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A SINGLE MONOCLONAL ANTI-Ia ANTIBODY INHIBITS ANTIGEN-SPECIFIC T CELL PROLIFERATION CONTROLLED BY DISTINCT Ir GENES MAPPING IN DIFFERENT H-2 I SUBREGIONS[1]

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A xenogeneic rat anti-mouse Ia monoclonal antibody, M5/114 (γ_{2b},κ), was studied for its effects *in vitro* on T cell proliferative responses. Strain distribution studies revealed that M5/114 could inhibit I-A subregion-restricted T cell responses of the H-$2^{b,d,q,u}$ but not the H-$2^{f,k,s}$ haplotypes, indicating that this xenoantibody recognizes a polymorphic determinant on mouse Ia molecules. This same monoclonal antibody was found to inhibit BALB/c (H-2^d) T cell proliferation to both $G^{60}A^{30}T^{10}$ and $G^{58}L^{38}\phi^4$. The *Ir* genes regulating responses to these antigens map to either the I-A subregion (GAT), or the I-A and I-E subregions (GLϕ), raising the possibility that M5/114 recognizes both I-A and I-E subregion-encoded Ia glycoproteins. It could be shown, using appropriate F_1 responding cells, that M5/114 does in fact affect GAT and GLϕ responses by interaction with both the I-A and the I-E subregion products, and not by any nonspecific effect resulting from binding to the I-A subregion product alone. These results are consistent with genetic and biochemical studies directly demonstrating that M5/114 recognizes $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecular complexes. The existence of a shared epitope on I-A and I-E subregion products suggests the possibility that these molecules arose by gene duplication. Finally, the precise correlation between the Ia molecules recognized by M5/114 and the ability of this antibody to block T cell responses under *Ir* gene control strengthens the hypothesis that Ia antigens are *Ir* gene products.

Monoclonal antibodies (Mab)[3] are powerful reagents for both preparative and analytic purposes. They have proven extremely useful in experiments aimed at elucidating the molecular basis of immune response (*Ir*) gene function and the mechanism of Ia-restricted T cell activation (1–4). Recent reports have demonstrated that anti-Ia Mab can selectively inhibit T cell proliferative responses under *Ir* gene control mapping to the same subregion as the genes coding for the Ia molecules recognized by the monoclonal antibodies. This is true of both I-A and I-E subregion-controlled responses (1, 4). In experiments using mouse-mouse hybridomas producing allospecific antibodies, this subregion selective blocking can be shown to occur despite binding of antibodies to I-A and to I-E products on the same Ia^+ antigen-presenting cell (APC), which can be lysed by either Mab (4).

During the screening of hybridomas produced by xenoimmunization of a (BN × Lewis) rat with C57BL/6 lymphoid cells (5), an antibody with strong binding to resting B cells but not T cells was detected. As documented in a separate report (6), this antibody was found to recognize mouse Ia molecules. Despite evidence that this antibody recognized a polymorphic determinant on mouse Ia molecules, and hence was not a general anti-framework antibody (in contrast to the mouse anti-HLA antibody W6/32 (7)), it was found that this monoclonal product inhibits T cell response to antigens controlled by *Ir* genes mapping to distinct I subregions. This report describes these functional studies and demonstrates that this activity results from recognition of the products of two different I subregions. A separate report examines this unique antibody in greater detail by biochemical and immunogenetic methods, and confirms the interpretation of the functional studies that this single antibody recognizes a determinant shared by I-A and I-E subregion-coded molecules. The complete correlation between *Ir* gene subregion assignment, the molecular specificity of M5/114 Mab, and inhibition of *Ir* gene-controlled T cell proliferation in a number of different H-2 haplotypes provides further support for the idea that Ia antigens are *Ir* gene products.

MATERIALS AND METHODS

Animals. BALB/c (H-2^d) mice were obtained from Charles River Breeding Laboratories, Wilmington, MA. A/J, (H-2^a), (C57BL/6J [H-2^b] × A/J)F_1 ((B6A)F_1), (BALB/c × A/J)F_1 (CAF_1), C57BL/6J, B10.D2 (H-2^d), B10.A (H-2^a), and B10.BR (H-2^k) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. All other mice were from our colony in the Department of Pathology. Animals were fed lab chow and chlorinated water *ad libitum* and used at 8 to 20 wk of age.

Antigens. The random terpolymer L-glutamic $acid^{60}$-L-$alanine^{30}$-L-$tyrosine^{10}$ (GAT), m.w. 30 to 50 × 10^3 was obtained from Vega-Fox Biochemicals, Tucson, AZ. The random terpolymer of L-glutamic $acid^{58}$-L-$lysine^{38}$-L-$phenylalanine^4$ (GLϕ), was obtained from Miles, Kankakee, IL. Both antigens were dissolved individually in saline with 5% Na_2CO_3, neutralized with HCl to pH 7 to 7.2, filtered, and stored as sterile solutions at either 4°C or −20°C, until used. Ovalbumin (Miles, Kankakee, IL) was dissolved in saline, sterile filtered, and stored at 4°C until used.

Hybridomas. The mouse-mouse B cell hybridoma 10.2.16 (IgG2b), which produces antibody directed against the I-A public determinant Ia.17, shared by H-2^k and H-2^r (8), was obtained from the Salk Institute, La Jolla, CA. The production and detailed characterization of the xenogeneic rat-mouse hybridoma M5/114 is described in a separate paper (6). Hybridomas were maintained by serial passage in Dulbecco's MEM with 4500 mg/ml glucose plus 5 to 10% heat-inactivated fetal calf serum (FCS). Supernatants of such cultures, taken just before cell death from medium exhaustion and containing 10 to 50 μg/ml specific antibody, were collected by centrifugation, sterile filtered, and stored in aliquots at −20°C. The other xenogeneic rat-

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[3] Abbreviations used in this paper: Mab, monoclonal antibody; *Ir*, immune response; GAT, random terpolymer of L-glutamic $acid^{60}$-L-$alanine^{30}$-L-$tyrosine^{10}$; GLϕ, random terpolymer of L-glutamic $acid^{58}$-L-$Lysine^{38}$-L-$phenylalanine^4$; APC, antigen-presenting cell; RPMI-5, RPMI 1640 medium containing 5% fetal calf serum; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

TABLE I

Monoclonal antibodies used in this study

MAb	Subclass	Target Antigen	Reference
M5/114	γ2b	I-$A^{b,d,q}$, I-$E^{d,k}$	6[a]
10.2.16	γ2b	I-$A^{k,f}$	8
M5/24	γ2b	Lyt 2, 3	Davignon *et al.*, unpublished observations
M5/49	γ2a	Thy-1	5
M5/54	γ2c	Thy-1	5

[a] These assignments are based on quantitative fluorescence measurements, cross-inhibition studies with subregion specific anti-Ia hybridomas, cytotoxicity studies, and two-dimensional gel electrophoresis analysis, as documented in Reference 6.

mouse hybridomas mentioned in Table I were prepared in the same manner (5).

Immunizations. Mice were immunized in the base of the tail and the four footpads with a total of 0.2 ml of an emulsion of antigen and complete Freund's adjuvant (Difco, Detroit, MI) containing 2 mg/ml H37Ra mycobacteria. The total antigen dose was 100 μg/animal.

In vitro T cell proliferation. Draining lymph nodes were removed from primed mice 4 to 10 days after immunization, teased in RPMI 1640 medium containing 5% heat-inactivated FCS and 10 mM HEPES[3] (MA Bioproducts) RPMI-5), filtered through nylon mesh, washed once, and resuspended in RPMI-5. T cell-enriched populations were prepared by nylon wool column filtration according to Julius *et al.* (9). The recovered T cells were washed and resuspended in RPMI 1640 containing 2× L-glutamine, 1× nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated FCS, 10 mM HEPES, and 5×10^{-5} 2-mercaptoethanol (complete medium). 4×10^5 viable cells were placed in the wells of flat-bottom microtiter plates (Linbo Chemicals, Hamden, CT) to which were added, as indicated, medium, GAT, ovalbumin, or GLϕ (final concentration 100 μg/ml) and hybridoma supernatants to the given final concentration. In some experiments, 2×10^5 1500-R gamma-irradiated Tris-NH_4Cl-treated spleen cells, anti-Thy 1.2 + C-treated spleen cells, or G10-passed spleen cells were added to cultures. All wells contained a total volume of 0.2 ml. Cultures were incubated for 3 days at 37°C in 95% air-5% CO_2, then pulsed for 18 hr with 1 μCi/well ^{3}H-thymidine in 25 μl medium. Cultures were harvested on a MASH II (MA Bioproducts), and the filters were processed for liquid scintillation counting. Data are expressed as Δcpm = (cpm of cultures containing all additions − cpm of cultures containing same additions without antigen). Inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 1 - \left[\frac{\Delta\text{cpm in the presence of Mab}}{\Delta\text{cpm without Mab}}\right] \times 100\%$$

SEM were approximately ≤15% of the given mean values and are omitted for clarity. On the basis of both this variation in replicate samples and control experiments with various irrelevent hybridoma supernatants and control medium, values of inhibition of ≤35% in any given experiment have proved to be insignificant and not reproducibly observed in repeat experiments, whereas inhibitions ≥45% invariably indicate reproducible and significant inhibition.

Antibody and C treatments. Nylon-passed lymph node cells or Tris-NH_4Cl-treated spleen cells were resuspended in culture supernatant of M5/114 at 40×10^6 cells/1 ml, or $(NH_4)_2SO_4$ fractionated monoclonal anti-Thy 1.2 (HO 13.4 (10)) at 10^8 cells/ml RPMI-5 containing 50 μg purified antibody, respectively, incubated for 30 min at 4°C, washed one time, resuspended to the original volume in 1:6 diluted Low-Tox rabbit C (Accurate Scientific, Hicksville, LI, NY) incubated 45 min at 37°C, washed three times, and resuspended in complete medium for use.

G10 treatment. Spleen cells were passed through Sephadex G-10 columns as described previously (11), using 10^7 cells/10 ml beads.

RESULTS

M5/114 recognizes mouse Ia molecules. As documented in detail elsewhere (6) M5/114 binds to mouse Ia cell surface glycoproteins. This is consistent with the strong fluorescence observed after staining resting B but not T lymphocytes with M5/114 antibody followed by fluoresceinated rabbit anti-rat immunoglobulin (6). Further, one-dimensional SDS-PAGE gel analysis of M5/114 antibody precipitates of detergent solubilized ^{125}I surface-labeled mouse lymphocytes revealed bands at 28K and 33K, consistent with the β- and α-chains of murine Ia glycoproteins (12) (data not shown).

Inhibition of T cell proliferation by M5/114-derived antibody. Previous work from this and other laboratories has documented the ability of monoclonal anti-Ia antibodies to inhibit I region-restricted T cell proliferative responses (1, 4). Table II presents data demonstrating that M5/114 antibody, as expected for an anti-I-A subregion-reactive antibody, completely blocks the *Ir* gene-controlled T cell proliferative response of H-2^d BALB/c mice to GAT. Three other rat-mouse hybridoma products, including one of the same heavy chain isotype as M5/114 and with the ability to bind to cells in the cultures, fail to show such blocking activity. Thus, the inhibitory activity of M5/114 is not simply due to nonspecific effects of xenogeneic hybridoma culture supernatant in this assay system.

The experiment shown in Table III indicates that, as expected, the anti-Ia M5/114 antibody appears to be directed toward determinants on the adherent accessory cells, and not the T cells in the proliferative cultures. Thus, treatment of the responding lymphoid population with M5/114 antibody plus C eliminates antigen-driven proliferation, as in the blocking experiment. The response can be reconstituted with unseparated and irradiated syngeneic spleen cells and anti-Thy 1.2 + C-treated syngeneic spleen cells, but not by adherent cell-depleted, T cell-containing irradiated spleen cells. These data suggest that at least before culture, the responding T cells are Ia^- with respect to determinants recognized by M5/114 antibody, and are consistent with the interpretation that blocking occurs by interference with T cell recognition of Ia on the APC in the culture.

The potency and specificity of the M5/114 antibody is illustrated in Table IV. Highly significant blocking is seen with as little as 50 to 100 ng/ml antibody added to cultures of GAT-primed BALB/c cells. The same antibody fails to have any significant effect on the proliferative response of A/J (H-2^a) T cells to GAT, even at 20 μg/ml, although this response can be easily inhibited by less than 0.5 μg/ml of the anti-I-A^k-directed 10.2.16 antibody. Thus, the inhibitory activity of M5/114 can be seen at extremely low antibody concentrations, and yet can be exquisitely specific even at high concentrations, as expected for a Mab. Further, the failure of M5/114 antibody to

TABLE II

Effects of rat monoclonal anti-mouse antibodies on GAT-specific T cell proliferation[a]

Responding Cells	MAb	Ig Class	Response (Δ cpm)	% Inhibition
BALB/c GAT primed T			62,350	
	M5/114	γ2b	621	99
	M5/24	γ2b	61,141	1
	M5/49	γ2a	90,047	0
	M5/54	γ2c	59,575	5

[a] Nylon wool-purified T cells from antigen (GAT)-primed BALB/c mice were incubated in the presence or absence of antigen (GAT) with or without added Mab. ^{3}H-thymidine incorporation and inhibition of proliferation were measured as described in *Materials and Methods.*

TABLE III

Treatment with M5/114 and C eliminates macrophages but not T cells required for GAT-induced T cell proliferation[a]

Responding Cells	Added Filler Cells	Response (Δcpm)
C treated		53,656
C treated	γR spleen	119,747
C treated	α-Thy 1 + C treated γR spleen	95,501
C treated	G10 passed γR spleen	64,619
M5/114 + C treated		722
M5/114 + C treated	γR spleen	36,146
M5/114 + C treated	α-Thy 1 + C treated γR spleen	56,505
M5/114 + C treated	G10 passed γR spleen	4,545

[a] Nylon wool-purified T cells from GAT-primed BALB/c mice were treated with C alone or M5/114 + C, then used as responder cells with soluble GAT as antigen in the absence or presence of the indicated filler cells.

TABLE IV

Titration of M5/114 MAb-mediated inhibition of GAT-specific T cell proliferation[a]

Responding Cells	Antigen	MAb	[MAb]	Response (Δcpm)	% Inhibition
BALB/c GAT-primed T	100 μg/ml GAT			58,535	
BALB/c GAT-primed T	100 μg/ml GAT	M5/114	25%	52	>99
BALB/c GAT-primed T	100 μg/ml GAT	M5/114	8.3%	484	>99
BALB/c GAT-primed T	100 μg/ml GAT	M5/114	2.8%	196	>99
BALB/c GAT-primed T	100 μg/ml GAT	M5/114	0.9%	678	98
BALB/c GAT-primed T	100 μg/ml GAT	M5/114	0.3%	11,108	81
BALB/c GAT-primed T	100 μg/ml GAT	M5/114	0.1%	29,676	49
BALB/c GAT-primed T	100 μg/ml GAT	10.2.16	25%	69,130	0
A/J GAT-primed T	100 μg/ml GAT			25,262	
A/J GAT-primed T	100 μg/ml GAT	M5/114	25%	25,981	0
A/J GAT-primed T	100 μg/ml GAT	M5/114	8.3%	32,143	0
A/J GAT-primed T	100 μg/ml GAT	M5/114	2.8%	28,989	0
A/J GAT-primed T	100 μg/ml GAT	10.2.16	25%	1,648	93
A/J GAT-primed T	100 μg/ml GAT	10.2.16	8.3%	6,020	76
A/J GAT-primed T	100 μg/ml GAT	10.2.16	2.8%	8,622	66
A/J GAT-primed T	100 μg/ml GAT	10.2.16	0.9%	21,119	16

[a] See footnote to Table II.

affect A/J T cell responses to GAT indicates that M5/114 antibody is directed to a polymorphic determinant of mouse Ia molecules.

Genetic control of the polymorphic expression of the determinant recognized by M5/114 was further explored in a strain distribution study, a summary of which is presented in Table V. These data show that M5/114 antibody blocks I-A subregion-controlled proliferative responses using cells from strains possessing $I\text{-}A^{b,d,q,u}$ but not $I\text{-}A^{f,k,s}$. These results are in complete agreement with quantitative binding, fluorescence, and cytotoxicity data (6), and indicate that this antibody does not recognize a species-specific framework determinant on mouse Ia molecules.

Activity of M5/114 antibody in blocking proliferation to antigens under the control of Ir genes mapping in I-A and I-E subregions. In previous reports of monoclonal anti-Ia inhibition of T cell responses, if the antibody recognized a polymorphic determinant of Ia and was not against a species framework determinant, the blocking activity was restricted to antigens whose presentation was associated with only one subset of I subregion products, e.g., only I-A- or only I-E-restricted responses (1, 4). Table VI reveals that in clear contrast to these data, including our own study in the same strain and with the same antigens, but with mouse-mouse subregion-specific hybridoma antibodies (4), M5/114 potently inhibits *both* the GAT (I-A-associated) and GLϕ (I-E-associated) T cell proliferative responses. Because the evidence that these antigens stimulate T cells only in the context of the indicated Ia products is extremely strong (13, 14), it would seem that two types of hypotheses could explain the present results: 1) because the APC have been shown to have both I-A and I-E products (4, 15, 16), if M5/114 recognized either Ia molecule, it could prevent responses to both by killing or in some manner 'inactivating' the APC; or 2) M5/114 could recognize a polymorphic determinant associated with *both* I-A and I-E subregion products, and by binding to each, inhibit the relevent response. Tables VII and VIII present experiments ruling out the first explanation. In Table VII, inhibition studies were carried out using F_1 mice able to respond to both GAT and GLϕ, but whose genotype and/or phenotype allowed us to determine whether M5/114 was eliminating all APC or selectively blocking responses to antigen seen in the context of a particular Ia molecule. In all cases, M5/114 could bind to the F_1 APC, as predicted genetically and shown experimentally. The results clearly reveal that merely binding to APC does not permit M5/114 antibody to interfere with responses to antigens not using M5/114 antibody-recognized Ia molecules for T cell activation. For example, $(B6A)F_1$ mice, although derived from parents that are both GAT responders, show preferential stimulation by GAT in association with $I\text{-}A^k$ rather than $I\text{-}A^b$ gene products (unpublished observations). As seen in Table VI, 10.2.16 antibody blocks the $(B6A)F_1$ response to GAT 88%, whereas M5/114 only blocks 23%. Nonetheless, when tested on $(B6A)F_1$ responses to GLϕ, which involve E^b_β-E^k_α complementation (14), M5/114 antibody, but not 10.2.16 antibody, inhibits significantly. Thus, M5/114 antibody can bind to $(B6A)F_1$ accessory cells without having inhibitory activity, indicating it does not lyse or inactivate the APC. Similar data were obtained using CAF_1 mice, although the reciprocal parental preference for GAT responses is seen in this case.

TABLE V

H-2 genetic regulation of inhibitory activity of M5/114 on T cell proliferation[a]

Expt. No.	Responding Cells	Antigen	H-2 haplotype	MAb	Response (Δcpm)	% Inhibition	Status
1	B10.D2	GAT	ddddddd		24,068		
	B10.D2	GAT	ddddddd	M5/114	1,954	92	+
	B10.D2	GAT	ddddddd	10.2.16	36,566	0	−
	B10.A	GAT	kkkkkdd		19,220		
	B10.A	GAT	kkkkkdd	M5/114	26,618	0	−
	B10.A	GAT	kkkkkdd	10.2.16	2,768	90	+
	B10.BR	GAT	kkkkkkk		20,619		
	B10.BR	GAT	kkkkkkk	M5/114	20,072	3	−
	B10.BR	GAT	kkkkkkk	10.2.16	3,743	82	+
2	B10	GAT	bbbbbbb		36,012		
	B10	GAT	bbbbbbb	M5/114	485	98	+
	B10	GAT	bbbbbbb	10.2.16	33,152	8	−
	B10.A(4R)	GAT	kkbbbbb		28,329		
	B10.A(4R)	GAT	kkbbbbb	M5/114	25,271	11	−
	B10.A(4R)	GAT	kkbbbbb	10.2.16	2,823	90	+
3	B10.AQR	OVA	qkkkkdd		23,085		
	B10.AQR	OVA	qkkkkdd	M5/114	31,873	0	−
	B10.AQR	OVA	qkkkkdd	10.2.16	847	96	+
	B10.G	OVA	qqqqqqq		11,789		
	B10.G	OVA	qqqqqqq	M5/114	−431	>99	+
	B10.G	OVA	qqqqqqq	10.2.16	15,154	0	−
	B10.PL	OVA	uuuuuuu		20,841		
	B10.PL	OVA	uuuuuuu	M5/114	81	>99	+
	B10.PL	OVA	uuuuuuu	10.2.16	17,028	18	−
	B10.S	OVA	sssssss		24,939		
	B10.S	OVA	sssssss	M5/114	16,355	34	−
	B10.S	OVA	sssssss	10.2.16	25,751	0	−
	B10.S(9R)	OVA	ss_kkdd		17,188	0	
	B10.S(9R)	OVA	ss_kkdd	M5/114	12,564	27	−
	B10.S(9R)	OVA	ss_kkdd	10.2.16	11,345	33	−
4	A.TRF4	GAT	ff____d		49,870		
	A.TRF4	GAT	ff____d	M5/114	40,470	19	−
	A.TRF4	GAT	ff____d	10.2.16	14,212	72	+
	B10.M	GAT	fffffff		23,815		
	B10.M	GAT	fffffff	M5/114	23,011	3	−
	B10.M	GAT	fffffff	10.2.16	6,565	72	+

[a] See footnote to Table II.

TABLE VI

M5/114 inhibition of BALB/c T cell proliferation to GAT and GLϕ[a]

Responder Cells	Antigen	MAb	Response (Δcpm)	% Inhibition
BALB/c	GLϕ		33,646	
	GLϕ	M5/114	1,301	96
BALB/c	GAT		57,220	
	GAT	M5/114	−21	100

[a] See footnote to Table II.

TABLE VII

Specificity of M5/114 inhibitory effect on T cell proliferation[a]

Expt. No.	Responder Cells	Antigen	MAb	Response (Δcpm)	% Inhibition
1	A/J	GAT		14,850	
	A/J	GAT	M5/114	10,564	29
	A/J	GAT	10.2.16	821	95
	B6	GAT		14,950	
	B6	GAT	M5/114	246	98
	B6	GAT	10.2.16	12,674	15
	(B6A)F_1	GAT		56,404	
	(B6A)F_1	GAT	M5/114	43,354	23
	(B6A)F_1	GAT	10.2.16	6,984	88
	(B6A)F_1	GLϕ		11,259	
	(B6A)F_1	GLϕ	M5/114	391	97
	(B6A)F_1	GLϕ	10.2.16	7,046	37
2	A/J	GAT		24,537	
	A/J	GAT	M5/114	35,807	0
	A/J	GAT	10.2.16	6,614	73
	B/C	GAT		58,998	
	B/C	GAT	M5/114	80	>99
	B/C	GAT	10.2.16	71,664	0
	CAF_1	GAT		14,941	
	CAF_1	GAT	M5/114	4,469	70
	CAF_1	GAT	10.2.16	20,832	0
	CAF_1	GLϕ		12,146	
	CAF_1	GLϕ	M5/114	129	98
	CAF_1	GLϕ	10.2.16	11,479	5

[a] See footnote to Table II.

TABLE VIII

Ir gene-related blocking of T cell proliferation by M5/114 MAb[a]

Responding Cell	Antigen	MAb	Response (Δcpm)	% Inhibition
(B10.T(6R) × B10.M)F_1	GAT		13,078	
(B10.T(6R) × B10.M)F_1	GAT	M5/114	8,951	32
(B10.T(6R) × B10.M)F_1	GAT	10.2.16	0	>99
(B10.T(6R) × B10.M)F_1	GLϕ		11,482	
(B10.T(6R) × B10.M)F_1	GLϕ	M5/114	3,998	65
(B10.T(6R) × B10.M)F_1	GLϕ	10.2.16	9,857	14

[a] See footnote to Table II.

The ability of M5/114 and reciprocally of 10.2.16 (8) to distinguish I-A^f from I-A^q (Table V) permitted an additional test of the reactivity of this antibody with I-A and I-E products. H-2^q mice are responders to GLϕ (17) but nonresponders to GAT (18), and in primed (responder × nonresponder)F_1 mice, presentation of GAT in the context of H-2^q is not observed. H-2^f mice are GAT responders (19) but GLϕ nonresponders (18), the reverse of H-2^q animals. F_1 mice between I^q and I^f mice, (B10.T(6R) × B10.M), were primed with *both* GAT and GLϕ, and their lymph node T cells were used for proliferation assays in the presence of M5/114 or 10.2.16 antibodies. As seen in Table VIII, both of these antibodies can be shown to react with the APC and block a T cell response, but each only blocks the response associated with the I subregion product it can recognize. Again, because products of both haplotypes are known to be on the same APC, this establishes that M5/114 activity is the result of specific blocking and not cell lysis, and that inhibition of both GAT and GLϕ responses observed using BALB/c T cells must result from recognition by M5/114 antibody of I-A *and* I-E subregion products on the APC.

DISCUSSION

The data presented above indicate that M5/114 xenogeneic rat anti-mouse Ia Mab is capable of potent *in vitro* blocking of antigen-specific T cell proliferation. This blocking occurs when the antigen being used is presented in the context of an I-A molecule recognized by M5/114, which detects a polymorphic determinant present in $I^{b,d,q}$- but not $I^{k,f,s}$-bearing strains. In addition, this Mab also inhibits proliferation to GLϕ, the response to which is controlled by gene complementation involving the I-A and I-E subregions, producing the 'I-E' subregion product (14, 17). Thus, this Mab recognizes both the I-A and I-E subregion-controlled Ia moelcules and inhibits *Ir* gene-regulated T cell responses associated with both these two I subregions.

Previous data from this and other laboratories have shown the ability of both conventional antisera (20) and Mab (1, 4) against Ia antigens to inhibit Ia-restricted T cell proliferation. In all of these studies, it was established that antibody against an Ia molecule on an APC only interfered with T cell responses when genetic considerations indicated the involvement of that Ia moelcule in the response being measured. Anti-Ia antibody to Ia moelcules on the APC that were not involved in stimulation of the T cell did not block proliferation. This was true for allelic variants of I-A subregion products on F_1 APC or for I-A *vs* I-E/C-encoded molecules on homozygous APC. Nonetheless, it is clear that most, if not all, APC possess both I-A- and I-E-encoded molecules (4, 15, 16) and can be lysed by anti-I-A *or* anti-I-E antibody plus C. It was therefore critical to establish that M5/114 blocked both GAT (I-A-associated) and GLϕ (I-E-associated) responses without killing the APC, for if this were the case, reactivity with only the I-A product could account for the blocking of BALB/c T cell responses to both GAT and GLϕ. The experiments with (B6A)F_1, CAF_1, and (6R × B10.M)F_1 mice clearly indicate that M5/114 inhibits responses by blocking via the relevent Ia product, and not by killing APC via a single I-A subregion associated determinant. These data are entirely consistent with biochemical and genetic evidence that M5/114 reacts with the $A_\alpha A_\beta$ product in b, d, and q, but not f, k, or s, haplotypes, and with an $E_\alpha E_\beta$ product in d and k, but not b, f, or s, haplotypes (6).

The I-A:I-E cross-reaction detected by M5/114 raises several interesting points concerning the relationship between Ia-restricted antigen presentation, *Ir* gene function, and Ia antigen structure. One explanation for the observations reported here would be the possibility that M5/114 recognizes a determinant on the E_β chain, which in the d and b haplotypes might associate with the A_α chain to produce the Ia molecule involved in presenting GAT. This would be consistent with the mapping of the GAT *Ir* gene to I-A (21), and still account for blocking GLϕ responses which involve $E_\alpha E_\beta$ products (14). However, the demonstration by Bhattacharya *et al.* (6) of precipitation of $A_\alpha A_\beta$ and $E_\alpha E_\beta$ by M5/114 rules out this interpretation and strongly suggests true sharing of a determinant between the classical I-A and I-E subregion products.

The hypothesis that Ia antigens are *Ir* gene products has received support from a variety of experimental approaches, including genetic mapping studies (13, 14), the ability of a mutation in an Ia gene to coordinately affect Ia structure, Ia restriction and *Ir* gene activity (22, 23), and antibody blocking experiments (1, 4, 20, 24, 25). This latter approach has shown that Mab to the $E_\alpha E_\beta$ product only blocks responses that require genetic complementation between A and E subregion genes for Ir responder status (1, 4). The present report extends these observations by showing in an extensive series of haplotypes a complete correlation for two anti-Ia Mab (M5/114 and 10.2.16) 1) between recognition of I-A molecules and inhibition of proliferation and 2) between *Ir* gene status, M5/114 A *vs* E subregion activity, and inhibition of proliferation.

It has been generally assumed that inhibition of APC-T cell interaction by anti-Ia antibody reflects steric inhibition of T cell recognition of APC surface Ia. This interpretation is consistent with a variety of data, including recent studies performed using guinea pigs that showed variations in the ability of monoclonal anti-Ia antibodies to a single molecule to inhibit T cell responses to distinct antigens with that molecule used as a restriction element (26). However, alternative explanations have also received some experimental support. Thus, Thomas *et al.* (27) showed that preculture of APC in anti-Ia antibody followed by washing before antigen exposure decreased the ability of these cells to stimulate T cells, suggesting the possibility that capping or complexing of surface Ia before antigen exposure prevented effective Ia antigen 'association.' More recently, Berzofsky and Richman (28), using murine cells, and Muchmore *et al.* (29), using human cells, have provided evidence for a suppressive effect of anti-Ia-treated APC. In the myoglobin system, this suppression could only be induced by antibody to responder-type Ia antigens, but could do so even when these molecules were not directly involved in T cell triggering (28). The failure of M5/114 to block when directed towards the uninvolved allelic I-A product on (responder × responder)F_1 APC is inconsistent with these latter results and suggests that such suppression either is not of importance under the present experimental conditions or involves subregion and allele specific mechanisms. Further study of the mechanism of anti-Ia blocking using both subregion-specific and cross-reactive antibodies seems warranted.

Finally, as discussed extensively by Bhattacharya *et al.* (6), the demonstration of a shared determinant on I-A- and I-E-encoded molecules, a finding also suggested by prior studies using conventional anti-Ia alloantisera (30, 31), strengthens the presumption that the I-A and I-E products arose by gene duplication. Nonetheless, it has been possible to demonstrate only A-A′ 'hybrid' Ia molecules (23, 32, 33). No $A_\alpha E_\beta$ or $E_\alpha A_\beta$ hybrid molecules have been recognized to date (32). This implies that despite the similarity of chain structure and probable common origin of the I-A and I-E molecules, the α- and β-chains of each subregion product have recognition potential for each other, and not for the complementary product of the other subregion. The molecular basis of this 'A'-ness and 'E'-ness will be of interest to elucidate.

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REFERENCES

1. Lerner, E. A., L. A. Matis, C. A. Janeway, P. P. Jones, R. H. Schwartz, and D. B. Murphy. 1980. Monoclonal antibody against an Ir gene product. J. Exp. Med. 152:1085.
2. Hodes, R. J., K. S. Hathcock, and A. Singer. 1980. Major histocompatibility complex-restricted self recognition. A monoclonal anti-I-A^k reagent blocks helper T cell recognition of self major histocompatibility determinants. J. Exp. Med. 152:1779.
3. Swain, S. L. 1980. Role of I-A positive cells in the production of T cell replacing factors: blocking of factor production with anti-I-A serum. J. Immunol. 125:1224.
4. Nepom, J. T., B. Benacerraf, and R. N. Germain. 1981. Analysis of Ir gene function using monoclonal antibodies: independent regulation of GAT and GLPhe T cell responses by I-A and I-E subregion products on a single accessory cell population. J. Immunol. 127:31.
5. Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, and T. A. Springer. 1981. Lymphocyte function-associated antigen 1 (LFA-1): a surface antigen distinct from Lyt-2,3 that participates in T-lymphocyte mediated killing. Proc. Natl. Acad. Sci. 78:4535.
6. Bhattacharya, A., M. E. Dorf, and T. A. Springer. 1981. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. J. Immunol. 127:2488.
7. Brodsky, F. M., P. Parham, C. J. Barnstable, M. J. Crumpton, and W. F. Bodmer. 1979. Monoclonal antibodies for analysis of the HLA system. Immunol. Rev. 47:3.
8. Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. Curr. Top. Microbiol. Immunol. 7:115.
9. Julius, M. F., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645.
10. Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. J. Immunol. 122:2491.
11. Ly, I. A., and R. I. Mishell. 1974. Separation of mouse spleen cells by passage through columns of Sephadex G-10. J. Immunol. Meth. 5:239.
12. Vitetta, E., and J. D. Capra. 1978. The protein products of the murine 17th chromosome: genetics and structure. Adv. Immunol. 26:148.
13. Schwartz, R. H., A. Yano, and W. E. Paul. 1978. Interaction between antigen-presenting cells and primed T lymphocytes: an assessment of Ir gene expression in the antigen-presenting cell. Immunol. Rev. 40:153.
14. Schwartz, R. H., A. Yano, J. H. Stimpfling, and W. E. Paul. 1979. Gene complementation in the T-lymphocyte proliferative response to poly($Glu^{58}Lys^{36}Phe^6$)n. A demonstration that both immune response gene products must be expressed in the same antigen presenting cell. J. Exp. Med. 149:40.
15. Cowing, C., S. H. Pincus, D. H. Sachs, and H. B. Dickler. 1978. A subpopulation of adherent accessory cells bearing both I-A and I-E or C subregion antigens is required for antigen-specific murine T lymphocyte proliferation. J. Immunol. 121:1680.
16. Pettinelli, C. B., G. B. Ahmann, and G. M. Shearer. 1980. Expression of both I-A and I-E/C subregion antigens on accessory cells required for *in vitro* generation of cytotoxic T lymphocyte against alloantigens or TNBS modified syngeneic cells. J. Immunol. 124:1911.
17. Dorf, M. E. 1978. Complementation of H-linked genes controlling immune responsiveness. Springer Semin. Immunopathol. 1:171.
18. Schwartz, R. H., and W. E. Paul. 1976. T lymphocyte enriched murine peritoneal exudate cells. II. Genetic control of antigen-induced T lymphocyte proliferation. J. Exp. Med. 143:529.
19. Benacerraf, B., and D. H. Katz. 1975. The histocompatibility linked immune response genes. Adv. Cancer. Res. 21:121.
20. Schwartz, R. H., C. S. David, D. H. Sachs, and W. E. Paul. 1976. T lymphocyte enriched murine peritoneal exudate cells. III. Inhibition of antigen induced T lymphocyte proliferation with anti-Ia antisera. J. Immunol. 117: 531.
21. Dorf, M. E., J. M. Plate, J. H. Stimpfling, and B. Benacerraf. 1975. Characterization of immune response and mixed leukocyte reactions in selected intra-H-2 recombinant strains. J. Immunol. 144:602.
22. McKenzie, I. F. C., G. M. Morgan, M. S. Sandrin, M. M. Michaelides, R. W. Melvold, and H. I. Kohn. 1979. B6.C-H-2^{bm12}: a new H-2 mutation in the I-region of the mouse. J. Exp. Med. 150:1323.
23. Kimoto, M., and C. G. Fathman. 1981. Antigen-reactive T cell clones. II. Unique homozygous and (high responder × low responder)F_1 hybrid antigen presenting determinants detected using poly(Tyr, Glu) poly-D,L-Ala-poly-Lys reactive T cell clones. J. Exp. Med. 153:375.
24. Shevach, E. M., W. E. Paul, and I. Green. 1972. Histocompatibility linked immune response gene function in guinea pigs. Specific inhibition of antigen-induced lymphocyte proliferation by alloantisera. J. Exp. Med. 136:1207.
25. Longo, D. L., and R. H. Schwartz. 1981. Inhibition of antigen-induced proliferation of T cells from radiation-induced bone marrow chimeras by a monoclonal antibody directed against an Ia determinant on the antigen presenting cell. Proc. Natl. Acad. Sci. 78:514.
26. Burger, R., and E. M. Shevach. 1980. Monoclonal antibodies to guinea pig Ia antigens. II. Effect on alloantigen, antigen and mitogen-induced T lymphocyte proliferation *in vitro*. J. Exp. Med. 152:1011.
27. Thomas, D. W., L. Clement, and E. M. Shevach. 1978. T lymphocyte stimulation by hapten-conjugated macrophages: a model system for the study of immunocompetent cell interactions. Immunol. Rev. 40:181.
28. Berzofsky, J. A., and L. K. Richman. 1981. Genetic control of the immune response to myoglobins. IV. Inhibition of determinant-specific Ir gene controlled antigen presentation and induction of suppression by pretreatment of presenting cells with anti-Ia antibodies. J. Immunol. 126:1898.
29. Muchmore, A. V., R. M. Blaese, J. M. Decker, D. L. Mann, and S. Broder. 1980. Inhibition of antigen-specific proliferation by antisera recognizing antigens encoded by the human DR locus may be due to active suppression. Clin. Res. 78:506A.
30. Murphy, D. B., and D. C. Shreffler. 1975. Cross-reactivity between H-2K and H-2D products. I. Evidence for extensive and reciprocal serological cross-reactivity. J. Exp. Med. 141:374.
31. Letarte, M., and Falk, J. 1980. Analysis of serologic cross-reactions between human and mouse Ia antigens. J. Immunol. 125:1210.
32. Kimoto, M., and C. G. Fathman. 1980. Antigen reactive T cell clones. I. Transcomplementing hybrid I-A region gene products function effectively in antigen presentation. J. Exp. Med. 152:759.
33. Spoviero, J. F., M. J. Imperiale, and M. Zauderer. 1980. Clonal analysis of F_1 hybrid helper T cells restricted to parental or F_1 hybrid major histocompatibility determinants. J. Exp. Med. 152:920.