ARSONATE-SPECIFIC MURINE T CELL CLONES

I. Genetic Control and Antigen Specificity*

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Thymus-derived lymphocytes exert a number of regulatory and effector activities in the immune system. Evidence has accumulated in recent years that these activities are largely executed by distinct subsets of T cells that can be distinguished by particular cell surface antigens (1, 2). This has led to the concept of a complex network of T cell types, each endowed with its own specific function. Rigorous proof of this concept has been difficult to achieve with heterogeneous cell populations. Recently, it has been possible to establish and maintain for prolonged periods in culture antigen-specific T cell lines (3, 4), paving the way to a more definitive approach to this question. Homogeneous populations of antigen-specific T cells should also prove extremely useful for studies of the chemical nature of the antigen in its activating form, as well as the nature of the T cell antigen receptor itself. In addition, comparison of these parameters between different functional subsets of T cells specific for the same antigenic determinant should be possible.

We report here the establishment, genetic control, and preliminary characterization of the antigen specificity of normal T cell lines specifically responsive to a structurally defined epitope, L-tyrosine-*p*-azobenzenearsonate (ABA-Tyr).[1] It had been established previously (5, 6) that this simple synthetic compound is immunogenic in guinea pigs and mice. Accordingly, A/J mice were immunized with ABA-Tyr, and lymph node cells from the immune animals were used to establish antigen-reactive T cell lines. Strain A/J mice were chosen for this purpose because their anti-ABA antibody response is dominated by a major cross-reactive idiotype (7), providing the possibility of useful markers for the purification and characterization of T cell antigen-specific molecules as well as studies of the regulation of idiotype expression in antibody responses. The functional activity of these ABA-Tyr-specific T cell lines will be the subject of another communication.

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[1] *Abbreviations used in this paper:* ABA-His, L-histidine-azobenzenearsonate; ABA-Tyr, L-tyrosine-*p*-azobenzenearsonate; CFA, complete Freund's adjuvant; Con A, Concanavalin A; FACS, fluorescence-activated cell sorter; Ir, immune response; LNC, lymph node cells; 2-ME, 2-mercaptoethanol; MHC, major histocompatibility complex; PPD, tuberculin-purified protein derivative; SR, stimulation ratio; *xid*, x-linked immune deficiency.

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Materials and Methods

Mice. A/J, A.BY/Sn, C3H.SW/Sn, C57BL/6 (B6), C57BL/10 (B10), B10.A, B10.BR, B10.D2/Sn, and B10.A(5R) strains of mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10.M, B10.S, B10.G, and B10.A(18R) strains of mice were kindly provided by Dr. J. Stimpfling (McLaughlin Institute, Great Falls, MT). B10.A(2R), B10.A(3R), B10.A(4R), and B10.MBR strains of mice were provided by Dr. Chella David (The Mayo Clinic, Rochester, MN). DBA/2 and (C57BL/6 × DBA/2)F_1 (BDF_1) mice were purchased from Diablo Laboratories, Berkeley, CA. C57BL/Ka mice were provided by Dr. Henry Kaplan (Dept. of Radiology, Stanford University, Palo Alto, CA), and (CBA/N × CAL.20)F_1 mice were either bred in our colony or were provided by Dr. R. T. Woodland. Female mice 6–10 wk of age were used in all experiments.

Antigens and Immunizations. The azobenzenearsonate derivatives of tyrosine and histidine were prepared as previously described (5, 6). Tuberculin purified protein derivative (PPD-CT68) from *Mycobacterium tuberculosis* was purchased from Connaught Laboratories, Ontario, Canada. Mice were primed with a total of 0.15 mg of ABA-Tyr emulsified in complete Freund's adjuvant (CFA) (H37Ra; Difco Laboratories, Detroit, MI) in the hind footpads and the base of the tail (8).

Antigen-specific T Cell Proliferative Assay. Lymph node cell suspensions were prepared from draining lymph nodes (inguinal, popliteal, and para-aortic) 7 d after immunization, and 5 × 10^5 viable cells were cultured in 0.2 ml of Click's medium containing 0.5% normal syngeneic mouse serum and supplemented with 5 × 10^{-5} M 2-mercaptoethanol (2-ME), 50 μg ml^{-1} gentamycin, and 2 mM L-glutamine in flat-bottomed microtiter plates (Microtest II; Falcon Labware, Becton, Dickinson & Co., Oxnard, CA). PPD (10 μg ml^{-1}) and concanavalin A (Con A) (1 μg ml^{-1}) were used in some cultures in all experiments as positive controls. The cultures were kept at 37°C/5% CO_2 for 3–4 d. 4 h before harvest, 1 μCi of [^{3}H]thymidine (New England Nuclear, Boston, MA) (20 Ci/mM) was added to each culture. Cells were harvested using a semiautomatic cell-harvester and counted in a Packard Liquid Scintillation Counter (Packard Instrument Co., Downers Grove, IL). The data were expressed as the arithmetic mean of triplicate cultures. In inhibition experiments using monoclonal antibodies, 1 μg of purified protein was added to each well upon initiation of culture.

Monoclonal Antibodies. The hybridoma cell lines used in this investigation are listed in Table I. The cell lines were either obtained from the originators or from the American Type Culture Collection, Bethesda, MD.

To obtain culture supernatants, cells were overgrown in an equal mixture of RPMI 1640 and Ham's F12 containing 10 μg/ml^{-1} each of insulin and transferrin, 2 mM L-glutamine, 10 mM Hepes, 50 μg/ml gentamycin, 5 × 10^{-5} M 2-ME, and in some instances 1% heat-inactivated

TABLE I

Monoclonal Antibodies

Clone	Subclass mouse hybridomas	Specificity	Reference
10-2.16	γ2a, κ	I-$A^{k,f,r,s}$	10
10-3.6	γ2a, κ	I-$A^{k,f,r,s}$	10
17-3-3	γ2a, κ	I-E^k	11
14-4-4S	γ2a, κ	I-E^{k}*	11
Y-17	γ2a, κ	I-E^{k}*	12
HO-13	μ, κ	Thy-1.2	13
	Rat hybridomas		
53.7.3	γ2a	Lyt-1	14
53.6.7	γ2a	Lyt-2	14

* Combinatorial determinant.

fetal calf serum. Hybridoma antibodies were purified from the supernatants by fractionation on protein-A Sepharose as described by Oi (15).

ABA-Tyr Reactive T Cell Lines. ABA-Tyr reactive T cell lines from A/J mice were established as described by Kimoto and Fathman (16). Briefly, the technique was modified as follows: Lymph node cells from ABA-Tyr primed mice were cultured with 100 $\mu g/ml^{-1}$ ABA-Tyr. 2 wk later cells were harvested and serially restimulated every 10-14 d with ABA-Tyr in the presence of irradiated (3,000 rad) A/J spleen cells. The bulk cultures were cloned by limiting dilution or by the fluorescence-activated cell sorter (FACS) after the sixth to ninth restimulation. Cells were cloned at limiting dilution by culturing 0.5-10 T cells per 0.2 ml well of a Falcon Microtest II plate. Each culture contained 2.5×10^5 irradiated A/J spleen cells and 25-250 $\mu g/ml$ of antigen in complete medium supplemented to 20% (vol/vol) with a supernatant from Lewis rat spleen cells stimulated for 24 h with Con A. Alpha methyl mannoside was added at 20 mg/ml to the Con A supernatant to prevent stimulation by Con A. FACS cloning was carried out under identical culture conditions. In this procedure, a single viable cell was deposited into each well of a 96-well Microtest II plate using a Becton Dickinson sorter cloning attachment for the FACS-IV (B-D FACS Systems, Sunnyvale, CA). Wells were positive for growth after 2-4 wk of culture. The cloned cell lines were raised and kept in 10% fetal calf serum/RPMI 1640 supplemented with 5×10^{-5} M 2-ME, 2 mM L-glutamine, penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), 10 mM Hepes (16), plus 0.1%-0.25% supernatant from rat spleen cells stimulated with Con A. The cloning efficiency was ~50% for the limiting dilution technique and 0.8% for the FACS. The reasons for this disparity are unknown, but two possibilities must be entertained. One is that FACS sorting impairs cell viability, and the other is that wells receiving more than one cell, which will occur at higher frequency with limiting dilution, have a higher probability of growth.

Results

Lymph node cells (LNC) from various strains of mice immunized 7 d previously with 0.15 mg of ABA-Tyr were tested for in vitro proliferation to ABA-Tyr (Table II). All strains responded significantly above background, but those of H-2^b haplotype were consistently lower than other H-2 haplotypes tested. The non-H-2 genetic background of the cells had no apparent influence on the response. Thus, C57BL/6, C57BL/10, C57BL/Ka, A.BY, and C3H.SW cells all proliferated only 3-8 times above background, compared with stimulation ratios of 10-42 by non-H-2^b haplotype cells. Dominance or recessiveness of the high responder trait was evaluated using F_1 hybrid offspring between low responder C57BL/6 and high responder DBA/2 parents. Responsiveness proved to be dominant inasmuch as LNC from primed F_1 animals proliferated to the same degree as LNC from DBA/2 mice (Table III).

The influence of the *xid* gene (17) on responsiveness to ABA-Tyr was studied using offspring of CBA/N female by CAL.20 male matings. Lymph node cells from male and female F_1 hybrid animals did not exhibit significant differences in their in vitro proliferative responses to ABA-Tyr (data not shown).

The possible role of suppressor T cells in the relatively poor response of cells from H-2^b mice was addressed in two ways. First, LNC from immune C57BL/6 mice were treated with anti-Lyt-2.2 antibody and complement just before in vitro culture. Depletion of Lyt-2-positive cells from the LNC failed to alter the magnitude of the subsequent proliferative response (data not shown), suggesting that the low responder state was not due to efferent suppression. Second, to explore the possibility that priming with ABA-Tyr induces afferent suppression, C57BL/6 mice were given 20 mg/kg^{-1} body weight of cyclophosphamide 2 d before antigen. Primed LNC from treated and control animals gave proliferative responses to ABA-Tyr that were 2.3- and 2.0-fold above background, respectively (data not shown). Thus, the findings

TABLE II

Proliferative Response to ABA-Tyr by LNC from Various Inbred Strains of Mice

Experiment number	Strain	H-2 haplotype	Δ cpm*	SR‡	Responder designation§
1	B10	b	4,714	6.3	L
	B10.A	a	28,897	42.3	H
	B6/Ka	b	6,646	8.5	L
2	A.BY	b	11,198	3.5	L
	C3H.SW	b	11,155	2.8	L
	B10.A	a	14,618	26.0	H
3	B10.A(18R)	i18	3,774	3.9	L
	B10.A	a	19,774	21.5	H
	B10.S	s	22,182	17.4	H
4	B6	b	4,604	3.4	L
	B10.A	a	16,483	17.9	H
	B10.BR	k	15,038	10.5	H
	B10.D2	d	12,437	9.2	H
5	B10	b	5,699	4.4	L
	B10.M	f	51,309	38.0	H
	B10.S	s	38,828	22.5	H
	B10.G	q	7,921	7.0	?

* Proliferation expressed as cpm obtained in the presence of 400 μg/ml of ABA-Tyr minus cpm obtained from cultures with the responder cells alone.

‡ Stimulation ratio: cpm in the presence of 400 μg/ml of ABA-Tyr divided by cpm in the absence of antigen.

§ L, low responder, H, high responder to ABA-Tyr.

TABLE III

Responsiveness to ABA-Tyr Is Dominant in F_1 Progeny of Low and High Responder Parental Strains

Strain	cpm*	SR‡	Responder designation§
B6	2,379	3.0	L
DBA/2	22,675	13.8	H
BDF1	18,851	19.1	H

*, ‡, § See Table II.

indicate that T suppressor cells were not solely responsible for the low responder status of H-2^b mice to ABA-Tyr. They do not exclude, however, the possible existence of ABA-Tyr-specific suppressor T cells in primed animals.

The genetic control of responsiveness to ABA-Tyr was investigated further using B10 congenic recombinant strains. Low responder status was manifested only by strains which were of b haplotype across the entire I region of the H-2 complex (Table IV). Thus, of the strains tested, only B10 and B10.A(18R) were low responders, whereas strains that were I-A^b [B10.A(3R) and B10.A(5R] or I-E^b [B10.A(4R)], but

TABLE IV
Genetic Restriction of T Cell Proliferation to ABA-Tyr

Experiment number	Strain	H-2 composition				cpm*	SR‡	Responder designation§
		K	A	E	D			
1	B10	*b*	*b*	*b*	*b*	3,711	3.7	L
	B10.A(2R)	k	k	k	*b*	9,748	10.6	H
	B10.A(3R)	*b*	*b*	k	d	23,673	15.6	H
2	B10.A(4R)	k	k	*b*	*b*	12,453	11.5	H
	B10.A(5R)	*b*	*b*	k	d	8,093	7.6	H
3	B10.A(3R)	*b*	*b*	k	d	23,224	13.7	H
	B10.A(4R)	k	k	*b*	*b*	10,485	6.2	H
	B10.BR	k	k	k	k	14,503	7.9	H
4	B10	*b*	*b*	*b*	*b*	7,043	1.6	L
	B10.MBR	*b*	k	k	k	30,850	4.7	H
	B10.D2	d	d	d	d	46,329	5.9	H
5	B10.A	k	k	k	d	14,618	26.0	H
	B10.A(18R)	*b*	*b*	*b*	d	1,111	2.4	L

*, ‡, § See Table II.

TABLE V
Inhibition of T Cell Proliferation to ABA-Tyr by Monoclonal Anti-I-A and Anti-I-E Antibodies

Strain	H-2 composition				Monoclonal antibody*	Average cpm‡	SR§	Percent inhibition
	K	A	E	D				
B10.A(4R)	k	k	b	b	None	4,549	7.2	
					10-2.16	577	0.9	87.3
					17-3-3	3,986	6.3	12.4
B10.A(5R)	b	b	k	d	None	4,740	5.5	
					10-2.16	4,816	5.6	0
					17-3-3	1,356	1.2	71.4
B10.A	k	k	k	d	None	5,970	10.4	
					10-2.16	847	1.5	85.8
					17-3-3	2,016	3.5	66.2

* 1 μg of protein A purified protein per culture.
‡ cpm in cultures of B10.A(4R), B10.A(5R), or B10.A LNC without ABA-Tyr were 642, 855, or 572 cpm, respectively.
§ See note to Table II.

k at the other respective locus, were of high responder phenotype. The indication that I-A and I-E loci were both implicated in the response to ABA-Tyr was further assessed by using monoclonal anti-Ia antibodies to block proliferation (Table V). The proliferative response of B10.A cells was significantly inhibited by both 10-2.16 (anti-I-A^k) and 17-3-3 (anti-I-E^k). On the other hand, B10.A(4R) cells were inhibited only by 10-2.16 and B10.A(5R) cells were inhibited only by 17-3-3, the appropriate monoclonal in each case. The combined results from these experiments support the

conclusion that the responding T cells in the proliferation assay recognize ABA-Tyr in conjunction with I-A and/or I-E gene products.

Cloned T cell lines were obtained by limiting dilution of T cells from long-term culture of A/J LNC. Using the proliferative assay, all clones responded to ABA-Tyr and did not respond to PPD, to which the original LNC were highly responsive. The same clones in different experiments gave markedly different cpm values, so cpm values themselves, rather than ratios, are given in the tables. Although the basis for the variation is unclear, it has been regularly observed in other laboratories working with cloned T cells (E. Sercarz, personal communication). Treatment of cloned cells with monoclonal anti-Thy-1 antibody and complement before the proliferative assay completely abrogated the response to ABA-Tyr, attesting to their Thy-1 positivity.

The proliferative responses to ABA-Tyr by 14 of 15 clones were inhibited to background levels by the 10-2.16 and 10-3.6 monoclonal antibodies (anti-I-A^k) and not by Y-17, 14-4-4S, or 17-3-3 (anti-I-E^k). The data for a representative clone, 16-4, are shown in Table VI. 1 out of the 15 clones tested (clone 16-5) had its proliferation inhibited to background levels by the anti-I-E^k, but not by the anti-I-A^k, monoclonal antibodies (Table VI). This indicated that T cells responsive to ABA-Tyr were either I-A or I-E region restricted. The results also established the validity of the blocking protocol, inasmuch as the antibodies were selective in their activity and were not cytotoxic. It was further shown that clone 16-4 recognized ABA-Tyr presented by B10.A(4R) but not by B10.A(3R) feeder cells, the response to which was totally inhibited by anti-I-A^k but not at all by anti-I-E^k, monoclonal antibody (Table VII).

The I-E^k restricted clone (16-5) only responded to ABA-Tyr in the presence of syngeneic feeder cells (Table VII). Neither B10.A(3R) nor B10.A(4R) feeders presented antigen to this clone. The inability of B10.A(3R) cells to present antigen to clone 16-5 is likely due to the A_e^b, E_β^k phenotype of this strain, inasmuch as the homologous A/J strain, from which the clone was derived, is A_e^k, E_β^k. The blocking

Table VI

Monoclonal Anti-Ia Antibodies Block Proliferation of Cloned T Cells to ABA-Tyr

Clone*	ABA-Tyr	Monoclonal antibody‡	cpm
	μg/ml		
16-4	None	None	77
	25	None	10,899
	25	10-2.16	212
	25	10-3.6	316
	25	14-4-4S	15,958
	25	17-3-3	13,440
16-5	None	None	320
	25	None	5,475
	25	10-2.16	5,295
	25	10-3.6	5,736
	25	14-4-4S	92
	25	17-3-3	271

* 2×10^4 cloned T cells plus 1×10^6 irradiated A/J spleen cells per culture.
‡ See *, Table IV.

TABLE VII
Response of T Cell Clones to ABA-Tyr in the Presence of Feeder Cells with Different H-2 Haplotypes

T cell clone*	Antigen μg/ml	Feeder cells‡	Monoclonal antibody§	Average cpm
16-4	None	A/J	None	434
	ABA-Tyr, 50		None	115,917
	ABA-Tyr, 50		10-2.16	410
	ABA-Tyr, 50		Y-17	156,364
	None	B10.A(4R)	None	68,966
	ABA-Tyr, 50		None	253,375
	ABA-Tyr, 50		10-2.16	1,421
	ABA-Tyr, 50		Y-17	252,767
	None	B10.A(3R)	None	170
	ABA-Tyr, 50		None	478
	ABA-Tyr, 50		10-2.16	179
	ABA-Tyr, 50		Y-17	906
16-5	None	A/J	None	1,643
	ABA-Tyr, 50		None	47,507
	ABA-Tyr, 50		10-2.16	26,203
	ABA-Tyr, 50		Y-17	5,218
	None	B10.A(4R)	None	677
	ABA-Tyr, 50		None	1,248
	ABA-Tyr, 50		10-2.16	198
	ABA-Tyr, 50		Y-17	846
	None	B10.A(3R)	None	517
	ABA-Tyr, 50		None	1,180
	ABA-Tyr, 50		10-2.16	435
	ABA-Tyr, 50		Y-17	1,380

* 2×10^4 cells per culture.
‡ 1×10^6 irradiated spleen cells per culture.
§ See *, Table IV.

studies with monoclonal antibody Y-17 suggest that clone 16-5 recognizes ABA-Tyr in the presence of a combinatorial determinant between A_e and E_β.

The fine antigen specificity of the clones was studied using structural analogs of the homologous antigen, ABA-Tyr, to induce proliferation. The most interesting data were obtained with histidine derivatives of azobenzenearsonate. The clones could be divided into three types with respect to responsiveness to ABA-histidine (ABA-His). One type responded about equally well to ABA-Tyr and 4-ABA-His (Table VIII, clones 16-4 and 16-F1). A second group responded less strongly to 4-ABA-His than to ABA-Tyr (Table VIII, clones 16-2 and 16-F2), whereas a third set gave no response above background to 4-ABA-His (data not shown). In all instances, the 4-ABA-His responsive clones discriminated exquisitely between the 2-azo and 4-azo histidine isomers, responding only to the 4-azo compound (2-azo responses not shown). The responses to 4-ABA-His were blocked by 10-2.16 monoclonal anti-I-A^k antibody, as were responses to ABA-Tyr (Table VIII). Thus, these T cell clones manifest distinctly

TABLE VIII
Fine Specificity of ABA-Tyr Responsive T Cell Clones

Clone*	Antigen 25 μg/ml	Antibody 10-2.16	cpm
16-2	None	None	1,620
	ABA-Tyr	None	117,043
	ABA-Tyr	+‡	387
	4-ABA-His	None	39,202
	4-ABA-His	+	347
16-4	None	None	8,310
	ABA-Tyr	None	60,560
	ABA-Tyr	+	257
	4-ABA-His	None	62,290
	4-ABA-His	+	150
16-F1	None	None	17,817
	ABA-Tyr	None	150,130
	ABA-Tyr	+	97
	4-ABA-His	None	146,897
	4-ABA-His	+	57
16-F2	None	None	6,550
	ABA-Tyr	None	138,290
	ABA-Tyr	+	257
	4-ABA-His	None	12,747
	4-ABA-His	+	97

* See note to Table VI.
‡ 1 μg of protein A purified protein per culture.

different response patterns to closely related compounds, providing extremely useful tools for investigation of T cell specificity, antigen receptors, and cell interaction phenomena.

Discussion

The results presented here demonstrate that (*a*) the murine T lymphocyte response to the simple synthetic antigen ABA-Tyr is under genetic control; (*b*) the genetic control is restricted to the I-A and/or I-E loci of the H-2 complex; (*c*) high-responsiveness is dominant in the hybrid progeny of high and low responder parents; (*d*) cloned lines of ABA-responsive T cells are either I-A or I-E restricted in their responses; and (*e*) clones show marked differences in antigen specificity.

All strains of mice used in this study mounted significant proliferative responses to ABA-Tyr, but H-2^b haplotype strains were markedly weaker than those of other haplotypes, regardless of non-H-2 genetic background. The "low" and "high" responses averaged 3 and 20 times above background in the proliferative assay, respectively (Table II). This genetic control was more precisely mapped using B10 recombinant strains as well as monoclonal anti-Ia^k antibodies to block antigen-induced proliferative responses. Thus, mice that were b haplotype at both the I-A and I-E loci were low responders, whereas those that were non-Ia^b at either locus were high responders (Table IV). Blocking experiments with monoclonal anti-I-A^k and anti-I-E^k reagents (Table IV) supported the conclusion that ABA-Tyr could be

presented to T cells in the context of either I-A or I-E. This conclusion was given more weight by analysis of the genetic restriction of antigen-reactive cloned T cell lines. Of 15 clones tested, only one (16-5) was I-E^k restricted on the basis of blocking with anti-Ia monoclonal antibody (Table VI). Furthermore, this clone did not respond to ABA-Tyr presented by B10.A(4R) feeder cells (I-A^k, I-E^b) (Table VII). However, the experiments using feeder cells of different H-2 haplotype as antigen presenters to clone 16-5 were inconclusive because a strain that was I-A^b_α,A^b_β,I-A^k_e,E^k_β was unavailable. The clone did not respond to antigen presented by B10.A(3R) cells, which are I-A^b_e,E^k_β and thereby differ from A/J in the I-E_β chain.

The cumulative evidence clearly indicates that T cells responsive to ABA-Tyr can "see" antigen in the context of either I-A or I-E, with a decisive statistical preference for I-A. This represents the first demonstration that a particular defined epitope (ABA-Tyr) can be presented to responding T cells in the context of more than one Ia product.

Analysis of the fine antigen specificity of the T cell clones revealed that they fell into three categories with respect to responsiveness to the structurally related compound 4-ABA-His (Table VIII). One group of clones, exemplified by 16-4 and 16-F1, responded to the tyrosine and histidine derivatives equally. A second set, exemplified by clones 16-2 and 16-F2, made responses to the histidine compound that were markedly weaker and readily distinguishable from those to the homologous tyrosine compound. The third group failed to mount responses significantly above background to 4-ABA-His. Thus, the clones manifest clearly differing specificities for nominal antigen, and a detailed structural comparison of their antigen receptors will be of great interest. It was also fascinating that none of the clones responded to 2-ABA-His, which at least appears to be chemically similar to the 4-isomer. It would be of obvious interest to determine whether T cells specific for 2-ABA-His can be induced, or if there is an inherent inability to present this compound to T cells in an activating form.

Summary

The antigen-induced proliferative response of lymph node cells (LNC) from mice sensitized to the monofunctional antigen L-tyrosine-*p*-azobenzenearsonate (ABA-Tyr) was used to monitor genetic control. All strains tested mounted significant responses, but those that were H-2^b at both the I-A and I-E loci [B10., B6., B10.A(18R), A.BY, and C3H.SW] gave consistently weaker responses than other haplotypes. The F_1 progeny of matings between high and low responder phenotype parents (DBA/2 and B6, respectively) were high responders, establishing the dominance of the responder trait. Proliferative responses of LNC to ABA-Tyr were blocked by the appropriate anti-Ia monoclonal reagents. For example, B10.A(4R) LNC (I-A^k, I-E^b) were blocked by anti-I-A^k, whereas B10.A(3R) LNC (I-A^b, I-E^k) were blocked by anti-I-E^k. Long-term cultures of T cell lines specifically reactive to ABA-Tyr were established from LNC of A/J mice immunized with ABA-Tyr and were cloned by limiting dilution. The proliferative responses to ABA-Tyr of 14 out of 15 clones tested were I-A restricted on the basis of activation by antigen-presenting cells from appropriate recombinant strains and the blocking activity of the monoclonal anti-Ia antibodies. The response of the other clone was I-E restricted. The fine antigen specificity of the clones was studied using structural analogs of the homologous antigen to induce proliferation.

The clones could be divided into three types with respect to responsiveness to ABA-histidine (ABA-His). One group responded about equally well to ABA-His and ABA-Tyr. A second set responded less strongly to ABA-His than to ABA-Tyr, while the third showed no response above background to ABA-His. In all instances, the ABA-His-responding clones discriminated exquisitely between the 2-azo and 4-azo histidine isomers, responding only to the 4-azo compound. These T cell clones provide extremely useful tools for studies of T cell specificity, antigen recognition and lymphoid cell interaction systems.

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