tube, b the background ^{51}Cr release, and s the maximal release in the presence of 5% saponin. Background release was taken to be the ^{51}Cr release seen in the presence of syngeneic nonactivated, cultured cells. For example, the background release for target cells from mouse strain A was the release seen after incubation of A target cells with A + A irradiated effector cells. The background release was always <23%, the maximal release varied from 82 to 100% crude lysis. All assays were performed in triplicate. The standard error of triplicate tests was always <3% specific lysis.

Determination of Lymphocyte Activation in MLC. Responding and stimulating cells from the same cell suspensions used for the generation of killer cells were each brought to a concentration of 5×10^6/ml. 100 μl of responding and 100 μl of stimulating cells were transferred to each well of a round-bottomed microculture plate (Cooke Engineering Co.). After 3 d of culture at 37°C in humidified air with 5% CO_2, 20 μl [^{3}H]thymidine (Amersham Corp., Arlington Heights, Ill.; 200 mCi/mmol, 20 μCi/ml) was added. The cultures were harvested 24 h later, and [^{3}H]thymidine incorporation was determined as described previously (26). All determinations were performed in triplicate.

Results

CML Reactions of Three New K^b Mutants. Three new K^b-locus mutants, all discovered by skin graft rejection and proven to be gain/loss mutations of the K^b locus (15, 19) were tested in CML after MLC with the strain of origin, C57BL/6. The results are shown in Table II.

As observed with all previously studied K^b-locus mutants, CML was strongly positive in both directions (Table II), illustrating the gain/loss nature of these mutations. Proliferative responses in MLC were also positive in both directions (Table II).

Kinetics of CML: CML Cross-Reactivity. The definition of CML target determinants specified by the mutations largely depends on the phenomenon of cross-reactivity, i.e., lysis of target cells of genotypes other than those of the stimulator or responder cell in MLC (third-party targets). An example of CML reactions against targets of

TABLE II

MLC and CML Reactions of Three H-2K^b Mutants (H-2 Haplotypes bm9, bm10, and bm11) with the Strain of Origin, B6-H-2^b

MLC					CML (percent specific lysis of target cell§), H-2 haplotype:											
H-2 haplotype		Proliferative response*			b			bm9			bm10			bm11		
Stimulator	Responder				1‖	2	3	1	2	3	1	2	3	1	2	3
		cpm × 10^{-2}	*SE* × 10^{-2}	*si*‡												
bm9	b	56	5	1.41	4	5	2	54	59	77						
bm10	b	108	10	2.75	4	3					67	63				
bm11	b	155	13	3.95	6	2								60	53	
b	bm9	55	4	2.18	79	37	27	9	1	0						
b	bm10	85	6	1.57	69	58					7	−1				
b	bm11	159	8	2.77	84	70								3	3	
b	b	39	7													
bm9	bm9	25	4													
bm10	bm10	54	5													
bm11	bm11	58	9													

* Measured by [^{3}H]thymidine uptake (Materials and Methods).

‡ si, stimulation index: $\frac{\text{counts per minute allogeneic MLC}}{\text{counts per minute syngeneic MLC}}$, using the same MLC responder.

§ 2 × 10^5 effector cells and 1 × 10^5 target cells (effector:target cell ratio, 2:1).

‖ Experiment number.

stimulator- or responder-cell genotypes and against third-party targets is shown in Fig. 1. The effector-cell number was varied while the target-cell number was kept constant.

Although strongest lysis was seen against targets of stimulator-cell genotype, extensive cross-killing of third-party targets occurred. Target cells of responder-cell genotype were not lysed (Fig. 1).

CML after MLC with Homozygous Responder Cells in All Possible Combinations of Seven K^b Mutants and the Wild-Type H-2^b. On the basis of the results shown in Fig. 1 and similar results with other cell combinations (data not shown), an effector-cell number of 2 × 10^5 was selected for optimal detection of the various levels of cross-reactivity. The results of CML tests after MLC with homozygous responder cells are shown in Table III. This analysis includes all possible unilateral MLC combinations between 7 K^b-locus mutants and the strain of origin (B6-H-2^b).

Table III shows that lysis of target cells of MLC responder genotype never exceeded 10%. Therefore, a CML result was scored as positive when ≥15% specific lysis occurred. With most MLC combinations, cross-killing of third-party targets was extensive, but usually the specific lysis of these targets fell below that of targets of stimulator-cell genotype. These results indicate CML specificity for target determinants of stimulator-cell genotype and extensive sharing of CML determinants between stimulator cells and third-party targets. Solid line boxes indicate lysis against target cells of responder genotypes (Table III). Broken line boxes indicate the only MLC

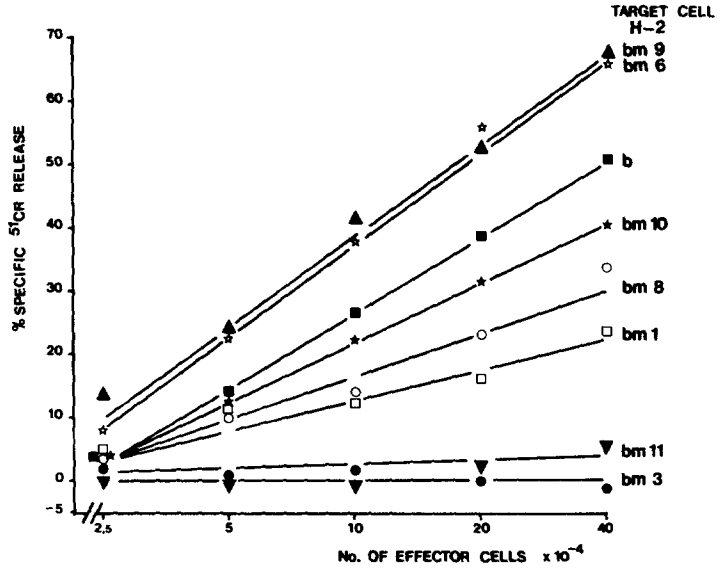

FIG. 1. Kinetics of CML cross-reactivity, bm3 anti-bm6 effector cells.

combinations in which no killer cells were generated: bm6-anti-bm9 and vice versa (Table III). This indicates that bm6 and bm9 are identical for CML determinants. This notion is supported by the finding that all effector-cell combinations kill bm6 and bm9 to a similar extent. Moreover, the CML killing patterns, observed with bm6 as stimulator or responder in MLC, were virtually identical to those observed with bm9 stimulator or responder cells (see below, Designation of CML Determinants; Relatedness among Different K^b Mutants).

CML after MLC with F_1 Hybrid Responder Cells in All Possible MLC Combinations of Four K^b Mutants and the Wild-Type H-2^b. Previous studies have indicated that CML cross-reactivity against third-party targets can be eliminated by introducing the third-party genotype into the responder cell, i.e., by using F_1 hybrid responder cells (25). The results of a series of CML experiments with F_1 hybrid responders in MLC are shown in Table IV. This analysis includes all possible MLC combinations with F_1 responders among four K^b mutants (bm1, bm3, bm6, and bm8) and the wild-type H-2^b.

Table IV shows that lysis of target cells from a parent of the F_1 hybrid responder was sometimes slightly higher than that of responder type targets in the case of homozygous responders (Table III), but never exceeded 18% specific lysis. A CML result achieved with F_1 hybrid responding cells was scored as positive when ≥20% specific lysis occurred. According to this criterion, a positive CML result was at least 11% specific lysis above the highest background lysis against parental-type targets seen with the same effector cell (Table IV).

In all MLC combinations, strong cytotoxicity was generated against cells of stimulator-cell genotype. Cross-killing against targets of the partner in the F_1 responder was eliminated, but cross-reactivity against other targets was still seen, though to a lesser extent than with homozygous MLC responders. In a previous study, strong specific cytotoxicity was reproducibly observed against target cells from one of the parents of two F_1 responders: (b × bm1)F_1 responder and (b × bm8)F_1 responder

TABLE III

CML by Effector Cells from MLC with Homozygous Responder Cells

MLC, H-2 haplotype		Specific lysis* (percent) of target cell, H-2 haplotype																												Determinant number‡ (Tables V and VI)
		b				bm1				bm3				bm6				bm8				bm9				bm10				bm11			
Stimulator	Responder	1§	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
bm1	b	3	3			77	70			14	33			10	12			12	21			6	9			12	15			16	23			3
bm3	b	5	1			9	11			85	60			36	22			28	18			33	17				6			78	56			4
bm6	b	2	2			6	22			35	78			61	90			8	21			55	83			8	20			30	66			17
bm8	b	3	3			6	27			15	55			9	26			63	83			8	22				29			19	47			18
bm9	b	4	5	2		7	5	24		38	42			53	56				12	26		54	59	77		8	7	23		31	42			17
bm10	b	4	3			19	16			25	11			14	5				6			13	6			67	63			21	15			26
bm11	b	6	2			14	11			52	39			18	16				11			18	13			8	7			60	53			27
b	bm1	82	60			6	2			76	48			77	55				35			79	52				60			31	50			1
bm3	bm1	74	38			5	2			88	55			81	46				24			78	36				18			84	53			28
bm6	bm1	83	34	40	38	8	0	1	1	88	27	33	37	92	53	51	52		15	45	11	91	44	50	24		16	27	16	83	25	44	40	29a
bm8	bm1	46				3				50				41				68				44				44				51				12
bm9	bm1	53	64	40		4	2	0		40	56	38		60	72	49		23	51	23		58	75	51			56	25			39	37		29b
bm10	bm1	34				2				25				24				16				30				46				31				30
bm11	bm1	23	22			2	4			34	43			23	30			13	22			18	27				15			45	57			31
b	bm3	93	62	64		46	17	16		7	4	3		82	56	51			28	29		82	47	48			54	42		10	10	7		9
bm1	bm3	25	36	17		76	88	64		6	6	4		27	32	16			29	5		30	32	7			27	8		13	12			10
bm6	bm3	21	58	52		2	11	14	23	0	1	4	5	33	76	71	71	8	26	27		41	66	67		13	34	58		4	11	12	8	9
bm8	bm3	63				23				10				54				75				60				64				24				11
bm9	bm3	63	66			20	21			7	6			68	82			25	38			70	74				47			11	14			9
bm10	bm3	55				26				3				41				28				38				64				13				32
bm11	bm3	3	5			5	10			1	6			2	9			2	8			4	7				4			46	58			33
b	bm6	79	53	47		26	8	7		42	15	12		5	3	1			17	16		5	2	1			27	25		20	13	17		8
bm1	bm6	14	17			77	80			15	23			7	5				20			9	5				21			24	32			34
bm3	bm6	19	21			21	8			87	60	74		4	1	2			10	15		1	1				8	18		83	60			7
bm8	bm6	27				18				26				0				68				2				30				34				15
bm9	bm6	0	0			0	0			0	4			0	2			0	3			0	1				0			0	0			
bm10	bm6	21				16				18				3				11				1				64				21				35
bm11	bm6	11	4				12			50	39	52		8	4	5		15	11	8		4	4	4		9	10			71	60	68		36

b	bm8	50	63	76		15	19	25		38	22	47		49	38	63		6	2	6		46	31	63		38	20	53		37	21	60		37
bm1	bm8	61	32			85	73			34	33			59	37			6	3			58	34				23			63	42			6
bm3	bm8	21	46			6	21			41	65			29	54			0	3			26	50			10				33	72			38
bm6	bm8	100	50	44	69	64	13	10	31	60	50	34	56	88	64	60	77	7	−1	2	4	86	66	57	77		28	31	62		53	35		2
bm9	bm8	81	61			37	21			67	70			80	78			6	7			77	80				50			16	66			2
bm10	bm8	56				21				38				41				4				41				71				46				39
bm11	bm8	44	15			24	13			63	42			48	22			6	4			38	22				10			68	53			38
b	bm9	79	37	27		22	3	3		45	6	5		11	3	1			15	8		9	1	0			13	15		29	4	8		8
bm1	bm9	23	15			91	78			36	22			6	3				19			7	3				21			37	33			34
bm3	bm9	23	21			20	18			88	73			8	6				19			5	4				18			76	73			7
bm6	bm9	1	0			1	2			2	−1			1	1			1	−1			0	0			1	0			2	1			—
bm8	bm9	26				21				37				10				81				7				42				42				15
bm10	bm9	35	25			16	20			20	19			4	2				12			5	1			64	65			19	20			35
bm11	bm9	12	8			13	14			52	50			5	5				15			6	4			12	10			63	67			36
b	bm10	69	58			21	19			48	45			62	49				24			59	44			7	−1			39	43			40
bm1	bm10	21	22			79	76			21	27			18	23			18	10			16	22			4	2			20	28			41
bm3	bm10	34				21				69				44				17				40				0				65				42
bm6	bm10	75	53			28	24			71	55			78	65			52	22			78	57			4	1			64	53			43
bm8	bm10	30	30			10	14			29	36			27	30			62	50			23	27			3	1			30	35			44
bm9	bm10	66	51			27	21			67	53			74	58				23			76	56			7	2			54	50			43
bm11	bm10	20	17			12	12			49	44			25	21			19	10			23	20			3	2			58	54			45
b	bm11	84	70			30	16			27	14			74	55				34			76	54				45			3	3			46
bm1	bm11	33	24	20		86	76	70		14	9	5		38	23	22			19	6		31	25	9			20	11		2	6	5		10
bm3	bm11	19	4	12		7	1	4		44	14	37		26	8	19			1	7		23	5	18			2	10		3	1	2		47
bm6	bm11	78	42	58	78	32	11	13	28	38	12	14	29	79	56	69	84	52	23	28	54	81	60	70	82	62	32	33	61	9	3	3	10	46
bm8	bm11	58				23				23				50				78				62				60				8				48
bm9	bm11	68	43	78		21	10	31		25	15	29		75	59	84			21	54		73	63	80		40	29	61		4	4	7		46
bm10	bm11	41	66			11	34			13	24			32	57				34			30	56			19	77			4	4			49

Solid line boxes indicate lysis against target cells of responder genotypes. Broken line boxes indicate the only MLC combinations in which no killer cells were generated.

* Results obtained with 2×10^5 effector cells and 1×10^5 target cells.

‡ For derivation of determinants, see Results: Designation of CML Determinants.

§ Experiment number. An identical experiment number refers to the same experiment only for targets tested with the same MLC effector-cell combination.

TABLE IV

CML by Effector Cells from MLC with F_1 Hybrid Responder Cells

MLC H-2 haplotype		Specific lysis* (percent) of target cell H-2 haplotype																								Determinant number‡ (Tables V and VI)
		b			bm1			bm3			bm6			bm8			bm9			bm10			bm11			
Stimulator	Responder	1§	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
bm3	b × bm1	9	10		2	17		89	98		50	75			45		45	55			29		87	84		50
bm6	b × bm1	5	12	9	5	11	2	46	73	48	81	79	68	38	38	6	79	79			27	8	76	77	49	51
bm8	b × bm1	4	15		4	10		18	50		19	45		93	99		22	38			33		44	50		19
bm1	b × bm3	5	13	7	77	75	75	3	16	9	14		15		28	7	14		7		39	10	10		16	52
bm6	b × bm3	10	11		16	12		7	17		83	81			29		78	76			18		20	26		53
bm8	b × bm3	15			17			13			32			90			30				31		29			54
bm1	b × bm6	4			78	77		11	22		3			32			4	10			42		20	50		3
bm3	b × bm6	2			20	12		80	53		6			18			5	5			19		81	66		55
bm8	b × bm6	1			9			15			3			70			7				21		29			20
bm1	b × bm8	4			78	74		17	23		14			4	11		13	18			33		39	40		56
bm3	b × bm8	9			29	13		87	58		55			7			60	23			20		85	62		57
bm6	b × bm8	14	10		35	7	11	55	28	48	86	66		11	2	2	86	31			9	6	87	50		58
b	bm1 × bm3	89	75		5	4		14	13		79	64			50		77	68			71		29	23		5a
bm6	bm1 × bm3	75	72		3	5		11	14		76	77			45		77	79			59		29	31		5b
bm8	bm1 × bm3	58			5			10			38			76			40			48			24			59
b	bm1 × bm6	79	81		6	4		53	40			18			47		16				63		47	47		14
bm3	bm1 × bm6	41	29		7	7		84	75			17			31		16	15			41			41		60
bm8	bm1 × bm6	53	47		6	8		56	45			12		81	77		11	15			52		51	55		61
b	bm1 × bm8	77	81		5	5		61	70		65	79		14			72	77			73		61	71		13
bm3	bm1 × bm8	55	72	40	4	4	3	78	82	74	55	75	50	13	12	2	64	71	23		57	21	82	81	71	62
bm6	bm1 × bm8	73	83		4	5		70	78		74	83		15			80	83			71		74	75		3

b	bm3 × bm6	71	65		18	55		0	8		11				26		12				48		6	11		63
bm1	bm3 × bm6	10			79	80		0	10		3	8			18		4	8			26		14	16		64
bm8	bm3 × bm6	30			55			10			10			70			9			26			18			65
b	bm3 × bm8	84	75		27	18		6	10		77	57			13		75	59			63		14	15		66
bm1	bm3 × bm8	34	25		81	75		9	6		36	30			8		39	32			30		21	28		67
bm6	bm3 × bm8	76	69		25	19		7	9		84	74			8		84	76			51		18			66
b	bm6 × bm8	72	73		15	12		26	30		7	9		10	8		11	7			50		28	27		16
bm1	bm6 × bm8	9	23	9	62	82	64	14	31	15	3	6	5	7	10	1	6	7	2		39	7	16	37	17	68
bm3	bm6 × bm8	12	26		11	22		63	80		4	9		4	8		7	9			34		68	81		69

Boxes indicate lysis of target cells from parents of F_1 responder.

* Results obtained with 2×10^5 effector cells and 1×10^5 target cells.

‡ For derivation of de.erminants, see Results: Designation of CML Determinants.

§ Experiment number. An identical experiment number refers to the same experiment only for targets tested with the same MLC effector-cell combination.

stimulated by bm5 or bm6 lysed not only bm5 or bm6 but also b targets (25). Subsequent analysis has revealed that these anomalous F_1 anti-P CML reactions were a result of segregation of a bm5/6-like mutation in the H-2^b stock used for raising these F_1 hybrids (C. J. M. Melief et al. Unpublished observations.). This has led to the identification of the first H-2 mutant picked up by CML, that was provisionally named KB-96 (19). In our study, in which H-2^b stock that was free of the bm5/6-like mutation was used for raising F_1 hybrids, these anomalous CML reactions were no longer seen (Table IV).

Designation of CML Determinants. The methodology by which CML determinants are identified has been published (25, 35, 36). If a particular MLC effector-cell combination lyses target cells of stimulator-cell genotype, then that target cell, and any other target cell killed to a similar extent, are considered to express determinant X. Target cells that are killed less intensely but still above background are considered to partially express determinant X, designated as (X). Target cells that are not killed are considered to lack the relevant antigenic determinant. We have arbitrarily defined expression of CML determinants as follows:

ABSENCE OF DETERMINANT. Lysis below threshold levels of positivity. These levels are 15% specific lysis (homozygous responders, section 3, Table III) and 20% specific lysis (F_1 hybrid responders, section 4, Table IV).

FULL EXPRESSION OF DETERMINANT. Lysis of target cells of stimulator-cell genotype. Lysis of other targets greater than four-fifths of this value.

PARTIAL EXPRESSION OF DETERMINANT. Lysis of other targets less than four-fifths of this value.

At the 1978 H-2-mutant workshop (19), a common nomenclature was agreed upon for the first 20 K^b CML determinants, 4 of which were detected independently by Forman and Klein (23), Klein and Forman (35), and Geib et al. (36); and by Melief et al. (24, 25). The remaining 16 determinants were identified in our laboratory. The designation of 15 of these and their expression on 4 K^b mutants and the strain of origin, B6-H-2^b, have been published (25).

Table V shows the expression of the first 20 determinants in seven K^b-locus mutants and B6-H-2^b. Each determinant is defined by at least one MLC effector combination (Tables III and IV). Further analysis of the CML data of Tables III and IV allowed the designation of 44 new determinants (determinants 26–69,[2] Table VI). In four instances, two patterns of expression of a particular determinant were distinguished (determinants 2, 5, 9, and 29; a and b). In three of these cases (determinants 2, 9, and 29; a and b), a slight difference of expression was found when either bm6 or bm9 stimulators were used in MLC. Because of the strong evidence that bm6 and bm9 are identical (Discussion), the difference in expression between determinant a and determinant b was likely a result of technical variability. In the fourth case (determinant 5), (bm1 × bm3)F_1 anti-b effector cells showed the same CML reaction pattern as (bm1 × bm3)F_1 anti-bm6 effector cells, except for bm10 target cells, which showed full and partial expression of the relevant determinant, respectively. Because the reactions against bm10 target were only performed once (Table IV) and could not be repeated because of the lack of available mice, the 5a and 5b determinants are considered identical until proven otherwise.

[2] CML determinants 21–25 were defined by J. Klein on the H-2 haplotypes f, fm_1, k, and km_1 (37).

TABLE V

H-2 Antigenic Determinants Defined by CML

H-2 haplotype	H-2 determinants*																						
	1	2		3	4	5		6	7	8	9		10	11	12	13	14	15	16	17	18	19	20
		a	b			a	b				a	b											
b	1	2	2	—	—	5	5	(6)	(7)	8	9	(9)	(10)	11	(12)	13	14	(15)	16	—	—	—	—
bm1	—	(2)	(2)	3	(4)	—	—	6	(7)	(8)	(9)	(9)	10	(11)	—	—	—	(15)	—	(17)	(18)	—	—
bm3	1	(2)	2	(3)	4	—	—	(6)	7	(8)	—	—	—	—	(12)	13	(14)	(15)	(16)	(17)	(18)	19	—
bm11	(1)	(2)	(2)	(3)	4	(5)	(5)	(6)	7	(8)	—	—	—	(11)	(12)	13	(14)	(15)	(16)	(17)	(18)	(19)	(20)
bm8	(1)	—	—	(3)	(4)	(5)	(5)	—	(7)	(8)	(9)	(9)	(10)	11	12	—	(14)	15	—	(17)	18	19	20
bm6	1	2	2	—	(4)	5	5	(6)	—	—	9	9	(10)	(11)	(12)	13	—	—	—	17	(18)	(19)	—
bm9	1	2	2	—	(4)	5	5	(6)	—	—	9	9	(10)	(11)	(12)	13	—	—	—	17	(18)	(19)	—
bm10	1	(2)	(2)	(3)	—	5	(5)	(6)	(7)	(8)	(9)	(9)	(10)	11	(12)	13	(14)	(15)	(16)	(17)	(18)	(19)	(20)

Defining effector cells
bm1 anti-b
bm8 anti-bm6
bm8 anti-bm9
b anti-bm1, b/bm6 anti-bm1
b anti-bm3
bm1/bm3 anti-b
bm1/bm3 anti-bm6
bm8 anti-bm1
bm6 anti-bm3, bm9 anti-bm3
bm6 anti-b, bm9 anti-b
bm3 anti-b, bm3 anti-bm9
bm3 anti-bm6
bm3 anti-bm1, bm11 anti-bm1
bm3 anti-bm8
bm1 anti-bm8
bm1/bm8 anti-b, bm1/bm8 anti-bm6
bm1/bm6 anti-b
bm6 anti-bm8, bm9 anti-bm8
bm6/bm8 anti-b
b anti-bm6, b anti-bm9
b anti-bm8
b/bm1 anti-bm8
b/bm6 anti-bm8

* Number alone indicates full expression of determinant; number in parentheses indicates partial expression of determinant (defined by weak CML reaction); —, indicates absence of determinant. Boxes indicate identity for determinants between two mutants.

TABLE VI

H-2 Antigenic Determinants Defined by CML

H-2 haplotype	H-2 determinants																							
	26	27	28	29 a	29 b	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
b	—	—	(28)	(29)	29	(30)	(31)	32	—	(34)	(35)	—	37	(38)	(39)	40	(41)	(42)	43	(44)	(45)	46	(47)	(48)
bm1	(26)	—	—	—	—	—	—	(32)	—	34	(35)	—	(37)	(38)	(39)	(40)	41	(42)	(43)	—	—	(46)	—	(48)
bm3	(26)	27	28	(29)	(29)	(30)	(31)	—	—	(34)	(35)	(36)	(37)	38	(39)	(40)	(41)	42	43	(44)	45	(46)	47	(48)
bm11	(26)	27	28	(29)	(29)	(30)	31	—	33	(34)	(35)	36	(37)	38	(39)	(40)	(41)	42	(43)	(44)	45	—	—	—
bm8	—	—	(28)	(29)	(29)	(30)	(31)	(32)	—	(34)	—	(36)	—	—	—	(40)	(41)	(42)	(43)	44	(45)	(46)	—	48
bm6	—	(27)	28	29	29	(30)	(31)	(32)	—	—	—	—	(37)	(38)	(39)	40	(41)	(42)	43	(44)	(45)	46	(47)	(48)
bm9	—	(27)	28	29	29	(30)	(31)	(32)	—	—	—	—	(37)	(38)	(39)	40	(41)	(42)	43	(44)	(45)	46	(47)	(48)
bm10	26	—	(28)	(29)	(29)	30	(31)	32	—	(34)	35	—	(37)	—	39	—	—	—	—	—	—	(46)	—	(48)

Defining effector cells
b anti-bm10
b anti-bm11
bm1 anti-bm3
bm1 anti-bm6
bm1 anti-bm9
bm1 anti-bm10
bm1 anti-bm11
bm3 anti-bm10
bm3 anti-bm11
bm6 anti-bm1, bm9 anti-bm1
bm6 anti-bm10, bm9 anti-bm10
bm6 anti-bm11, bm9 anti-bm11
bm8 anti-b
bm8 anti-bm3, bm8 anti-bm11
bm8 anti-bm10
bm10 anti-b
bm10 anti-bm1
bm10 anti-bm3
bm10 anti-bm6, bm10 anti-bm9
bm10 anti-bm8
bm10 anti-bm11
bm11 anti-b, bm11 anti-bm6, bm11 anti-bm9
bm11 anti-bm3
bm11 anti-bm8

H-2 haplotype	H-2 determinants																				
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
b	49	—	—	—	—	—	—	—	—	—	(59)	(60)	(61)	(62)	63	—	(65)	66	(67)	(68)	(69)
bm1	(49)	—	—	52	—	—	(55)	56	(57)	(58)	—	—	—	—	(63)	64	(65)	(66)	67	68	(69)
bm3	(49)	50	(51)	—	—	—	55	(56)	57	(58)	—	60	(61)	62	—	—	—	—	—	(68)	69
bm11	—	50	51	—	(53)	(54)	55	(56)	57	58	(59)	60	(61)	62	—	—	—	—	(67)	(68)	69
bm8	(49)	(50)	(51)	(52)	(53)	54	—	—	—	—	59	(60)	61	—	(63)	—	65	—	—	—	—
bm6	(49)	(50)	51	—	53	(54)	—	—	(57)	58	(59)	—	—	(62)	—	—	—	66	(67)	—	—
bm9	(49)	(50)	51	—	53	(54)	—	—	(57)	58	(59)	—	—	(62)	—	—	—	66	(67)	—	—
bm10	49	(50)	(51)	(52)	—	(54)	—	(56)	(57)	—	(59)	(60)	(61)	(62)	(63)	(64)	(65)	(66)	(67)	(68)	(69)

Defining effector cells
bm11 anti-bm10
b/bm1 anti-bm3
b/bm1 anti-bm6
b/bm3 anti-bm1
b/bm3 anti-bm6
b/bm3 anti-bm8
b/bm6 anti-bm3
b/bm8 anti-bm1
b/bm8 anti-bm3
b/bm8 anti-bm6
bm1/bm3 anti-bm8
bm1/bm6 anti-bm3
bm1/bm6 anti-bm8
bm1/bm8 anti-bm3
bm3/bm6 anti-b
bm3/bm6 anti-bm1
bm3/bm6 anti-bm8
bm3/bm8 anti-b, bm3/bm8 anti-bm6
bm3/bm8 anti-bm1
bm6/bm8 anti-bm1
bm6/bm8 anti-bm3

For explanatory notes, see legend to Table V.

TABLE VII

Number of CML Mismatches and Matches with H-2^b

A. Matching for CML determinants defined by MLC with homozygous responders

			H-2 haplotype						
			bm1	bm3	bm6	bm8	bm9	bm10	bm11
CML mismatches	Type of mismatch								
	+*	−	2	4	2	3	2	3	7
	(+)‡	−	11	6	7	9	7	9	7
	+§	(+)	13	11	5	11	5	7	13
Total number of mismatches			26	21	14	23	14	19	27
CML matches	Type of match								
	+‖	+	0	2	5	1	5	4	0
	(+)¶	(+)	7	11	13	9	13	9	9
	−**	−	3	1	4	3	4	4	0
Total number of matches			10	14	22	13	22	17	9
Undecided			2	3	2	2	2	2	2
Total number of CML determinants			38	38	38	38	38	38	38

B. Matching for all CML determinants including those defined by MLC with F_1 hybrid responders

			H-2 haplotype			
			bm1	bm3	bm6	bm8
CML mismatches	Type of mismatch					
	+*	−	9	11	7	9
	(+)‡	−	18	11	17	17
Total number of mismatches	+§	(+)	17	17	5	16
			44	39	29	42
CML matches	Type of match					
	+‖	+	0	3	8	1
	(+)¶	(+)	10	13	16	11
	−**	−	8	6	9	9
Total number of matches			18	22	33	21
Undecided			2	3	2	3
Total number of CML determinants			64	64	64	64

Data compiled from Tables V and VI.

* Difference between full expression of determinant (full number in Tables V and VI) and absence of determinant.

‡ Difference between partial expression of determinant (number in parentheses) in Tables V and VI and absence of determinant.

§ Difference between full and partial expression of determinant.

‖ Shared fully expressed determinant.

¶ Shared partially expressed determinant.

** Common absence of determinant.

Relatedness among Different K^b Mutants. Two pairs of mutants showed complete (bm6 and bm9) or extensive (bm3 and bm11) sharing of CML determinants. Sharing of determinants or common absence of a determinant is indicated by boxes in Tables V and VI. The identity of bm6 and bm9 for all CML antigens is in agreement with the observation that CML between these two strains is negative (section 3, Table III) and that skin grafts exchanged between these mutants are permanently accepted (R. W. Melvold. Unpublished observations.).

The mutants bm3 and bm11 are closely related although not identical with respect to CML antigens (Tables V and VI). This relationship is also reflected in the finding that most MLC combinations with either bm3 or bm11 stimulator cells yielded strong CML activity against both types of target cells (Tables III, V, and VI). Furthermore, CML generated in reciprocal MLC between these two mutants showed little (bm11 anti-bm3) or no (bm3 anti-bm11) cross-reactivity against third-party targets (Tables III, V, and VI; specificities 47 and 33). A complete absence of CML cross-reactivity was not observed with any other MLC combination. The nonidentity of bm3 and bm11 is in agreement with the observation that these mutants rapidly reject each others skin grafts (R. W. Melvold. Unpublished observation.).

Distance of Each K^b Mutant from H-2^b. From the CML antigen chart presented in Tables V and VI, it was determined to which extent each mutant differed from the strain of origin (H-2^b) by scoring antigen matches and mismatches. In addition, it was determined how many antigens were commonly lacking in both mutant and H-2^b.

Matching was performed in two ways. First, by matching only for those CML antigens defined by MLC with homozygous responders. Second, by matching in addition for those antigens defined by MLC with F_1 hybrid responders.

The mutants bm9, bm10, and bm11 were excluded from the second analysis, because in the distinction of CML determinants by F_1 hybrid responders, they were not used as stimulators nor as one of the parents of the F_1 hybrid responder. The results of both ways of CML matching with H-2^b are shown in Table VII A and B.

Table VII shows that from the total number of matches and mismatches the sequence of relatedness with H-2^b is as follows: bm6/bm9-bm10-bm3-bm8-bm1-bm11; bm6 and bm9 being the closest to H-2^b. The two mutants differing most from H-2^b, bm1 and bm11, do not share a single fully expressed determinant with H-2^b, whereas all other mutants share one to eight fully expressed determinants with H-2^b. H-2^{bm11} appears to differ most from H-2^b, not only because it shows the largest total number of mismatches with H-2^b, but also because seven of these mismatches are of the $+ \leftrightarrow -$ type versus only two mismatches of this type in the H-2^{bm1} mutant.

Discussion

Three new K^b mutants, bm9, bm10, and bm11, behave in MLC and CML like other known K^b mutants. In MLC with the strain of origin, proliferative responses occur in both directions and CTL are generated. H-2^b anti-H-2^{bm1} CTL were phenotypically Lyt23 and arose as a result of cooperation between Lyt1 helper T cells and Lyt23 killer T cell precursors with possibly a minor contribution by Lyt123 cells (38). The CTL are specifically directed against the stimulator cells but, in addition, show cross-killing against several other members of the series of K^b mutants. This indicates that H-2^b shares CML target determinants with the cross-killed mutant targets which are lacking in the new mutant (MLC mutant anti-H-2^b) and that each new mutant shares CML target determinants with other mutants that are lacking in H-2^b (MLC H-2^b anti-mutant). Extensive CML cross-reactivity also occurred with all possible MLC effector-cell combinations among the new mutants and of the new mutants with other K^b mutants. Exceptions were the MLC combinations bm6 anti-bm9 and vice versa, in which no CTL were generated, and MLC bm3 anti-bm11, in which only CTL against targets of stimulator-cell genotype arose.

The chart of the first 20 K^b CML specificities, arrived at during the 1978 H-2 mutant workshop (19), was confirmed with minor modifications and the three new mutants were typed for these determinants (Table V). In addition, 44 new K^b CML determinants were identified and all seven available K^b mutants were typed for these determinants (Table VI).

As discussed previously (23–25, 35, 36), homozygous MLC responders in many instances react to multiple CML determinants. This is illustrated by comparing CML by homozygous responders with CML by F_1 hybrid responders in MLC. For example, b anti-bm8 effector cells kill not only bm8 target cells, but also target cells from all other haplotypes except that of the MLC responder (determinant 18, Table V). Effector cells (b × bm1)F_1 anti-bm8 kill the same targets except for the reaction against bm1 which is eliminated (determinant 19, Table V). A further reduction of cross-kill is seen with (b × bm6)F_1 anti-bm8 effector cells (determinant 20, Table V) in which, apart from bm8, only bm10 and bm11 targets are lysed. Therefore, b anti-bm8 effector cells recognize at least three CML determinants: one present on bm8 and partially shared by all other mutants (determinant 18, Table V), a second expressed on bm8 and bm3 and partially expressed on all other mutants with the exception of bm1 (determinant 19, Table V), and a third expressed on bm8 and partially on bm10 and bm11 (determinant 20, Table V). Cross-killing against third-party targets can also be eliminated by adsorption to third-party monolayers (36) but, like the use of F_1 hybrid responders, this approach is unlikely to eliminate all cross-killing, unless multiple subsequent absorptions are done. This follows from the following set of data. Effector cells bm1 anti-b kill not only b target cells, but also targets from all other mutant lines except bm1 (determinant 1, Table V). Geib et al. (36) have shown that the cross-reactivity against bm3 can be eliminated by absorbing bm1 anti-b effector cells to bm3 monolayers (determinant 5, Table V). Determinant 5 was found to be unique for b of the targets tested (b, bm1, and bm3). We achieved the same result using (bm1 × bm3)F_1 responders (determinant 5, Table V). However, when the other targets were tested, (bm1 × bm3) anti-b effector cells still lysed bm6, bm9, and bm10 targets and to a smaller extent, bm8 and bm11 targets (Table V). It can be predicted that bm1 anti-b effectors absorbed to bm3 monolayers show the same killing pattern.

The F_1 hybrid and absorption experiments indicate that multiple clones of CTL are generated, but the actual number and specificity of all CTL clones generated in a given MLC can best be studied by producing monoclonal cytolytic T-cell lines (39–41). The possibility that even in MLC with F_1 responders multiple CML determinants are recognized indicates that the current chart of determinants is only an approximation of the actual situation. The complexity of K^b CML determinants calls for a mathematical model by which the CML reaction patterns can be analyzed in a systematic fashion. Recently, such a model was developed by Wohlgemuth (42) and was applied to the data from which the first 19 CML determinants were deduced (43).

Although development of monoclonal cytolytic T cell lines as CML-typing reagents and mathematical analysis of the system of K^b CML determinants can be expected to enhance our insight into the organization of this complex immunogenetic system, the current chart of determinants is operationally useful for a number of deductions. First, the chart of determinants allows conclusions concerning the functional relat-

edness among different mutants and concerning the correlation function/structure. The mutants bm6 and bm9 show identical typing for all 64 CML determinants (Tables V and VI). Two sets of additional data indicate that bm6 and bm9 are indeed identical. CML between these two mutants is negative in both directions (Table III) and both mutants share AA substitutions in the K^b molecule (see above: K. Yamaga et al. Unpublished observations.). Three other K^b mutants are phenotypically identical with bm6: bm5 and bm7 and the K^b mutant picked up by CML, KB-96 (19). Together, these mutations form the bg-series. (The bm5, bm6, and bm7 mutations were formerly called bg1, bg2, and bg3, respectively [19]).

The close relationship of the mutants bm3 and bm11 with respect to CML determinants (Tables V and VI) is likewise matched by a structural relationship. Others (see above: B. M. Ewenstein et al. Manuscript in preparation; K. Yamaga et al. Unpublished observations.) found that bm3 and bm11 share an identical AA substitution at position 77 of the K^b molecule. A second AA substitution at position 89 in the K^{bm3} glycoprotein (see above: B. M. Ewenstein et al. Manuscript in preparation; K. Yamaga. Unpublished observations.) explains why bm3 and bm11 also show CML differences. The relationship between these AA substitutions and CML determinants is not direct. If that were the case, bm3 anti-b effector cells should show strong cross-killing of bm11 targets and bm3 anti-bm11 effector cells should show strong cross-killing of b targets, because b and bm11 share an AA residue at position 89 which is substituted in bm3. These predictions are not substantiated by our data, however (Table III). Also, it might be expected that bm3 with two AA substitutions differs more from H-2^b than bm11 with only one AA substitution. The actual analysis of relatedness indicates, however, that bm11 differs more from H-2^b than does bm3 (Table VII). These findings could signify that CML determinants reflect tertiary protein structure rather than direct AA sequences. Thus, the effects of the AA substitutions on the CML specificities are difficult to predict.

The sequence of relatedness of the mutants bm6, bm3, bm8, and bm1 with H-2^b (Table VII) is the same as that published earlier (25), except that it is now clear that bm3 differs more from H-2^b than does bm6. In studies on the role of the K^b molecule in associative recognition and lysis of virus-infected target cells by virus-specific T cells, it was found that the difference b ↔ bm1 completely precluded lysis in T cell-target combinations that shared all other genes in the K, I-A, or I-B regions of the H-2 complex (28, 29). In case of a b ↔ bm8 or b ↔ bm5/6 difference, target-cell lysis was impaired but not prevented (29). We have noted earlier that these findings correspond with the relatedness sequence by CML typing with alloreactive T cells (25).

At first sight, it seems unexpected that the difference b ↔ bm1 completely precludes H-2K-restricted killing in the virus systems, because bm1 shares 10 matches with H-2^b (Table VII). However, there is not a single match for fully expressed determinants of the type + ↔ + between b and bm1 (Table VII), whereas bm8 and bm6 show one and five such matches, respectively. A match for partially expressed determinants of the type (+) ↔ (+) (Table VII) does not necessarily indicate antigenic identity because the partial lysis could be directed against different portions of the fully expressed determinant(s) or against altered determinants. As a working hypothesis, we postulate that for the specificity of H-2 restriction in virus-directed T cell killing, matching for fully expressed determinants, recognized by alloimmune CTL, is crucial,

whereas matching for partially expressed determinants is not important. The hypothesis predicts that in H-2K-associated killing by virus-directed T cells, a more complete failure to lyse would be seen with the combinations b ↔ bm1 and b ↔ bm11, than with the other b ↔ b mutant combinations, a hypothesis which remains to be tested with the bm11, bm3, bm9, and bm10 mutants, but so far holds for the bm1, bm5/6, and bm8 mutants (29). This hypothesis implies that at least some of the CML determinants recognized by alloreactive CTL are identical to the H-2 determinants involved in associative recognition by virus-directed T cells, as postulated elsewhere (44).

Different compatibility requirements prevail in H-2-associated recognition of trinitrophenyl (TNP)-modified targets, where b ↔ bm1 or b ↔ bm3 differences between attackers and targets did not preclude H-2K-associated lysis (45), in contrast to the virus system. Perhaps in T cell killing of TNP-modified targets, the sharing of partially expressed determinants is sufficient.

That limited AA changes in the K^b molecule have such drastic effects on K^b-directed alloreactivity points out how sensitive the T lymphocyte system is to subtle changes in the K^b molecule. Indeed, the complexity at the target-cell and receptor levels reported here may be a reflection of the important biological role of this category of H-2 molecule in associative recognition and elimination of virus-infected cells (5–7). In addition, this complexity may help to explain the observation that virus-directed cytotoxic T lymphocytes cross-react with alloantigens (46, 47).

Recently, it was argued that anti-H-2 CTL are less specific than the H-2-restricted response to the male antigen H-Y, because they show more cross-reactivity (48). However, the intensity of killing in the anti-H-2 response was in many instances greater than in the anti-H-Y response. Therefore, cross-reactivity may have been less conspicuous in the anti-H-Y system. Also cross-killing in the anti-H-Y response might show up if more haplotypes were tested.

The availability of the K^b series of mutants has allowed recognition of individual determinants by exquisitely specific CTL. The extensive cross-reactivity appears to indicate complexity, not lack of specificity. Comparison with the biochemical analysis of Brown and Nathenson (20), Nathenson et al. (21), and others (see above: B. M. Ewenstein et al. Manuscript in preparation; and K. Yamaga et al. Unpublished observations.) shows a remarkable correlation between function and structure and provides further incentives for study of the biological role of K and D (and L) molecules in alloreactivity and associative recognition.

Summary

The fine specificity of alloimmune cytotoxic T lymphocytes (CTL) was investigated in CTL responses across the smallest known H-2 differences, those based on mutation at a single H-2 locus. CTL were generated in all possible mixed lymphocyte culture (MLC) combinations among seven H-2K^b mutants and the mouse strain of origin, C57BL/6 (B6-H-2^b). CTL were also generated in all F_1 hybrid responder/homozygous stimulator-cell combinations among four K^b mutants and B6-H-2^b. CTL activity was measured in cell-mediated lympholysis (CML) against target cells from all K^b mutants and B6-H-2^b. Cross-reactivity against targets other than the MLC stimulator was extensive and led to the distinction of 64 CML target determinants. Two K^b mutants (B6-H-2^{bm6} and B6.C-H-2^{bm9}) showed identical typing for all 64 CML determinants.

CML reactions after MLC between these two haplotypes were mutually negative. The mutants B6-H-2^{bm3} and B6.C-H-2^{bm11} showed identical typing for 47 of the 64 determinants. Their close relationship is in agreement with the finding that H-2^{bm3} anti-H-2^{bm11} CTL were the only ones that exclusively lysed target cells of stimulator-cell genotype.

On the basis of CML typing, the sequence of relatedness of the mutants with H-2^b is as follows: bm6/bm9-bm10-bm3-bm8-bm1-bm11, bm6/bm9 being the closest to, and bm11 the most distant from H-2^b. The extensive cross-reactivity of alloimmune CTL appears to reflect immunogenetic complexity rather than lack of specificity. Comparison with other reports supports the notion that the system of K^b CML determinants, recognized by alloimmune CTL, is at least partially overlapping with the H-$2K^b$ specificity repertoire involved in the associative T cell recognition of virus-infected cells.

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