

T CELL CLONES WITH DUAL SPECIFICITY FOR
M1s AND VARIOUS MAJOR HISTOCOMPATIBILITY
COMPLEX DETERMINANTS*

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M1s gene products, like those of the major histocompatibility complex (MHC), stimulate strong primary mixed lymphocyte culture (MLC) responses, and the precursor frequency of T cells reactive to M1s determinants appears to be as high as those to MHC antigens (1-3). Unlike MHC determinants, however, M1s determinants do not evoke cytotoxic T cell responses (4, 5) nor lethal graft-vs.-host disease (6, 7), fail to act as transplantation antigens (8, 9), and cannot be detected serologically (10, 11). In the case of the strongly stimulatory M1s^a and M1s^d gene products, these determinants do not show demonstrable polymorphism in our hands and do not appear to be recognized in an MHC-restricted fashion (12-14) (although others have reported differently [3]).

Recently, we reported (14) that uncloned mouse spleen cell populations positively selected for reactivity to M1s determinants by repeated stimulation in bulk MLC cultures also show strong proliferative responses to any of five different MHC haplotypes tested. Further analysis showed that these lines of M1s, pan-H-2-reactive T cells could be diverted by subsequent repeated stimulation with cells of a particular H-2 haplotype; this diverted line would lose its reactivity for other H-2 haplotypes but would maintain responsiveness to the selecting MHC haplotype and also to stimulating cells bearing the original, priming M1s determinants. These findings suggested the possibility that individual T cells may be capable of recognizing allogeneic gene products of both the MHC and the *M1s* locus. The present studies provide direct support for this possibility with the use of cloned lines of M1s- and H-2-reactive T cells.

Materials and Methods

Animals. C3H/HeJ, AKR/J, B10.D2, B10.BR, DBA/2, CBA/J, and C57BL/10 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. D1.C, D1.LP, B10.M, B10.RIII, B10.S, and B10.P mice were bred in our own colony.

Cloned T Cell Lines. 20×10^6 B10.D2 lymph node cells were stimulated for 14 d in 10-ml bulk cultures with an equal number of irradiated (3,000 rad) spleen cells. 1×10^5 to 5×10^5 responder cells were serially restimulated at 2-wk intervals with 20×10^6 irradiated stimulator cells also in 10-ml bulk cultures. The medium used was RPMI 1640 with glutamine (0.25 μ g/ml), antibiotics, 2-mercaptoethanol (5×10^{-5} M), indomethacin (10^{-6} M; Sigma Chemical Co.,

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St. Louis, Mo.), and heat-inactivated fetal calf serum (56°C, 30 min, 10% vol/vol). Clones were obtained by limiting dilution 24–48 h after restimulation in flat-bottomed microtiter plates containing 1×10^6 irradiated stimulator cells and medium supplemented with supernatant (25% vol/vol) from Lewis rat spleen cell cultures (5×10^6 cells/ml) stimulated with concanavalin A (5 μ g/ml). 8–10 d later, wells positive for growth were expanded into bulk cultures. Clones derived in this way were maintained by restimulating the cells every 10–14 d at low cell density with fresh irradiated stimulators, medium, and growth factor. The cloning efficiency was ~30%. Subclones were derived similarly.

Assay of Cellular Proliferation. 10–14 d after routine subculture, T cell clones were assayed for proliferation by culturing 10^4 cells and 3×10^5 irradiated spleen cells in 0.2-ml round-bottomed microculture wells. After 2 d, the cultures were pulsed with 0.5 μ Ci [3 H]thymidine and harvested 16–18 h later. No exogenous growth factor was added in these assays.

Results

A test of our cloning procedures is shown in Table I. Lymph node cells from B10.D2 ($H-2^d$, $M1s^b$, herein referred to as d,b) mice were restimulated in bulk cultures five times over a 2-mo period with irradiated (3,000 rad) $H-2$ -incompatible, $M1s$ -incompatible D1.LP (b,a) spleen cells. Several clones were derived from this line and tested in analytical microcultures for their ability to proliferate in response to a panel of irradiated stimulator cells expressing a variety of different $M1s$ and MHC determinants. One of these clones, D5.16, proved to be reactive to $M1s^{a,d}$ and $H-2^b$, with some degree of cross-reactive proliferation with $H-2^f$ as well; this was recloned by limiting dilution to derive four independent subclones that were then tested with the same panel of stimulators. All four subclones showed the same dual specificity for $M1s^{a,d}$ and $H-2^{b,f}$, as expressed by the parent clone.

Table II shows the results with T cell clones generated in the $H-2$ -incompatible, $M1s$ -compatible B10.D2 (d,b) anti-C57BL/6 (b,b) combination. Seven clones were derived from this line and tested as before with a panel of different stimulators. Some of them (4/7), for example C1.3, were reactive only to $H-2^b$ stimulator cells and were unreactive to $H-2^{k,r,s,f}$ or d (self) or to $M1s^{a,d}$. Others (3/7), for example C2.2, could respond to $H-2^b$ and also to stimulators expressing either $M1s^a$ or $M1s^d$ determinants, a finding that confirms at the clonal level an earlier suggestion of ours (13) and of others (12) that $M1s^a$ and $M1s^d$ are probably identical alleles.

TABLE I
Specificity of One Clone and Four Subclones of B10.D2 ($H-2^d$, $M1s^b$) Anti-D1.LP ($H-2^b$, $M1s^a$) T
Cells Tested with Various Stimulator Cells Bearing Different MHC and $M1s$ Determinants

Stimulators*	$H-2$, $M1s$	$[^3\text{H}]$ Thymidine incorporation by clones ($\bar{X} \pm \text{SD}$)				
		D5.16 (Parent clone)	D5.16/1	D5.16/2	D5.16/3	D5.16/4
B10.D2	d,b	299 \pm 89	413 \pm 81	90 \pm 6	104 \pm 12	82 \pm 24
B10	b,b	10,552 \pm 342	4,952 \pm 308	6,620 \pm 942	5,528 \pm 444	11,800 \pm 1,227
B10.BR	k,b	203 \pm 10	253 \pm 73	70 \pm 14	99 \pm 16	86 \pm 24
B10.M	f,b	4,837 \pm 806	1,317 \pm 445	669 \pm 106	470 \pm 99	831 \pm 238
B10.S	s,b	447 \pm 67	513 \pm 20	189 \pm 33	209 \pm 62	138 \pm 31
B10.RIII	r,b	317 \pm 43	339 \pm 48	145 \pm 39	186 \pm 62	112 \pm 26
C3H/HeJ	k,c	396 \pm 45	389 \pm 38	173 \pm 28	346 \pm 34	144 \pm 41
DBA/2	d,a	11,547 \pm 636	4,585 \pm 373	3,436 \pm 303	4,948 \pm 396	4,549 \pm 554
D1.LP	b,a	25,041 \pm 3,125	5,946 \pm 546	4,604 \pm 275	10,024 \pm 1,012	13,557 \pm 1,955
CBA/J	k,d	14,718 \pm 40	4,773 \pm 672	2,331 \pm 114	4,897 \pm 521	7,579 \pm 533
AKR/J	k,a	6,677 \pm 746	1,950 \pm 651	1,934 \pm 270	1,415 \pm 655	3,301 \pm 307

* In this and in subsequent experiments, controls were included to show that all stimulators were able to evoke strong responses by normal B10.D2 lymph node cells.

TABLE II
Specificity of Two B10.D2 ($H-2^d$, $M1s^b$) Anti-C57BL/6 ($H-2^b$, $M1s^b$) T Cell Clones Tested with a Variety of Stimulator Cells Bearing Different MHC and $M1s$ Determinants

Stimulators	$H-2$, $M1s$	$[^3H]$ Thymidine uptake by clones ($\bar{X} \pm SD$)	
		C1.3	C2.2
B10.D2	d,b	52 \pm 11	117 \pm 29
B10	b,b	5,522 \pm 1,178	10,071 \pm 1,745
B10.BR	k,b	119 \pm 78	107 \pm 25
B10.S	s,b	88 \pm 3	87 \pm 15
B10.R.III	r,b	162 \pm 41	118 \pm 5
B10.M	f,b	90 \pm 23	149 \pm 46
D1.LP	b,a	6,509 \pm 1,457	30,066 \pm 2,756
D1.C	d,a	151 \pm 14	23,681 \pm 3,689
DBA/2	d,a	160 \pm 43	31,436 \pm 4,378
CBA/J	k,d	305 \pm 42	27,348 \pm 5,128
AKR/Cum	k,a	149 \pm 29	37,729 \pm 4,164

TABLE III
Specificity of B10.D2 ($H-2^d$, $M1s^b$) Anti-DBA/2 ($H-2^d$, $M1s^a$) T Cell Clones*

Stimulators	$H-2$, $M1s$	$[^3H]$ Thymidine incorporation by clones ($\bar{X} \pm SD$)					
		E7	E4	E8	E19	E26	E21
B10.D2	d,b	162 \pm 10	178 \pm 50	216 \pm 52	287 \pm 119	3,620 \pm 69	2,618 \pm 279
B10	b,b	246 \pm 65	162 \pm 84	169 \pm 28	170 \pm 64	218 \pm 43	184 \pm 57
B10.BR	k,b	204 \pm 21	162 \pm 19	278 \pm 83	289 \pm 95	135 \pm 64	173 \pm 63
B10.R.III	r,b	265 \pm 9	682 \pm 218	19,514 \pm 1,886	1,631 \pm 75	536 \pm 167	292 \pm 40
B10.M	f,b	153 \pm 46	152 \pm 38	531 \pm 356	161 \pm 15	137 \pm 46	100 \pm 13
B10.S	s,b	163 \pm 33	124 \pm 8	2,863 \pm 1,042	173 \pm 15	159 \pm 41	163 \pm 73
B10.P	p,b	170 \pm 50	5,508 \pm 1,587	280 \pm 42	223 \pm 9	146 \pm 25	117 \pm 21
D1.LP	b,a	7,932 \pm 610	6,676 \pm 81	11,210 \pm 1,846	44,788 \pm 5,299	7,105 \pm 825	311 \pm 45
DBA/2	d,a	6,729 \pm 862	4,475 \pm 606	15,442 \pm 1,886	24,075 \pm 4,743	8,455 \pm 414	2,873 \pm 471
CBA/J	k,d	6,799 \pm 450	3,628 \pm 478	13,426 \pm 1,460	35,517 \pm 6,501	16,869 \pm 1,812	179 \pm 49
AKR/J	k,a	4,700 \pm 778	2,813 \pm 727	3,870 \pm 1,031	24,165 \pm 3,225	7,013 \pm 1,610	227 \pm 109
D1.C	d,a	8,858 \pm 274	3,565 \pm 357	24,172 \pm 1,117	22,723 \pm 5,281	10,654 \pm 191	4,748 \pm 341
C3H/HeJ	k,c	242 \pm 49	127 \pm 46	157 \pm 24	684 \pm 297	157 \pm 49	189 \pm 29
Reactivity Pattern	$M1s$	a,d	a,d	a,d	a,d	a,d	—
	$H-2$	—	p,r	r,s	r	d	d

* The proliferative specificity of these various clones was examined in three independent experiments with the same results; in the experiment reported here (B10.A(4R) \times B10.P) F_1 stimulator cells were substituted for B10.P.

A final series of 15 T cell clones was generated in the H-2-compatible, $M1s$ -incompatible B10.D2 (d,b) anti-DBA/2 (d,a) strain combination and tested against five different H-2 haplotypes and also against $M1s^{a,d}$ -disparate stimulators (Table III). 6 of these 15 clones, for example clone E7, showed specificity for $M1s^{a,d}$ only; four others, E4, E8, E19, and E26, were reactive to $M1s^{a,d}$ and to one or another of the H-2 haplotypes tested; significantly, each of these clones showed a different pattern of anti-MHC reactivity. One, clone E26, responded to a self-H-2^d gene product and also to $M1s^{a,d}$. Clone E21 was reactive to self-H-2^d but showed no response to the selecting $M1s^a$ determinants. For four other clones not shown, it was not possible to determine what their apparent specificity might be using this particular panel of stimulators. It should be mentioned that the reactivity patterns of the clones shown in Table III were constant in three consecutive experiments.

Discussion

To date, we have observed a total of eight distinct anti-H-2 reactivity patterns expressed by $M1s^{a,d}$ -responsive T cell clones (Table IV). The point to be emphasized is that in the case of T cells selected for reactivity to $M1s^{a,d}$ determinants, the clones

TABLE IV
Summary of Anti-H-2 Reactivity of M1s^{a,d}-reactive B10.D2 T Cell Clones*

Clone	Selecting determinants [§]	Reactivity to [‡]							M1s ^{a,d}
		H-2 ^b	H-2 ^k	H-2 ^l	H-2 ^r	H-2 ^s	H-2 ^p	H-2 ^d (self)	
D5.16	H-2 ^b ,M1s ^a	+++		++					+++++
D5.14	H-2 ^b ,M1s ^a		++						+++++
C2.2	H-2 ^b	++							+++++
E4	M1s ^a				+		+++++		+++++
E8	M1s ^a				+++++	+			+++++
E19	M1s ^a				+				+++++
E26	M1s ^a							++	+++++

* Data drawn from Tables I, II, and III, except for clone D5.14 (unpublished results).

[‡] For the H-2 responses each (+) indicates the ratio of proliferative responses relative to the M1s response, which is given an arbitrary value of +++++.

[§] M1s or H-2 determinants on stimulators used in bulk cultures before cloning.

showed apparent random anti-H-2 reactivity; the reactivity patterns of these clones were stable upon repeated stimulation. This finding places constraints on the most straightforward explanation for the data, namely that M1s and H-2 determinants cross-react. To sustain this view, one is forced to argue that M1s^{a,d} molecules express a number of different H-2 determinants. Considering the diversity of H-2-reactivity by M1s-reactive clones (Table IV), the apparent nonpolymorphism of M1s^{a,d} products, and the failure to detect these products serologically this possibility seems unlikely; moreover, there is no evidence that genetic tolerance or negative selection to M1s^{a,d} determinants impairs anti-H-2 reactivity (1).

A second, and in our view, more likely possibility, is that the dual reactivity for M1s and H-2 gene products is controlled by two different sets of receptors that function independently from one another. The obvious question here is whether the data bear on the controversial issue of whether one or two receptors are involved in responses to conventional non-MHC antigens (antigen X). Extrapolation to this issue obviously depends on whether M1s^{a,d} determinants fall under the antigen X category. If M1s determinants fall under this category, the data might be taken to support the dual-recognition hypothesis. The definition of antigen X thus becomes crucial. Because H-2-restriction is the hallmark of anti-X responses, the key question is whether anti-M1s^{a,d} responses are H-2 restricted. The evidence here is equivocal. Janeway et al. (3) have reported that under certain conditions, responses to M1s^{a,d} determinants do show H-2-restriction. Our findings are to the contrary; nevertheless, we cannot exclude the possibility that anti-M1s^{a,d} responses are restricted by public H-2 determinants shared between a variety of different H-2 haplotypes. Until this issue is resolved, further speculation on the relevance of the data to the one vs. two receptor controversy seems pointless.

Our own feeling is that M1s^{a,d} determinants might fall under neither the antigen X nor the H-2 alloantigen category. In view of the unusual properties and evident lack of polymorphism of M1s^{a,d} antigens (*vide supra*), one might envisage that responses to these determinants are more akin to reactions to mitogens than to conventional antigen. If so, the receptors for the M1s determinants might be entirely unrelated to the receptors that recognize either allo H-2 or self plus X.

Summary

A high proportion of T cell clones derived from bulk cultures selected to M1s^{a,d} determinants were found to have joint specificity for allo-H-2 determinants, and vice

versa. Significantly, the patterns of H-2 alloreactivity shown by clones selected to M1s^{a,d} determinants appeared to be random. The possible implications of these findings are discussed.

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