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A SHARED ALLOANTIGENIC DETERMINANT ON Ia ANTIGENS ENCODED BY THE I-A AND I-E SUBREGIONS: EVIDENCE FOR I REGION GENE DUPLICATION[1]

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Two monoclonal antibodies to mouse Ia antigens were produced by fusion of xenoimmune rat spleen cells with the NSI myeloma. These monoclonal antibodies detect polymorphic determinants present on B cells and activated T lymphocytes from mice carrying the H-2^b, H-2^d, H-2^k, H-2^r, and H-2^q haplotypes but not from mice carrying the H-2^s or H-2^f haplotypes. Antigenic site number determinations showed the positive haplotypes can be divided into 2 groups. Mice bearing the H-2^b, H-2^d, and H-2^q haplotypes express a high number—40,000 to 80,000—of antigenic sites per B lymphocyte, and monoclonal antibody plus complement can lyse B cells from these mice. In contrast, mice bearing the H-2^k and H-2^r haplotypes express a low number of antigenic sites—about 5000 per cell. Spleen cells from mice carrying the latter haplotypes are not lysed with monoclonal antibody and complement. Genetic mapping demonstrated that high and low expression map to the I-A and I-E subregions, respectively. The monoclonal antibodies detect an Ia specificity on I-A^b, I-A^d, I-E^d, and I-E^k molecules. These observations were confirmed using several different experimental approaches, i.e., cytotoxicity, fluorescent staining, competitive inhibition of monoclonal antibody binding, and 2-dimensional gel electrophoresis of immunoprecipitates. The avidity for $A_\alpha{}^bA_\beta{}^b$ and $E_\alpha{}^kE_\beta{}^k$ is 5 to 7 × 10^{-9} M^{-1}. The antigenic determinant is heat labile, which suggests that it is not carbohydrate. The results imply that Ia antigens encoded by distinct subregions share sequence homology, which may be a consequence of ancestral gene duplication.

The I region of the major histocompatibility complex contains genes that control immune response and immune suppression to certain antigens, lymphocyte-activating determinants, and histocompatibility determinants (1, 2). Studies on these genes have led to the definition of a number of I subregions (3). A set of membrane glycoproteins have also been described that map in the I-A and I-E subregions and are called I region-associated antigens (Ia). Recent 2-dimensional gel and peptide mapping experiments have shown that I-A codes for 3 polypeptide chains: A_α, A_β, and E_β (or A_e), and I-E for one: E_α (4, 5). The α and β chains are of 32,000 to 34,000 and 25,000 to 28,000 m.w., respectively. Two types of Ia antigen bimolecular complexes have been found expressed on cell surfaces, an $A_\alpha A_\beta$ complex and an $E_\alpha E_\beta$ complex, which are separable by immunoprecipitation (6). Tryptic peptide mapping and N-terminal sequencing have shown that polymorphism is primarily associated with the β chains (5, 7–14 and reviewed in 15). Thus far, these studies have not revealed homologies between $A_\alpha A_\beta$ and $E_\alpha E_\beta$ products. The similarities in function, size, and subunit structure, however, suggested that the I-A and I-E products might nonetheless be closely related.

In this report, we characterize 2 rat monoclonal antibodies (MAb),[2] M5/114 and M7/81, which have a very unusual type of cross-reactive specificity for murine I region products. The MAb recognize an allodeterminant present on either $A_\alpha A_\beta$ only, $E_\alpha E_\beta$ only, both $A_\alpha A_\beta$ and $E_\alpha E_\beta$, or neither, depending on the H-2 haplotype. The M5/114 MAb has equal affinity for $A_\alpha{}^bA_\beta{}^b$ and $E_\alpha{}^kE_\beta{}^k$. This study provides immunologic evidence for homology between I-A and I-E Ia antigens, and hence for gene duplication within the I region. The Mab have also been used to quantitatively study I-A and I-E Ia antigen cell surface expression.

MATERIALS AND METHODS

Animals. Adult mice of the strains C57BL/6 (B6), C57BL/10 (B10), BALB/c, B10.D2, B10.BR, B10.M, B10.A(5R), B10.A, and SJL were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.S(8R) mice were kindly provided by Dr. J. Stimpfling (Great Falls, MT). All other strains were bred in our own colony.

Derivation of anti-Ia MAb. The M5/114.15.2 and M7/81.3.2 hybridomas (M5/114 and M7/81 for brevity) are stable, subcloned lines derived after immunization of (BN × Lewis)F_1 rats with secondary B6 mouse spleen cell anti-irradiated BN rat lymphoma mixed lymphocyte cultures and fusion with the NSI myeloma line, as described elsewhere (16). Screening was performed by indirect binding assay on concanavalin A-activated spleen cells and spleen cells from nu/nu mice and subsequently by SDS-PAGE analysis of immunoprecipitates. Large-scale spinner cultures were grown in Dulbecco's modified Eagle's medium (DME) supplemented with 5% fetal bovine serum (FCS; GIBCO, Grand Island, NY).

Other cell lines. Hybridoma lines secreting the M1/69 (HLK) and M1/9.3 MAb (17), the M7/21 and M7/85 MAb (16), and the M1/69 HK inactive MAb of the IgG2b subclass, used as a control (18), have been previously described. The monoclonal anti-I-A^d producing cell line MKD-6 (19) was kindly supplied by Drs. Kappler and Marrack. 14-4-4 (anti-I-E^{k,d,r,p}) (20) was generously provided by Dr. David Sachs, NIH. All other cell lines were obtained from the Cell Distribution Center, Salk Institute, P.O. Box 1809, San Diego, CA.

MAb purification and immunoadsorbent preparation. MAb were purified from spent culture supernatants as previously described (21). Purified antibodies were coupled to Sepharose CL-4B (Pharmacia) at a concentration of 2 mg protein per ml of bed volume, following the method of Cuatrecasas (22). Protein concentration was determined by optical density (1.5 A_{280} − A_{310} units = 1 mg/ml).

Iodination of antibodies. For the indirect binding assay, rabbit anti-rat IgG reagent was prepared and iodinated as previously described (18, 23). Murine MAb 14-4-4 and MKD-6 were iodinated on an affinity column coupled with the mouse cross-reactive portion of rabbit anti-rat IgG (9.9 mg protein/ml bed vol of Sepharose CL-4B). A 5-µl bed of the affinity absorbent was saturated with 0.8 ml of culture supernatant. The antibodies were iodinated with 1 to 2 mCi of NaI and chloramine T as described elsewhere (18, 23). Iodination of purified M5/114 by Iodo-gen to known specific activity was carried out as described (21).

Cell binding assays. The indirect cell binding assay with ^{125}I-rabbit anti-rat Fab was carried out as previously described (23). The direct binding assay with ^{125}I-M5/114 was performed according to Mason and Williams

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[2] Abbreviations used in this paper: FI, fluorescence intensity; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MAb, monoclonal antibody(s); NEPHGE, nonequilibrium pH gradient electrophoresis; PMSF, phenylmethylsulfonyl fluoride; GAT, poly-(glutamic acid^{60}-alanine^{30}-tyrosine^{10}); GLPhe, poly-(glutamic acid^{58}-lysine^{38}-phenylalanine^{4}).

(24) with the following modifications. All washes were carried out in phosphate-buffered saline containing 0.5% bovine serum albumin (BSA). The final incubation mixture contained, in a total vol of 100 μl, 4.0 mg/ml human IgG, 2.0 to 4.0% BSA, target cells, the indicated ^{125}I-MAb, and in some cases 25 μl of appropriate hybridoma supernatant.

The data from saturation curves were plotted according to Scatchard (25). The affinity constant (Ka) was calculated from the negative slope of Scatchard plots and the number of binding sites, by extrapolating the straight line to the *x*-axis. In each case, the number of binding sites was also calculated from the saturation curve (24). The values obtained from each calculation were similar.

Microcytotoxicity assays. Microcytotoxicity was assayed as detailed elsewhere (26). The undiluted antibody in tissue culture supernatants was at a concentration of 100 μg/ml.

Immunofluorescence flow cytometry. The procedures were essentially as previously described (27). Briefly, spleen cells (50 μl, 2.5 × 10^7/ml in Leibovitz's L-15 medium, 10 mM HEPES, 0.5% BSA, 0.01 M NaN_3) were mixed with equal vol of hybridoma supernatants in microtiter plates for 30 min and washed 3 times, and the pellets were suspended in pure FITC-rabbit $F(ab')_2$ anti-rat IgG absorbed with mouse IgG, incubated for a further 30 min, and washed 3 times. For controls, the M1/69 HK inactive MAb was substituted in the first step. Titrations of both MAb and FITC reagents showed they were in saturation. Analysis was on a Becton Dickinson FACS II equipped with a Nuclear Data log amplifier. The fraction of labeled cells was calculated as $\frac{e - c}{1 - c}$ where e and c are the fraction of experimental and control cells, respectively, brighter than the threshold marker. Median fluorescence intensity (FI) of labeled cells is expressed relative to the FI of glutaraldehyde-fixed sheep red blood cell standards, and after subtraction of the FI (0.05) of cells labeled with the control MAb.

Metabolic labeling and detergent extraction of cells. Splenic cells of appropriate strain were internally labeled with (^{35}S)-methionine (New England Nuclear, Billerica, MA) as described by Jones *et al.* (4), except that DME medium was buffered with 10 mM HEPES, pH 7.0. Incorporation of label was stopped with 3 to 4 ml of cold PBS containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma). The labeled cells were washed 3 times with the same medium. Washed cells were suspended in solubilization buffer (10 mM Tris-HCl, pH 7.5, 1% Triton X-100, 1% hemoglobin, 0.75 mM PMSF, and 0.05% sodium azide) at 1.25 × 10^7 cells per ml for 30 to 45 min, and then centrifuged for 1 hr at 100,000 × G.

Immunoprecipitation. Labeled cell extract (40 to 200 μl) was incubated with 10 to 50 μg of M5/114 coupled to Sepharose CL-4B for 1 hr at 4°C. The beads were washed and eluted with 30 μl Laemmli sample buffer (28) containing 2% SDS and no bromophenol blue.

For murine MAb, 50 to 100 μl supernatant were used. Immune complexes were separated either using fixed *Staph a* bacteria or with mouse cross-reacting rabbit anti-rat IgG Sepharose CL-4B (29).

Gel electrophoresis. One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (28).

Two-dimensional gel electrophoresis was carried out as described by Jones (4, 29) with the following modifications. The eluted antigens were lyophilized and then dissolved in 30 μl of isoelectrofocusing (IEF) buffer containing 16% Triton X-100. The first dimension, nonequilibrium pH gradient electrophoresis (NEPHGE), was on 150-mm long gels. Second-dimension gels were dried and fluorographed according to Laskey and Mills (30).

Miscellaneous techniques. Quantitation of MAb and determination of subclass have been described previously (17). Glutaraldehyde-fixed cells were prepared as described (23).

RESULTS

Characteristics of the M5/114 and M7/81 MAb. The M5/114 and M7/81 rat anti-mouse MAb were initially selected for study on the basis of immunoprecipitation of 35,000, 31,000, and 28,000 m.w. Ia antigen subunits, as determined in 1-dimensional SDS-PAGE (data not shown, but see 2-dimensional gels below). Both MAb are of the IgG2b subclass and lyse a subpopulation (50 to 75%) of B6 spleen cells in the presence of complement. The hybridoma lines were derived from 2 different rats, and secrete specific heavy (H) and light (L) chains of differing mobility in SDS-PAGE (Fig. 1), showing that the hybridomas arose from distinct B cell clones. M5/114 also secretes the myeloma kappa chain, whereas M7/81 does not, as shown in Figure 1 and confirmed by a radioimmunoassay for NSI kappa chain (18).

Expression of M5/114 and M7/81 on tumor cells. Over 20 tumor lines carrying the H-2^b, H-2^d, or H-2^k haplotype were examined by indirect radioimmunoassay. M5/114 and M7/81 determinants were present only on the BC-3A leukemic BALB/c (H-2^d) cell line (Table I). This line was previously shown to be Ia.8 positive

Figure 1. SDS-PAGE analysis of immunoglobulin chains secreted by M5/114 and M7/81. Hybridoma lines were labeled with ^{14}C-leucine, and proteins secreted into culture supernatants were treated with SDS-sample buffer and electrophoresed on 10% polyacrylamide gels as previously described. Bands were visualized by autoradiography and identified by comparison to those secreted by the P3X63Ag8 myeloma line. *G, K*: myelome γ_1 and kappa chains: *H, L*: specific heavy and light chains.

TABLE I

M7/81 and M5/114 expression on tumor cells[a]

Tumor	Lines Tested	Antibody Bound (e/c)[b]	
		M5/114	M7/81
T lymphoma	R1.1, R1E/TL8x.1 S49.1, WEHI-7, EL-4 TIMI.4G1.3, BW5147, RDM-4	0.85 (0.7–1.0)	0.9 (0.7–1.1)
Myeloma	SP2/0, S194/5.3, P1.17	1.0 (0.6–1.4)	1.0 (0.8–1.2)
Macrophage	PU-5-1R, RAW264.7, J774, P388D1, WR19M-1	0.75 (0.5–1.0)	1.15 (0.6–1.7)
Myeloid leukemia	C1498.3	0.7	0.9
Ia⁺ leukemia	BC-3A	<u>6.7</u>	<u>4.9</u>
Myelomonocytic leukemia	WEHI-3A	1.3	1.5
Erythroid leukemia	BB88	0.7	1.0
Mastocytoma	P815	1.0	0.8

[a] MAb were tested on glutaraldehyde-fixed tumor cells (5 × 10^5) in the indirect binding assay.

[b] Experimental/control ratio of ^{125}I-anti-rat IgG bound in the presence of M5/114 or M7/81 MAb-containing culture supernatants or NSI culture supernatant plus 50 μg/ml normal rat IgG as control.

(31). No T-lymphoma or macrophage lines that were tested expressed the antigen. Neither could the antigen be detected on erythroid, mastocytoma, myeloma, or monocytic leukemia lines.

M7/81 and M5/114 MAb recognize similar and proximal antigenic determinants. M7/81 and M5/114 were found to have identical strain specificities. Both MAb react with spleen cells from mice of H-2 haplotypes b, d, and k but not f or s (see indirect binding assays and complement-mediated lysis assay on different haplotypes and recombinants below). This raised the possibility that the MAb recognized the same determinant. Therefore, cross-competition experiments were carried out (Fig. 2). Both M7/81 and M5/114 unlabeled MAb were able to completely inhibit the binding of ^{125}I-labeled M5/114 MAb to BALB/c (H-2^d) spleen cells. This result can be rationalized only if the determinants recognized by these MAb are identical or very close spatially so that binding of 1 MAb inhibits the binding of the other. Therefore, M5/114 was chosen as a representative MAb for most experiments described below.

Immunofluorescence flow cytometry. Spleen cells were labeled under saturation conditions with M5/114 MAb, then FITC-rabbit $F(ab')_2$ anti-rat IgG absorbed with mouse IgG, and analyzed by immunofluorescence flow cytometry. Representative fluorescence histograms from 1 of 4 experiments are shown in Figure 3. Spleen cells from B10, B10.D2, and B10.BR were 61 to 70% M5/114⁺

(Fig. 3*A*–3*C*), whereas B10.S (Fig. 3*D*) and B10.M (data not shown) spleen cells were completely negative. M5/114 labeled purified splenic B cells but not purified normal splenic T cells (data not shown), which showed that the labeled subpopulation in spleen is predominantly B lymphocytes. T lymphocytes became 50% M5/114 positive after stimulation with Con A (data not shown). The major histocompatibility complex-linked reactivity, and specificity for normal B and activated but not normal T lymphocytes, is consistent with the conclusion that M5/114 is an anti-Ia MAb.

In contrast to M5/114, the M7/21 rat anti-mouse H-2 MAb of the same IgG2b subclass labeled 99% of normal splenic lymphocytes (Fig. 3*I*). This MAb is also allospecific, as shown by its absence of reactivity with B10.D2 (Fig. 3*J*). Comparisons of the shapes of the Ia and H-2 fluorescence distributions could be made when they were of similar average intensity. This revealed greater heterogeneity in number of Ia sites per cell (only considering positive cells) (Fig. 3*A*, *B*, and *F*) than in number of H-2 sites per cell (Fig. 3*I*).

Site number and affinity of M5/114 MAb for different H-2 haplotypes. The number of binding sites and affinity of M5/114 MAb for spleen cells of different H-2 haplotypes were determined with purified, ^{125}I-labeled MAb. Scatchard analysis showed that M5/114 MAb bound to B10 (H-2^b), B10.D2 (H-2^d), and B10.BR (H-2^k) spleen cells with very similar association constants of 5 to 7 × 10^9 M^{-1} (Fig. 4). B10.S (H-2^s) spleen cells were completely negative (Fig. 4).

The number of binding sites for M5/114 MAb was determined by Scatchard analysis for 8 independent H-2 haplotypes (Table II). Since whole spleen cells were used for these determinations, the percentage of positive cells as determined by immunofluorescence flow cytometry was used to calculate the average number of binding sites per Ia-positive spleen cell. Mice of s and f haplotypes expressed no binding sites. Among positive haplotypes, 2 groups expressing low and high numbers of M5/114 MAb sites could be distinguished. The H-2^r and H-2^k haplotypes can be classified in the low group, based on expression of 4700 and 3400 sites/cell, respectively. The other haplotypes (H-2^d, H-2^b, H-2^p, and H-2^q) belong to the high group. They express 82,000 to 45,000 M5/114 MAb sites per cell, an order of magnitude more than the low group. This 10-fold difference was highly significant, since variation in site number estimates on different days and with different ^{125}I-M5/114 MAb preparations was always less than 2-fold.

Expression of markedly different levels of M5/114 binding sites in the 2 haplotype groups was also reflected in their susceptibility to MAb and complement-dependent lysis (Table II). A threshold effect was evident, in that haplotypes with high levels of expression were lysed while those with low levels were not. Negative haplotypes were not lysed either.

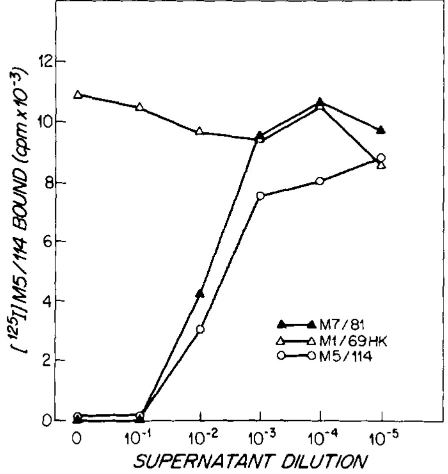

Figure 2. Cross-competition between M7/81 and M5/114 MAb. BALB/c spleen cells (5 × 10^5 in 50 μl) were incubated with 25 μl of hybridoma supernatant of dilutions thereof for 15 min, then ^{125}I M5/114 MAb was added and the direct binding assay carried out as described in *Materials and Methods*. The inactive M1/69 HK MAb was used as a control.

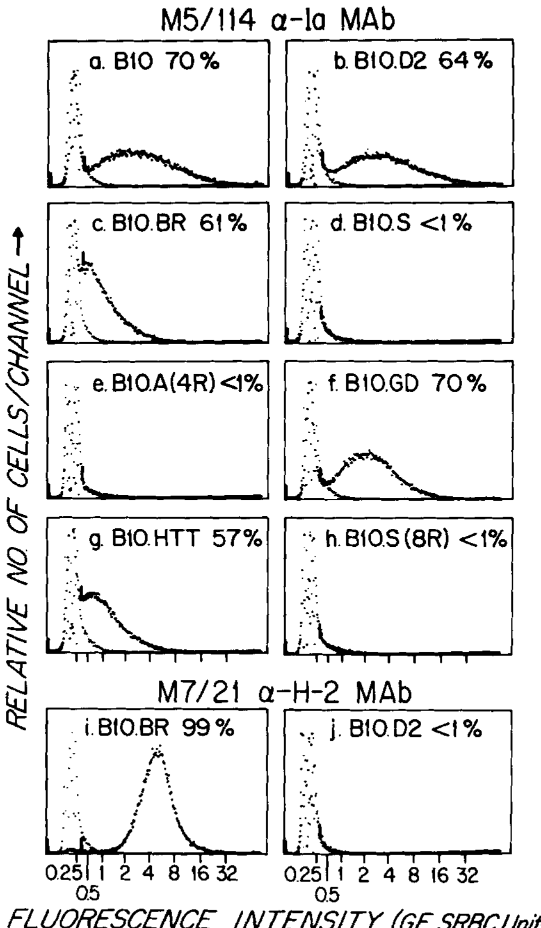

Figure 3. Allospecific immunofluorescent labeling of spleen cells by M5/114 anti-Ia and M7/21 anti-H-2 MAb. Spleen cells were labeled with M5/114 MAb (*a–h*, dark dots), M7/21 MAb (*i*, *j*, dark dots), or control M1/69 HK inactive MAb of the same IgG2b subclass (*a–j*, dim dots) culture supernatants, then with FITC-F(ab')$_2$ anti-rat IgG absorbed with mouse IgG as described in *Materials and Methods*. Cells were scatter-gated to exclude red and dead cells. Markers indicate thresholds for positive cells. GF SRBC = glutaraldehyde-fixed sheep red blood cells used as standards for fluorescence intensity. Cells/channel and fluorescence intensity are plotted on linear and logarithmic scales, respectively.

Quantitative differences between haplotypes were also confirmed by immunofluorescence flow cytometry. For example, the M5/114 MAb gave much brighter labeling of H-2^b and H-2^d spleen cells (Fig. 3*A*, *B*) than H-2^k spleen cells (Fig. 3*C*). Quantitation of median FI (Table II) showed differences of about 5-fold between low and high expression haplotypes, while site number quantitation showed about a 10-fold difference. This discrepancy was probably due to the incomplete resolution of labeled from unlabeled cells in low-expressing haplotypes (see Fig. 3*C*, *G*).

High expression of M5/114 sites maps to the I-A region in H-2^b and H-2^d haplotypes. Genetic mapping studies were facilitated by the finding that recombinants, like the independent haplotypes, fell into clear groups with high, low, and negative M5/114 expression, as determined by site number estimation and quantitative immunofluorescence (Fig. 3*E–H*). Likewise, all recombinants with low M5/114 expression were negative for complement-mediated lysis. Hence lysis could also be used to distinguish high expression. Criteria for placement into groups are described in the Table III legend.

One of the most interesting recombinants studied is B10.A(4R). This recombinant between high-expressing H-2^b (Fig. 3*A*) and low-expressing H-2^k (Fig. 3*C*) is negative in immunofluorescence (Fig. 3*E*). This was also confirmed by site number estimation and lysis (Table III). The 4R recombinant shows that high expression of M5/114 sites in H-2^b is controlled by a locus in the I-A region or to its left (and that low expression in H-2^k maps to the right of I-A). The 3R and 5R recombinants also show that high reactivity maps to

the left of the I-E region. B10.MBR is K^b $I\text{-}A^k$ and a low expressor, mapping high expression to I-A.

In the $H\text{-}2^d$ haplotype, high expression of M5/114 sites also maps to the I-A region. The C3H.OH, C3H.OL, A.TH, and B10.BSVS recombinants map high expression to the left of the S region. Comparison of B10.GD to B10.A(4R), which differ only in K and I-A, maps high reactivity to the left of I-B. The natural recombinant LG/Ckc is K^d $I\text{-}A^f$ and negative, mapping high reactivity to the right of K^d and hence to $I\text{-}A^d$. No recombinants were available with $I\text{-}E^d$ in the absence of $I\text{-}A^d$. Thus these studies did not bear on whether $I\text{-}E^d$ also coded for low M5/114 reactivity, which would have been masked by high reactivity mapping to $I\text{-}A^d$.

In the $H\text{-}2^q$ haplotype, high reactivity maps in the region between K and D, as shown by B10.AQR and DA.

Low expression of M5/114 sites maps to the I-E region in the $H\text{-}2^k$ haplotype. B10.BR and B10.A express low numbers of M5/114 binding sites, while the B10.A(4R) recombinant is negative (Table IV). This shows that the $A_\alpha^k A_\beta^k$ molecule is M5/114 negative, and that low reactivity maps to the right of I-A. B10.A(2R) maps reactivity to the left of D. The B10.HTT (Fig. 3G), B10.S(8R) (Fig. 3*H*), and B10.S(9R) recombinants with the $H\text{-}2^s$ haplotype map reactivity to the right of I-J. Comparison of the natural recombinant B10.BSVS to B10.S(9R) and B10.A maps reactivity to the left of S and hence to the I-E/C region. The B10.TFR5 and A.TFR4 recombinants with $H\text{-}2^f$ also map low reactivity to the I-E (or I-E/C) region in the $H\text{-}2^k$ haplotype.

In $H\text{-}2^d$, M5/114 determinants are expressed on both $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecules. Mapping experiments showed that $A_\alpha^d A_\beta^d$ molecules expressed the M5/114 determinant but left open the possibility that $E_\alpha^d E_\beta^d$, like $E_\alpha^k E_\beta^k$, might also be positive. Therefore, competition experiments with the MKD-6 mouse anti-$I\text{-}A^d$ MAb (19) were carried out (Fig. 5). Target cells were B10.GD and B10.HTG, identical at K, I-A, and D, but differing at I-E (Table III). Control experiments showed that both M5/114 MAb and MKD-6 MAb completely inhibit the binding of the ^{125}I-MKD-6 anti-$I\text{-}A^d$ MAb to B10.GD and B10.HTG (Fig. 5*C*, *D*). This showed both MAb bind to

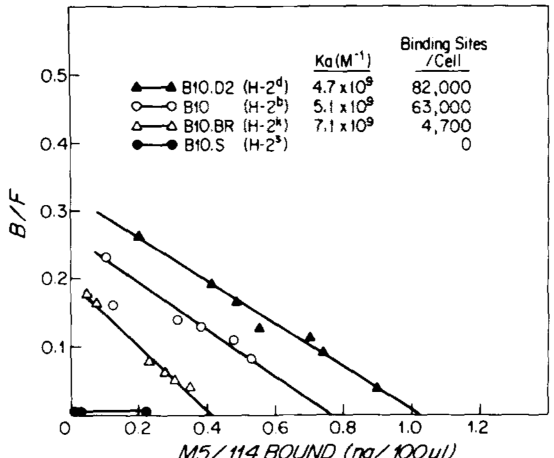

Figure 4. Scatchard analysis of M5/114 binding to spleen cells. Equilibrium binding of ^{125}I M5/114 to 7×10^4 B10 and B10.D2 or 5×10^5 B10.BR spleen cells was determined and plotted as described in *Materials and Methods*. Affinity and number of binding sites were determined from the negative slope and x-axis intercept, respectively.

TABLE II

Reactivity of the M5/114 MAb with independent H-2 haplotypes: site number quantitation, fluorescence intensity, and complement-mediated lysis

Strain	H-2	Binding[a] Sites/ Cell	FI[b]	Lysis[c]	Expression Phenotype
B10.D2	d	82,000	3.1	>320	High
B10	b	63,000	3.0	>320	High
C3H.NB	p	66,000	ND[d]	>320	High
B10.G	q	45,000	ND	>320	High
B10.BR	k	4,700	0.7	<5	Low
B10.RIII	r	3,400	ND	<5	Low
B10.S	s	0	0.0	<5	Negative
B10.M	f	0	0.0	<5	Negative

[a] From Scatchard analysis of ^{125}I-M5/114 MAb binding to spleen cells and corrected for the percentage of M5/114$^+$ cells as determined by immunofluorescence.
[b] Median fluorescence intensity.
[c] Reciprocal of M5/114 and M7/81 cytotoxic titer, tested on splenocytes.
[d] Not done.

TABLE III

High expression of M5/114 sites maps to I-A in $H\text{-}2^b$ and $H\text{-}2^d$ haplotypes and to between K and D in the $H\text{-}2^q$ haplotype

Strain	Haplotype	H-2 Region								Expression Phenotype[a]	Binding Sites/ Cell[b]	FI[b]	Lysis[c]	Indirect Binding Assay[d]
		K	A	B	J	E	C	S	D					
B10	b	b	b	b	b	b	b	b	b	H	63,000	3.0	+	+
B10.BR	k	k	k	k	k	k	k	k	k	L	4,700	0.7	−	+
B10.A	a	k	k	k	k	k	d	d	d	L	ND[e]	0.7	−	+
B10.A(4R)	h4	k	k ↰	b	b	b	b	b	b	N	0	0.0	−	ND
B10.A(2R)	h2	k	k	k	k	k	d	d ↰	b	L	4,800	ND	−	ND
B10.A(3R)	i3	b	b	b	b ↰	k	d	d	d	H	ND	3.0	+	ND
B10.A(5R)	i5	b	b	b ↰	k	k	d	d	d	H	ND	2.5	+	+
B10.MBR	bq1	b	↱ k	k	k	k	k	k	q	L	6,800	0.4	−	ND
B10.D2	d	d	d	d	d	d	d	d	d	H	82,000	3.1	+	+
B10.HTG	g	d	d	d	d	d	d	d	b	H	38,000	ND	+	ND
B10.GD	g2	d	d ↰	b	b	b	b	b	b	H	28,000	2.5	+	ND
C3H.OH	o2	d	d	d	d	d	d	d ↰	k	H	ND	ND	+	ND
C3H.OL	o1	d	d	d	d	d	d ↰	k	k	H	ND	3.1	+	ND
B10.S	s	s	s	s	s	s	s	s	s	N	0	0.0	−	−
A.TH	t2	s	s	s	s	s	s	s ↰	d	N	ND	ND	−	−
B10.BSVS	t5	s	s	s	s	s	s ↰	d	d	N	0	0.0	−	ND
B10.M	f	f	f	f	f	f	f	f	f	N	0	0.0	−	−
LG/Ckc	ar1	d	↱ f	f	f	f	f	f	?	N	ND	ND	−	−
B10.G	q	q	q	q	q	q	q	q	q	H	45,000	ND	+	+
B10.AQR	y1	q	↱ k	k	k	k	d	d	d	L or N	ND	ND	−	ND
DA	qp1	q	q	q	q	q	q	q ↰	s	H?	ND	ND	ND	+

[a] Criteria for placement into groups expressing quantitatively differing amounts of M5/114 antigen are as follows:

Expression Phenotype	Binding Sites/Cell	FI	Lysis	Indirect Binding Assay
H, high	28,000–82,000	2.5–3.1	+	+
L, low	3,400–6,800	0.4–0.8	−	+
N, negative	<100	0.0	−	−

[b] See Table II for details
[c] +, indicates cytolysis of splenocytes, with reciprocal titer of >160; −, indicates no cytolysis with a 1/3 dilution of antibody. Identical results were obtained with M7/81 MAb.
[d] Negative strains did not bind above background with up to 2×10^6 cells/assay.
[e] Not done.

TABLE IV

Low expression of M5/114 sites maps to I-E in the H-2^k haplotype[a]

Strain	Haplotype	H-2 Region								Expression Phenotype	Binding Sites/Cell	FI	Lysis[b]	Indirect[b] Binding Assay
		K	*A*	*B*	*J*	*E*	*C*	*S*	*D*					
B10.BR	k	k	k	k	k	k	k	k	k	L	4,700	0.7	–	+
B10.A	a	k	k	k	k	k	d	d	d	L	ND	0.7	–	+
B10	b	b	b	b	b	b	b	b	b	H	63,000	3.0	+	+
B10.A(4R)	h4	k	k	↱b	b	b	b	b	b	N	0	0.0	–	ND
B10.A(2R)	h2	k	k	k	k	k	d	d↰	b	L	4,800	ND	–	ND
B10.S	s	s	s	s	s	s	s	s	s	N	0	0.0	–	–
B10.HTT	t3	s	s	s	s	↱k	k	k	d	L	ND	0.6	–	+
B10.S(9R)	t4	s	s	↱?	k	k	d	d	d	L	ND	0.8	–	+
B10.S(8R)	as1	k	k	↱s	s	s	s	s	s	N	0	0.0	–	–
B10.BSVS	t5	s	s	s	s	s	?↰	d	d	N	0	0.0	–	ND
B10.M	f	f	f	f	f	f	f	f	f	N	0	0.0	–	–
B10.TFR5	ap5	f	f	f	f	↱k	k	k	d	L	6,600	0.4	–	ND
A.TFR4	ap4	f	f	f	f	f	f↰	s	d	N	ND	0.0	–	–

[a] See Table III for details.

[b] Identical results were obtained with M7/81.

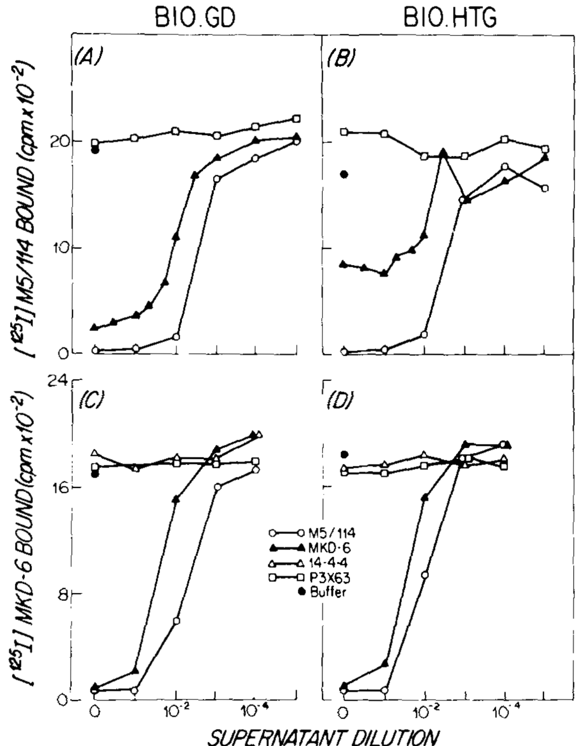

Figure 5. Competitive inhibition of binding of ^{125}I-M5/114 and ^{125}I-MKD-6 (anti-I-A^d) MAb to B10.GD and B10.HTG by unlabeled MAb. B10.GD or B10.HTG spleen cells (4×10^5 in 75 µl) were mixed with 25 µl MAb-containing culture supernatants at appropriate dilutions. P3X63 and 14-4-4 supernatants and buffer alone were used as controls. After shaking 30 min at 4°C, 5µl of ^{125}I-MAb was added, shaking continued for 30 min, and cells washed and γ-counted as described in *Materials and Methods*.

proximal determinants on $A_\alpha{}^dA_\beta{}^d$ molecules, and confirmed reactivity of M5/114 MAb for $A_\alpha{}^dA_\beta{}^d$. Next, inhibition of ^{125}I-M5/114 binding was examined. MKD-6 inhibited by 90% binding of ^{125}I-M5/114 to B10.GD (I-A^d, E^b, Fig. 5*A*). However, MKD-6 inhibited binding to B10.HTG (I-A^d, E^d) by only 60%, and inhibition plateaued at this level (Fig. 5*B*). The same result was obtained in 2 (out of 2) other experiments. This suggests that in B10.HTG, M5/114 binds to I-E^d as well as to I-A^d products.

M5/114 MAb did not compete with the 14-4-4 anti-I-E^d MAb (20) for binding to B10.D2 cells, and in fact in 2 (out of 2) experiments enhanced its binding (Fig. 6). Thus the determinants on I-E recognized by 14-4-4 and M5/114 MAb are spatially separated.

Biochemical characterization of the I region products defined by M5/114. To determine the biochemical nature of the molecules defined by M5/114, material from [^{35}S]-methionine labeled spleen

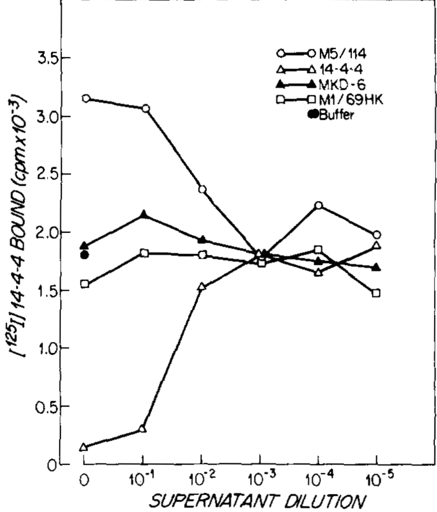

Figure 6. Effect on binding of ^{125}I-14-4-4 (anti-I-E^d) MAb to B10.D2 spleen cells by unlabeled MAb. B10.D2 spleen cells (5×10^5 in 75 µl) were preincubated with unlabeled MAb, then with ^{125}I-14-4-4 MAb as described in the Figure 5 legend.

cells was immunoprecipitated and analyzed by 2-dimensional gel electrophoresis (Fig. 7). M5/114 immunoprecipitated Ia subunits from B10.D2 (Fig. 7*A*), B10 (Fig. 7*D*), and B10.GD (Fig. 7*E*), but not from B10.M (Fig. 7*F*) spleen cells, which confirmed the findings on allospecificity. Three chains were precipitated from B10 (H-2^b): A_α, A_β, and the invariant (I_i) chain. This confirms mapping to I-A in H-2^b. From B10.D2 (H-2^d), 5 chains were precipitated: A_α, A_β, E_α, E_β (A_e), and I_i. Identity of the $E_\alpha{}^d$ and $E_\beta{}^d$ (A_e) chains precipitated by M5/114 was confirmed by comparison to the same chains precipitated by 14-4-4 (Fig. 7*B*). Identity of the $A_\alpha{}^dA_\beta{}^d$ chains was confirmed by comparison to B10.GD (I-A^{d}E^b, Fig. 7*E*), from which only A_α, A_β, and I_i chains were precipitated. These experiments thus confirm that M5/114 recognizes a determinant present on both $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecules in the H-2^d haplotype.

Heat lability of the M5/114 and M7/81 antigenic determinant. We next investigated the possibility that the antigenic determinant shared between $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecules was carbohydrate in nature (Table V). Carbohydrate antigenic determinants are stable to boiling, as has been demonstrated, for example, for the Forssman antigen (32). After 10 min at 100°C, the antigenicity of the M5/114 and M7/81 determinants was 95 to 97% destroyed (Table V). Antigenicity of the determinant on the Ly 5 glycoprotein defined by M1/9.3 was lost to a similar extent. In contrast, carbohydrate determinants recognized by the M7/85 and M1/69 MAb remained stable on the same cells. These experiments suggest the M5/114 and M7/81 antigenic determinants are not carbohydrate structures.

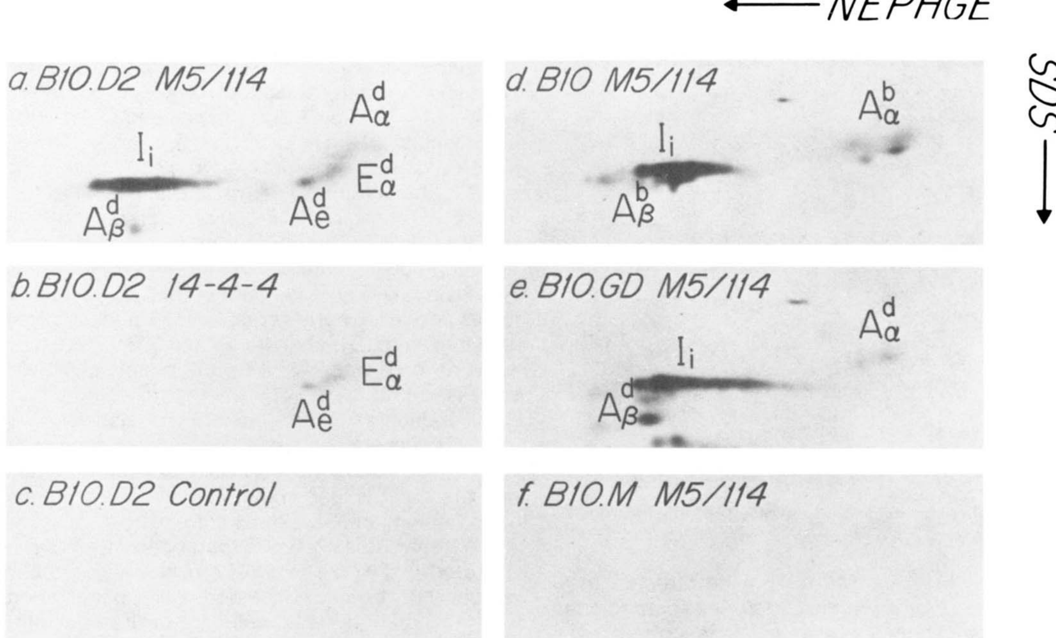

Figure 7. NEPHGE-SDS 2-dimensional PAGE of Ia antigen immunoprecipitates. Spleen cells (*a–c*, B10.D2; *d*, B10; *e*, B10.GD; *f*, B10.M) were labeled with ^{35}S-methionine. Detergent lysates were immunoprecipitated with M5/114 MAb (*a*, *d–f*), 14-4-4 MAb) (*b*), or normal rat IgG (*c*) and subjected to NEPHGE-SDS 2D PAGE and fluorography as described in *Materials and Methods*. Only the relevant portion of the gels containing Ia polypeptides is shown. Precipitated spots were identified by precipitation by different anti-Ia MAb, and by comparison to previous 2D PAGE analyses of Ia polypeptides carried out under the same conditions (4).

TABLE V

Heat lability of M5/114 and M7/81 determinants[a]

MAb	Cpm Bound (e − c)		Antigenicity Remaining (%)
	Control	Heated	
M5/114	14644	476	3
M7/81	9364	440	5
M7/85	9390	11800	126
M1/69	1265	2274	180
M1/9.3	8376	418	5

[a] Concanavalin A-stimulated B6/J spleen cells (10^8/ml in PBS) were either incubated in a boiling water bath or held on ice for 10 min. Cells were washed, resuspended to the same concentration, and assayed in the indirect binding assay as described in *Materials and Methods*.

DISCUSSION

The major histocompatibility complex (MHC) codes for 2 classes of cell surface molecules (1, 2, 15). Class I molecules, the H-2K, D, and L antigens, contain an α subunit of 44,000 m.w. coded for in the MHC and a 12,000 m.w. β_2-microglobulin subunit coded for on a different chromosome. In contrast, class II molecules, or the Ia antigens, contain α subunits of ~34,000 to 32,000 m.w. and β subunits of 28,000 to 25,000 m.w. Both α and β subunits are coded within the MHC; A_α, A_β, and E_β (or A_e) are coded in I-A, and E_α in I-E. These 4 polypeptides associate into the 2 distinct Ia antigen bimolecular complexes expressed on cell surfaces, $A_\alpha A_\beta$ and $E_\alpha E_\beta$, which are separable by immunoprecipitation. An invariant chain, I_i, of 31,000 m.w. has also been found to be associated with class II molecules but does not appear to be expressed on the cell surface (33, 34).

Complete amino acid sequencing has shown that the Class I H-2K and D MHC-encoded α chains are 85 to 90% homologous. Several public allodeterminants defined by alloantisera are also shared between H-2K and D molecules. This serologic cross-reactivity between K and D provided the first evidence for the proposal that K and D are homologous proteins and hence encoded by duplicated genes (35, 36). In contrast, no chemical evidence for homology between the $A_\alpha A_\beta$ and $E_\alpha E_\beta$ Class II molecules has yet been obtained. However, their chemical characterization is much less advanced than Class I molecules. Only the *N*-terminal ~10% of the amino acid residues have been sequenced (5, 9, 11, 12, 14, 15). Peptide mapping studies thus far have concentrated on comparisons between the same subunit in different haplotypes or between α and β chains of the same bimolecular complex. In the few cases where A and E chains were compared, only 10 to 15% of the tryptic peptides were found to coelute. It was unclear whether this was fortuitous or signified sequence homology (7, 10).

Two rat anti-mouse Ia MAb have been described in this paper that define an allodeterminant shared between both types of Class II molecules. By a number of criteria, the MAb do not have reactivity for any other types of cell surface components. Of many tumor cell lines tested, M5/114 and M7/81 MAb reacted only with a known Ia positive line, BC-3A, of BALB/c (H-2^d) origin. The immunoprecipitated molecules had size and charge characteristics identical to those previously described for Ia antigens (4). In immunofluorescent studies on b, d, and k haplotypes, a subpopulation of normal spleen cells corresponding to B lymphocytes was labeled. In addition, Con A-activated T cells but not normal T lymphocytes were labeled with these MAb.

The specificity of the M5/114 and M7/81 MAb in different H-2 haplotypes is summarized in Figure 8. In H-2^b, mapping studies and 2-dimensional gels show that the determinant is present on the $A_\alpha A_\beta$ complex. In H-2^d, mapping, MAb cross-competition, and biochemical data show that the determinant is present on $A_\alpha A_\beta$. MAb competition and 2-dimensional gel comparisons between B10.D2 and B10.GD show that the determinant is also present on the $E_\alpha E_\beta$ complex in H-2^d. H-2^f and H-2^s are completely negative. In H-2^k, mapping studies show that the determinant is absent from $A_\alpha A_\beta$ and presen ı $E_\alpha E_\beta$. In H-2^q, the determinant maps between K and D. Since $E_\alpha E_\beta$ is not expressed in H-2^q, and H-2^q mice express a high number of cell surface sites that map to I-A in other haplotypes, we have tentatively assigned the M5/114 determinant to $A_\alpha A_\beta$ in H-2^q. All possible combinations of M5/114 determinant expression, i.e., on $A_\alpha A_\beta$ only, $E_\alpha E_\beta$ only, both $A_\alpha A_\beta$ and $E_\alpha E_\beta$, and neither, are observed in different haplotypes (Fig. 8). However, it should be noted that all haplotypes examined that express $E_\alpha E_\beta$ on the surface bear the M5/114 antigenic determinant on this molecule. For $A_\alpha A_\beta$, both M5/114 positive and negative allelic products are expressed on the cell surface. A similar cross-reaction by B10.A (4R) anti-B10 sera between products of different I subregions was noted in an early review (37). However, the molecules involved were not defined, and gene complementation or sharing of a single subunit was not ruled out.

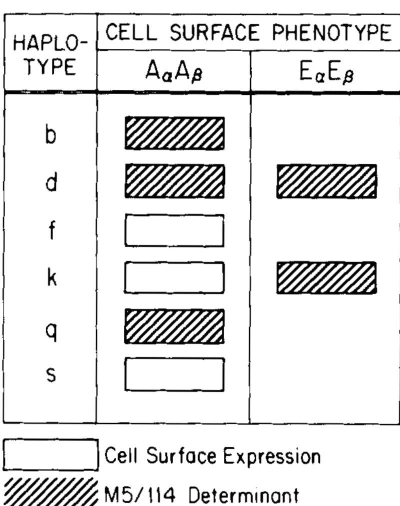

Figure 8. Expression of Ia molecules bearing the M5/114 antigenic determinant in different H-2 haplotypes.

The possibility that the M5/114 MAb might be directed to a single type of subunit, shared by products of different I subregions, was ruled out in the present study. If previously undescribed chain associations were involved, such as $E_\alpha A_\beta$ in addition to $A_\alpha A_\beta$, then only 2 α and 1 β, or 1 α and 2 β subunits should have been precipitated from B10.D2. However, all 4 α and β subunits were precipitated. The MAb could also not be directed to the invariant chain, since this would be inconsistent with mapping to the E but not A subregion in the k haplotype. The invariant chain is known to be associated with I-A products in k haplotypes (33, 34), and is also expressed in the M5/114 negative s and f haplotypes. The possibility that the MAb reacts with still another subunit, which can associate only with $E_\alpha E_\beta$ and $A_\alpha A_\beta$ antigens bearing a particular genetically controlled determinant, succumbs to Occam's razor. Furthermore, no subunits other than A_α, A_β, E_α, E_β, and I_i have been detected in 2-dimensional gel electrophoresis.

We therefore conclude that M5/114 recognizes a cross-reaction between the $A_\alpha A_\beta$ and $E_\alpha E_\beta$ heterodimers; i.e., monoclonal antibodies are not necessarily monospecific. This is a true cross-reaction, rather than fortuitous "multiple specificity" (38) of a MAb for dissimilar determinants, since another MAb, M7/81, has an identical specificity. M7/81 was derived from a different rat, and by the criterion of chain mobility in SDS-PAGE, from a different B cell clone. M7/81 and M5/114 have identical specificity for independent haplotypes and recombinants, and complete cross-competition of the MAb on B10.D2 shows they recognize identical or proximal determinants on $A_\alpha A_\beta$ as well as on $E_\alpha E_\beta$ molecules. The fact that both MAb that detect this shared determinant were derived from rat spleen cells may reflect the relative immunogenicity of this determinant in different species. The avidity of M5/114 for $A_\alpha{}^b A_\beta{}^b$ and $E_\alpha{}^k A_\alpha{}^k$ is identical within experimental error (5 to 7 $\times$ 10^9 M^{-1}), which suggests that the determinants recognized on $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecules are identical. The heat lability of the determinant strongly suggests it is not carbohydrate.

Thus, the cross-reactions noted in this report strongly suggest that $A_\alpha A_\beta$ and $E_\alpha E_\beta$ have a region of identity or high similarity in their protein structure, i.e., homology in at least 1 polypeptide chain. The M5/114 determinant shared between I-A and I-E antigens is completely analogous to the public allodeterminants shared between K and D antigens, which provided the first evidence that K and D are products of duplicated genes. We propose that either the α or β polypeptides of the I-A and I-E antigens are also products of duplicated genes. This is not suprising, since $A_\alpha A_\beta$ and $E_\alpha E_\beta$ are functionally homologous in that they both appear to mediate *Ir* gene-restricted responses. Mutations or gene conversion events subsequent to gene duplication during haplotype divergence might account for the allospecific nature of the M5/114 determinant.

Cell surface expression of the M5/114 determinant on $A_\alpha A_\beta$ and $E_\alpha E_\beta$ was separately quantitated by MAb binding; due to MAb bivalency, the number of molecules per cell may be 2-fold higher. The M5/114 determinant appeared to be expressed on $A_\alpha A_\beta$ and $E_\alpha E_\beta$ in amounts quantitatively similar to other Ia determinants defined by subregion-specific MAb. This was shown by cross-inhibition studies using MK-D6 for $A_\alpha A_\beta$ and by immunoprecipitation by 14-4-4 of similar quantities of $E_\alpha E_\beta$. The determinant detected by M5/114 MAb on $A_\alpha A_\beta$ is expressed in 28,000 to 63,000 sites per B lymphocyte in haploptypes b, d, and q, while the determinant detected on $E_\alpha E_\beta$ is expressed in 4700 to 6800 sites in haplotype k and its recombinants with s and f. The 4- to 10-fold higher cell surface expression of the shared determinants detected by M5/114 MAb on $A_\alpha A_\beta$ than $E_\alpha{}^k E_\beta{}^k$ was also confirmed by immunofluorescence flow cytometry, and by the fact that these determinants were expressed on $A_\alpha A_\beta$ but not on $E_\alpha E_\beta$ in a sufficient number of copies per cell to permit complement-mediated lysis. Complement-mediated lysis by antisera to Lyt-1 antigen has similarly been reported to be dependent on the quantity of cell surface antigen expressed (39).

Immunofluorescence comparisons also showed that $A_\alpha A_\beta$ and $E_\alpha E_\beta$ antigens are expressed less densely than H-2 on spleen cells. Furthermore, there was considerably more variation among cells from the same spleen in the quantity of Ia antigen expressed than in the quantity of H-2 expressed.

In a separate series of experiments, Germain *et al.* (40) demonstrated that M5/114 MAb blocks antigen-specific proliferative responses by primed lymph node cells, either when included in the culture medium or when cells are pretreated with MAb and complement before culture. Blockade of responses in the absence of complement occurred at concentrations as low as 0.1 μg/ml, while higher concentrations of mouse anti-mouse Ia MAb were required to achieve the same inhibition. This is probably due to the high affinity of M5/114 MAb of 5 $\times$ 10^9 M^{-1}. The response to GAT, under I-A control (41), was blocked in H-2 haplotypes b and d but not in f or k, correlating with the strain specificity of the MAb for $A_\alpha A_\beta$ molecules. The response to GLPhe, under complementing I-A and I-E control (41), was blocked in H-2^d mice, in agreement with reaction of M5/114 MAb with $E_\alpha{}^d E_\beta{}^d$. The response to GLPhe was also blocked by 14-4-4 MAb (42). M5/114 and 14-4-4 MAb do not inhibit the binding of each other and thus appear to bind to distinct domains on $E_\alpha E_\beta$ molecules. Thus, binding to an Ia molecule, but not to a specific topographic region on it, is required for immune response blockade. Blockade by M5/114 of immune responses controlled by the I-A and I-E regions (40) in all cases correlated with reactivity for $A_\alpha A_\beta$ and $E_\alpha E_\beta$ molecules as described here. These 2 studies taken together thus provide further evidence that Ia antigens are the products of immune response genes.

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REFERENCES

1. Benacerraf, B. 1981. The role of MHC gene products in immune regulation and its relevance to alloreactivity. Science 212:1229.
2. Klein, J., A. Juretic, C. N. Baxevanis, and Z. A. Nagy. 1981. The traditional and a new version of the mouse H-2 complex. Nature 291:455.
3. Shreffler, D. C., C. S. David, S. E. Cullen, J. A. Frelinger, and J. E. Niederhuber. 1976. Serological and functional evidence for further subdivision of the I regions of the H-2 gene complex. Cold Spring Harbor Symp. Quant. Biol. 41:477.
4. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two-gene control of the expression of a murine Ia antigen. J. Exp. Med. 148:925.
5. Uhr, J. W., D. Capra, E. S. Vitetta, and R. G. Cook. 1979. Organization of the immune response genes. Science 206:292.
6. Cullen, S. E., C. S. Kindle, and D. R.Littman. 1979. Structural comparison of murine Ia antigens determined by the I-A and I-E subregions. J. Immunol. 122:855.
7. McMillan, M., J. M. Cecka, L. Hood, D. R. Murphy, and H. O. McDevitt. 1979.Peptide map analyses of murine Ia antigens of the I-E subregion using HPLC. Nature 277:663.
8. Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra. 1979. Structural studies on the murine Ia alloantigens. III. Tryptic peptide comparisons of the allelic products of the IE/C subregions. Mol. Immunol. 16:29.
9. Allison, J. P., L. E. Walker, W. A. Russell, M. A. Pellegrino, S. Ferrone, R. A. Reisfeld, J. A. Frelinger, and J. Silver. 1978. Murine Ia and human Dr antigens: homology of amino-terminal sequences. Proc. Natl. Acad. Sci. 75: 3953.

10. Freed, J. H., C. S. David, D. C. Shreffler, and S. G. Nathenson. 1978. Structural studies of the protein portion of the H-2-linked Ia glycoprotein antigens of the mouse: tryptic peptide comparison of products from the I-A and I-C subregions of B10.HTT. J. Immunol. 121:91.
11. Silver, J., W. A. Russell, B. L. Reis, and J. A. Frelinger. 1977. Chemical characterization of murine Ia alloantigens determined by the I-E/I-C subregions of the H-2 complex. Proc. Natl. Acad. Sci. 74:5131.
12. Cecka, J. M., M. McMillan, D. B. Murphy, H. O. McDevitt, and L. Hood. 1979. Partial N-terminal amino acid sequence analyses and comparative tryptic peptide maps of murine Ia molecules encoded by the I-A subregion. Eur. J. Immunol. 9:955.
13. McMillian, M., J. A. Frelinger, P. P. Jones, D. B. Murphy, H. O. McDevitt, and L. Hood. 1981. Structure of murine Ia antigens. Two-dimensional electrophoretic analyses and high-pressure liquid chromatography tryptic peptide maps of products of the I-A and I-E subregions and of an associated invariant polypeptide. J. Exp. Med. 153:936.
14. Cook, R. G., M. H. Siegelman, J. D. Capra, J. W. Uhr, and E. S. Vitetta. 1979. Structural studies on the murine Ia alloantigens. IV. NH_2-terminal sequence analysis of allelic products of the I-A and I-E/C subregions. J. Immunol. 122:2232.
15. Strominger, J. L., V. H. Engelhard, A. Fuks, B. C. Guild, F. Hyafil, J. F. Kaufman, A. J. Korman, T. G. Kostyk, M. S. Krangel, D. Lancet, J. A. Lopez de Castro, D. L. Mann, H. T. Orr, P. R. Parham, K. C. Parker, H. L. Ploegh, J. S. Pober, R. J. Robb, and D. A. Shackelford. 1981. Biochemical analysis of products of the MHC. *In* "The role of the major histocompatibility complex in immunobiology." Edited by M. E. Dorf. Garland STPM Press, New York. Pp. 115–172.
16. Davignon, D., E. Martz, T. Reynolds, K. Kürzinger, 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.
17. Springer, T. A. 1980. Cell-surface differentiation in the mouse. Characterization of 'jumping' and 'lineage' antigens using xenogeneic rat monoclonal antibodies. *In* "Monoclonal Antibodies," Edited by R. H. Kennett, T. J. McKearn, and K. B. Bechtol. Plenum, New York. Pp. 185–217.
18. Springer, T. A. 1980. Quantitation of light chain synthesis in myeloma x spleen cell hybrids and identification of myeloma chain loss variants using radioimmunoassay. J. Immunol. Methods 37:139.
19. Kappler, J. W., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, H-2 restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J. Exp. Med. 153:1198.
20. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J. Immunol. 124:533.
21. Ault, K. A., and T. A. Springer. 1981. Cross-reaction of a rat-anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. J. Immunol. 126:359.
22. Cuatrecasas, P. 1970. Protein purification by affinity chromatography. J. Biol. Chem. 245:3059.
23. Springer, T., G. Galfre, D. S. Secher, and C. Milstein. 1978. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur. J. Immunol. 8:539.
24. Mason, D. W., and A. F. Williams. 1980. The kinetics of antibody binding to membrane antigens in solution and at the cell surface. Biochem. J. 187:1.
25. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. 51:660.
26. Dorf, M. E., E. K. Toth, and H. Balner. 1972. Cross-reactions of HL-A antibodies. V. Relationship between the human HL-A11 and chimpanzee ChW-11 specificities. Tissue Antigens 2:461.
27. Kürzinger, K., T. Reynolds, R. N. Germain, D. Davignon, E. Martz, and T. A. Springer. 1981. A novel lymphocyte function-associated antigen (LFA-1): cellular distribution, quantitative expression, and structure. J. Immunol. 127: 596.
28. Laemmli, U. K., and M. Favre. 1973. Maturation of the head of bacteriophage T4. J. Mol. Biol. 80:575.
29. Jones, P. P. 1980. Analysis of radiolabeled lymphocyte proteins by one- and two-dimensional polyacrylamide gel electrophoresis. *In* "Selected methods in cellular immunology," Edited by B. B. Mishell and S. M. Shiigi. Pp. 398–440.
30. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of 3H and 14C in polyacrylamide gels by fluorography. Eur. J. Biochem. 56:335.
31. Chesebro, B., K. Wehrly, K. Chesebro, and J. Portis. 1976. Characterization of Ia8 antigen, Thy-1.2 antigen, complement receptors, and virus production in a group of murine virus-induced leukemia cell lines. J. Immunol. 117: 1267.
32. Stern, P. L., K. R. Willison, E. Lennox, G. Galfre, C. Milstein, D. Secher, A. Ziegler, and T. Springer. 1978. Monoclonal antibodies as probes for differentiation and tumor-associated antigens: a Forssman specificity on teratocarcinoma stem cells. Cell 14:775.
33. Jones, P. P., D. B. Murphy, D. Hewgill, and H. O. McDevitt. 1979. Detection of a common polypeptide chain in I-A and I-E subregion immunoprecipitates. Mol. Immunol. 16:51.
34. Moosic, J. P., A. Nilson, G. J. Hämmerling, and D. J. McKean. 1980. Biochemical characterization of Ia antigens. I. Characterization of the 31K polypeptide associated with I-A subregion Ia antigens. J. Immunol. 125: 1463.
35. Shreffler, D. C., C. S. David, H. C. Passmore, and J. Klein. 1971. Genetic organization and evolution of the mouse H-2 region: a duplication model. Transplant. Proc. 3:176.
36. 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.
37. McDevitt, H. O., T. L. Delovitch, J. L. Press, and D. E. Murphy. 1976. Genetic and functional analysis of the Ia antigens: their possible role in regulating the immune response. Transplant. Rev. 30:197.
38. Richards, F. F., W. H. Konigsberg, R. W. Rosenstein, and J. M. Varga. 1975. On the specificity of antibodies. Science 187:130.
39. Ledbetter, J. A., R. V. Rouse, H. S. Micklem, and L. A. Herzenberg. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens: two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. J. Exp. Med. 152:280.
40. Germain, R. N., A. Bhattacharya, M. E. Dorf, and T. A. Springer. 1981. A single monoclonal anti-Ia antibody inhibits antigen-specific T cell proliferation controlled by distinct Ir genes mapping in the I-A and the I-E subregions. Manuscript submitted.
41. Dorf, M. E. 1981. Genetic control of immune responsiveness. *In* "The role of the major histocompatibility complex in immunobiology," Edited by M. E. Dorf. Garland STPM Press, New York. Pp. 221–254.
42. 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.