

A NEW *I* SUBREGION (*I-J*) MARKED BY A LOCUS
(*Ia-4*) CONTROLLING SURFACE
DETERMINANTS ON SUPPRESSOR T LYMPHOCYTES*

By DONAL B. MURPHY,‡ LEONORE A. HERZENBERG, KO OKUMURA, LEONARD A.
HERZENBERG, AND HUGH O. McDEVITT

*(From the Department of Genetics and Department of Medicine/Division of Immunology, Stanford
University School of Medicine, Stanford, California 94305)*

The immune response to a variety of antigens is controlled by loci in the *I* region of the murine *H-2* gene complex (1-3). Strains of mice carrying different immune response (*Ir*) genes are classified as high or low responders according to their capacity to produce antibody against synthetic and naturally occurring protein antigens. Crossovers within the *I* region have permitted its division into three subregions, designated *I-A*, *I-B*, and *I-C*, which are marked by separate *Ir* loci (*Ir-1A*, *Ir-1B*, *Ir-1C*).

Initial studies on the mechanism of *Ir* gene action suggested that the gene defect in low responder animals might involve T-cell function. This led to the suggestion that *Ir* genes coded for T-cell surface determinants (antigen receptors?) which determined responsiveness to a given antigen (1). It was therefore surprising to find that antibody prepared against *I*-region-associated (*Ia*) determinants reacted predominantly with B lymphocytes (4-6). These antigens are controlled by loci (*Ia-1*, *Ia-3*) which map in the *I-A* and *I-C* subregions (2). Both traits, *I*-region-associated membrane determinants and the capacity to mount an immune response, could be controlled by separate loci, or they could be different phenotypic manifestations of the same gene product.

Recent reports of *I*-region-controlled determinants detectable on T cells have reopened the question of *Ir* gene expression in this cell population. These include cytotoxicity studies which show that at least some peripheral T cells (6, 7) and concanavalin A (Con A)¹ responsive cells (8) or Con A blasts (9, 10) are killed by *I*-region antisera; studies on stimulation of mixed lymphocyte reactions which indicate that T cells alone can stimulate between *I*-region incompatible donors, and that these cells bear *Ia* determinants (11); fluorescent studies with thymus cell preparations and anti-*Ia* sera which suggest that a subpopulation of these cells express *I*-region antigens (12); and blocking studies which show that antigen-induced stimulation of T lymphocytes from genetic high responder strains (13) and the binding of antigen-antibody complexes to the Fc receptor on T lymphocytes (14) are both specifically inhibited by the presence of anti-*Ia* antibody. But, perhaps the most convincing evidence for the presence of *I*-region determinants on T cells comes from functional studies which show that suppressor (14-16) and helper T cells (14, 16) are killed by exposure to anti-*I*-region sera in the presence of complement.

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¹ Abbreviations used in this paper: Con A, concanavalin A; DNP, 2,4-dinitrophenyl; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cells; Ts, suppressor T cells; TsF, soluble suppressive factor.

In an accompanying publication, we present evidence showing that *I*-region antisera kill allotype suppressor T cells (17). These cells suppress production of IgG_{2a} antibodies carrying the *Ig-1b* allotype in (BALB/c × SJL)F₁ mice. Absorption studies show that the determinants detected by anti-*I*-region sera are also present on spleen cells from mice of several different mouse strains which are not allotype suppressed. These determinants are not found on helper T lymphocytes and thus identify a subpopulation of T lymphocytes with suppressor function. Data in the same publication also show that different *I*-region-controlled determinants are present on carrier-primed helper cells.

In this report, we define a locus, *Ia-4*, which controls the expression of determinants on a subclass of T lymphocytes which includes allotype suppressor T cells. These determinants are not found on B lymphocytes and cannot be readily detected by conventional serotyping (cytotoxicity) assays. Evidence presented here will also show that the *Ia-4* locus marks a new *I* subregion, designated *I-J*, which maps between the *I-B* and *I-C* subregions. This evidence is consistent with studies of carrier-specific suppression which show that the *I-J* subregion also codes for determinants on soluble factors which suppress antibody responses (18).

Materials and Methods

Mice. (BALB/cNHx × SJL/JHz)F₁ hybrids (*Ig-1a/Ig-1b*, *H-2^d/H-2^s*) and BALB/cNHx mice were used for the suppressor assay. Strains used to prepare anti-*Ia* sera and to conduct absorption studies are listed in Table I, together with the haplotype origin of the *H-2* regions they carry.

Antisera. Three anti-*Ia* antisera were used in these studies: A.TL anti-A.TH (anti-*I^s*, *S^s*, *G^s*, TL); (B10.A × A.TL)F₁ anti-B10.HTT (anti-*I-A^s*, *I-B^s*, *I-J^s*); (B10.T(6R) × B10.D2)F₁ anti-B10.AQR (anti-*I-A^k*, *I-B^k*, *I-J^k*). The immunization regimens have been described (17).

Absorption of Antiserum. In vivo: mice were injected i.p. with 0.3 ml undiluted antiserum and bled 3–4-h later. In vitro: (a) Unfractionated cells – 1.0 ml of antiserum diluted 1:5 was added to a pellet comprised of spleen and lymph node cells from four mice and incubated for 1 h at room temperature; (b) Purified T or B cells – 0.4 ml of antiserum diluted 1:5 was absorbed twice with 10⁶ purified cells for 45 min at room temperature.

Dye Exclusion Microcytotoxic Assay. A two-stage dye exclusion microcytotoxic test was performed, as described previously (22), with some modifications. Lymph node or spleen cell suspensions were prepared in Dulbecco's phosphate-buffered saline fortified with 5% heat-inactivated fetal calf serum and adjusted to a final cell concentration of 1 × 10⁶ cells/ml. Data are reported as percent cell death, with no correction for complement-mediated background cytotoxicity.

Preparation of Purified Lymphocyte Populations. T cells: nylon wool purified spleen cells were prepared according to Julius et al. (23). B cells: enriched B-cell preparations were obtained by treating spleen with a specific anti-T-cell reagent (rabbit anti-mouse brain T antigen [anti-BAT]) (24) plus complement.

Suppression of the Adoptive Ig-1b Secondary Response to DNP-KLH. Details of this assay have been published elsewhere (17, 25). Briefly, 2,4-dinitrophenyl (DNP) keyhole limpet hemocyanin (KLH)-primed (BALB/c × SJL)F₁ spleen (*Ig-1^a/Ig-1^b*) are cotransferred with *Ig-1b*-suppressed hybrid spleen cells into irradiated (600 R) BALB/c recipients and subsequently challenged with DNP-KLH. 7 days after transfer, the number of indirect IgG_{2a} DNP plaque-forming cells (DNP-PFC) enumerated in the spleen are determined. Under these conditions, the *Ig-1b* response is specifically suppressed, while the *Ig-1a* response is unaffected. Allotype suppressor T lymphocytes (Ts) are generated by perinatal exposure to anti-*Ig-1b* immunoglobulin in BALB/c mothers which bear (BALB/c × SJL)F₁ offspring. (BALB/c × SJL)F₁ donor animals had no circulating *Ig-1b* at the time of transfer.

Mass Kills for Adoptive Transfers. The presence of *I*-region determinants on allotype suppressor T cells can be tested by treating this cell population with anti-*I*-region sera plus complement and looking for alleviation of suppression. Details of this cytotoxic treatment are described in an accompanying paper (17).

TABLE I
Haplotype Origin of *H-2* Regions Carried by Strains in This Study

Strain	Haplotype	Region*							Allele†
		<i>I</i>							<i>Tla</i>
		<i>K</i>	<i>A</i>	<i>B</i>	<i>J</i>	<i>C</i>	<i>S</i>	<i>G D</i>	
B10/Sn	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
BALB/cNH ₂ , B10.D2/nSn	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>c</i>
SJL/JHz	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	?
B10.A/Sn	<i>a</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>a</i>
B10.A(4R)/Sg	<i>h4</i>	<i>k</i>	<i>k</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>
B10.A(3R)/Sg	<i>i3</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	?
B10.A(5R)/Sg	<i>i5</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>a</i>
A.TL/Sf	<i>t1</i>	<i>s</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>
A.TH/Sf, B10.S(7R)/Sg	<i>t2</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>d</i>
B10.HTT/Ph	<i>t3</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>
B10.S(9R)/Sg	<i>t4</i>	<i>s</i>	<i>s</i>	?	<i>k</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
B10.AQR(N4)/Klj	<i>y1</i>	<i>q</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
B10.T(6R)/Sg	<i>y2</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	?	<i>d</i>	<i>d</i>

* Haplotype origin of regions according to Shreffler and David (2), Klein et al. (19), and David et al. (20). The *I-J* subregion is postulated on the basis of evidence presented in this manuscript. The haplotype origin of this region is determined by the *Ia-4* allele which marks it.

† *Tla* allele according to Frelinger et al. (21).

Results

Allotype Ts carry surface determinants detectable by cytotoxic treatment with anti-*I*-region sera (14, 16). In the sections that follow, absorption studies demonstrate that these determinants are controlled by a new locus, *Ia-4*, which marks a new *I* subregion, tentatively designated *I-J*. We show (*a*) that independently derived intra-*I*-region recombinants previously reported to have crossovers in the same position (2, 26, 27) differ in their ability to absorb the antibody that kills Ts; and (*b*) that B lymphocytes previously shown to carry the known *I*-region determinants (2, 4-6) do not absorb Ts killing activity.

Control of Ts Determinants by a New Locus, Ia-4. Table I shows the known haplotype designations for the *H-2* regions carried by the strains used in these studies. These assignments are based on the haplotype origins of the loci that mark each subregion. The crossovers in strains B10.HTT, B10.A(5R), and B10.A(3R) have been localized to the segment of *I*-region chromosome between the *I-B* and *I-C* subregions (2, 27). The crossover in strain B10.S(9R) also appears to have occurred in this interval (2), although questions concerning the *I-B* subregion haplotype assignment in this strain only allow unambiguous positioning of this crossover between *I-A* and *I-C* (27) (see below).

The *Ia-4* allele carried by each of these strains is identified by the ability to absorb antibody that kills Ts. The (BALB/c × SJL)_F₁ hybrids in which Ts are generated are *H-2^d/H-2^s* heterozygotes, and therefore carry *I^d* and *I^s* chromosomal segments. The antisera used react with determinants controlled by *I^s*. Ts activity after cytotoxic treatment with absorbed or unabsorbed sera is measured

by the ability of the treated Ts to suppress Ig-1b anti-DNP production in a secondary adoptive cotransfer assay with syngeneic splenic cell populations containing primed helper T and B lymphocytes. Transfer of DNP-KLH-primed (BALB/c × SJL)_F₁ spleen (*Ig-1^a/Ig-1^b*) cells without Ts into irradiated BALB/c recipients and subsequent challenge with DNP-KLH results in roughly equal Ig-1a and Ig-1b responses. When Ts are cotransferred, the Ig-1b allotype response is specifically suppressed, while the Ig-1a response is not affected. Cytotoxic treatment of Ts with anti-*I* region sera before transfer eliminates the suppression, i.e., a high Ig-1b response is obtained, because Ts are removed. Absorption of the antibody reactive with Ts results in a low Ig-1b response because Ts are not killed.

Absorptions with intra-*I*-region recombinant strains show that B10.HTT carries the *Ia-4^s* allele, while B10.S(9R) does not (Table II). This work was conducted with two antisera against the *H-2^s* haplotype: A.TL anti-A.TH and (B10.A × A.TL)_F₁ anti-B10.HTT. Data presented below will show that B10.S(9R) carries the *Ia-4^k* allele.

A similar dichotomy is observed with a second pair of intra-*I*-region recombinant strains, B10.A(5R) and B10.A(3R) (Table III). In this case, the determinants on Ts were detected with a cross-reacting antiserum (B10.T(6R) × B10.D2)_F₁ anti-B10.AQR which was produced against the *H-2^k* haplotype. Whether the determinants detected on Ts with this cross-reactive antiserum are controlled by *Ia-4^s*, or a second, closely linked locus remains to be determined; however, since we presently have neither genetic nor serologic evidence indicating that these determinants are controlled by a second locus, we assume that they are controlled by *Ia-4^s*.

Recombinant strain B10.A(3R) carries the *Ia-4^b* allele, since, like its B10 parent, it does not absorb antibody reactive with Ts (Table III). In contrast, recombinant strain B10.A(5R) completely absorbs this antibody and therefore must carry *Ia-4^k* determinants. (This conclusion is surprising because strain B10.A(5R) has been described as carrying determinants controlled only by the *H-2^b* and *H-2^d* haplotypes (2). The absorption cannot be due to *H-2^d* determinants, since the serum producer carries the *H-2^d* haplotype, and cannot be due to *H-2^b* determinants, since, as indicated above, a strain carrying the *H-2^b* haplotype does not absorb antibody reactive with Ts.) The portion of *H-2^k*-derived chromosome in B10.A(5R) comes from parental strain A/WySn. Since B10.A was one of the parental strains which gave rise to B10.S(9R), this recombinant line also carries the *Ia-4^k* allele.

Data with the antisera discussed above define two antigenic specificities. Assuming these are both controlled by *Ia-4*, one specificity is unique to *Ia-4^s* determinants and is not shared with *Ia-4^k* determinants (A.TL anti-A.TH, (B10.A × A.TL)_F₁ anti-B10.HTT). The second specificity is shared by *Ia-4^s* and *Ia-4^k* determinants ((B10.T(6R) × B10.D2)_F₁ anti-B10.AQR).

Ia-4 Marks a New Subregion, I-J. The crossovers in the intra-*I* region recombinant strains discussed above indicate that the *Ia-4* locus is distinct from *Ir-1B* or *Ia-3*, which mark the *I-B* and *I-C* subregions, respectively. These crossover positions therefore define a new *I* subregion, designated *I-J*, which is intercalated between the *I-B* and *I-C* subregions (see Table I, Fig. 1).

Both B10.S(9R) and B10.HTT obtained the *K* end of the *I* region from the same

TABLE II
Mapping the *Ia-4* Locus to a New *I* Subregion, *I-J*: Recombinant Strains B10.HTT and B10.S(9R)

(BALB/c × SJL) _F ₁ spleen cells transferred (× 10 ⁶)		Treatment of Ts			IgG _{2a} DNP-PFC/10 ⁶		Conclu- sion: anti- <i>Ia-4</i> ^k absorbed
DNP-KLH primed	Ig-1b sup- pressed spleen (Ts)*	Serum	Absorbed with‡		Ig-1b	Ig-1a	
			Strain	<i>I</i> subregion			
				A B J C			
7	—	—	—	—	1,160	1,100	—
"	4	NMS	—	—	147	1,080	—
"	"	A.TL anti-A.TH	—	—	1,240	1,220	—
"	"	"	B10.A	<i>k k k d</i>	1,190	1,270	No
"	"	"	B10.S(7R)	<i>s s s s</i>	167	974	Yes
"	"	"	B10.HTT	<i>s s s k</i>	207	1,130	Yes
"	"	"	B10.S(9R)	<i>s ? k d</i>	1,450	1,310	No
"	"	(B10.A × A.TL) _F ₁ anti-B10.HTT	—	—	1,120	1,220	—
"	"	"	B10.A	<i>k k k d</i>	1,110	1,200	No
"	"	"	B10.HTT	<i>s s s k</i>	127	1,080	Yes
"	"	"	B10.S(9R)	<i>s ? k d</i>	1,180	1,350	No

* Number of cells before complement-dependent cytotoxicity. No adjustment in cell number made after this treatment.

‡ A.TL anti-A.TH absorbed in vivo; (B10.A × A.TL)_F₁ anti-B10.HTT absorbed in vitro with lymph node and splenic cell populations.

§ Haplotype origin of *I-J* subregion based on *Ia-4* allele which marks it. Vertical bars indicate crossover positions.

TABLE III
Mapping the *Ia-4* Locus to a New *I* Subregion: Recombinant Strains B10.A(5R) and B10.A(3R)

(BALB/c × SJL) _F ₁ spleen cells transferred (× 10 ⁶)		Treatment of Ts			IgG _{2a} DNP-PFC/10 ⁶		Conclu- sion: anti- <i>Ia-4</i> ^k absorbed
DNP- KLH primed	Ig-1b sup- pressed spleen (Ts)*	Serum	Absorbed with‡		Ig-1b	Ig-1a	
			Strain	<i>I</i> Subregion			
				A B J C			
8	—	—	—	—	123	133	—
"	4	NMS	—	—	0	113	—
"	"	(B10.T(6R) × B10.D2) _F ₁ anti-B10.AQR	—	—	115	109	—
"	"	"	B10.A	<i>k k k d</i>	6	118	Yes
"	"	"	B10	<i>b b b b</i>	100	109	No
"	"	"	B10.A(4R)	<i>k b b b</i>	96	115	No
"	"	"	B10.A(5R)	<i>b b k d</i>	16	111	Yes
"	"	"	B10.A(3R)	<i>b b b d</i>	109	120	No

* Number of cells before complement-dependent cytotoxicity. No adjustment in cell number made after this treatment.

‡ Antiserum absorbed in vivo.

§ Haplotype origin of *I-J* subregion based on *Ia-4* allele which marks it. Vertical bars indicate cross-over positions.

H-2^s haplotype parent (2). Since B10.HTT carries *Ia-4^s*, the crossover in this strain must have occurred to the right (*D* end) of the *Ia-4* locus, while the crossover in B10.S(9R), which carries *Ia-4^k*, occurred to the left (*K* end) of *Ia-4* (Fig. 1). The same conclusion may be drawn from the data with B10.A(3R) and B10.A(5R). These recombinant lines were derived from (A × B10)_F₁ hybrids, and, according to known *I*-subregion typing, appear to be identical (26, 27). Strain B10.A(5R), however, carries the *Ia-4^k* allele, while B10.A(3R) carries *Ia-*

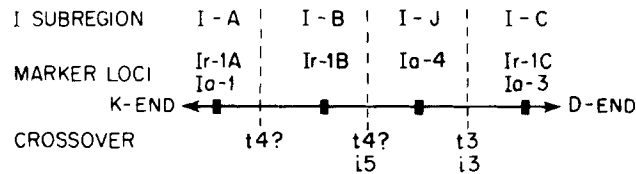


FIG. 1. Crossover positions which define the *I-J* subregion. B10.S(9R) (*H-2^d*), B10.HTT (*H-2^b*), B10.A(5R) (*H-2^b*), and B10.A(3R) (*H-2^b*).

4^b. Therefore, the crossover positions in these strains, as in B10.S(9R) and B10.HTT, occurred on opposite sides of *Ia-4* (Fig. 1).

At present, the crossover position in B10.A(3R) cannot be distinguished from that in B10.HTT. The crossover in B10.S(9R) initially appeared to be indistinguishable from the crossover in B10.A(5R). The localization of the recombinatory event in B10.S(9R), however, was based on the assignment of the *H-2^s* haplotype to the *I-B* subregion carried by this strain (2). This assignment was made because B10.S(9R) lacked specificity *Ia.3*. Although control of this specificity was thought to map in the *I-B* subregion, it now appears to map in *I-A* (28). Since B10.S(9R) has never been typed for its capacity to produce antibody against any of the antigens whose responses are controlled by genes in *I-B*, the haplotype origin of this subregion is currently unknown (27). Therefore, the crossover in B10.S(9R) cannot be equated with that in B10.A(5R), although, in both cases, this recombinatory event occurred to the left (*K* end) of the *Ia-4* locus.

In sum, the absorption studies presented above demonstrate that the *Ia-4* locus, which controls determinants present on Ts, can be separated from other known *I*-region genetic markers by recombinatory events. The *Ia-4* locus marks a chromosomal segment intercalated between the *I-B* and *I-C* subregions. We tentatively designate this new subregion *I-J*, and define its outer boundaries by the crossover positions in strains B10.A(5R) (*K* end) and B10.HTT (*D* end).

Antibody to I-J Determinants Cannot Readily Be Detected By Conventional Serotyping Assays. As shown above, absorption of A.TL anti-A.TH or (B10.A × A.TL)_{F₁} anti-B10.HTT with strain B10.S(9R) does not remove cytotoxicity for Ts, (i.e., anti-*Ia-4^s* activity). Nevertheless, as the data in Fig. 2 show, these absorptions completely remove the cytotoxic activity detectable with unabsorbed sera in the conventional dye exclusion microcytotoxicity assay. The splenic target cells used were from normal, nonsuppressed (BALB/c × SJL)_{F₁} mice. Similar results were obtained with hybrid lymph node targets and with A.TH or B10.HTT lymph node or splenic targets. In addition, an antiserum produced in strains that differ only in the *I-J* subregion (B10.A(3R) anti-B10.A(5R)) kills Ts, but does not give measurable cytotoxicity in the dye exclusion assay (data not shown). Thus, no cytotoxicity is detectable by standard serotyping procedures either with a specific *I-J* antiserum or with other antisera rendered specific for *I-J* determinants by absorption. Since in vitro absorption studies have shown that at least a subpopulation of spleen and lymph node cells carry *I-J* determinants (Table II) (17), these data suggest that the *I-J*-bearing subpopulation comprises less than 10% of spleen or lymph node cells, the lower limit of detectable killing with this assay.

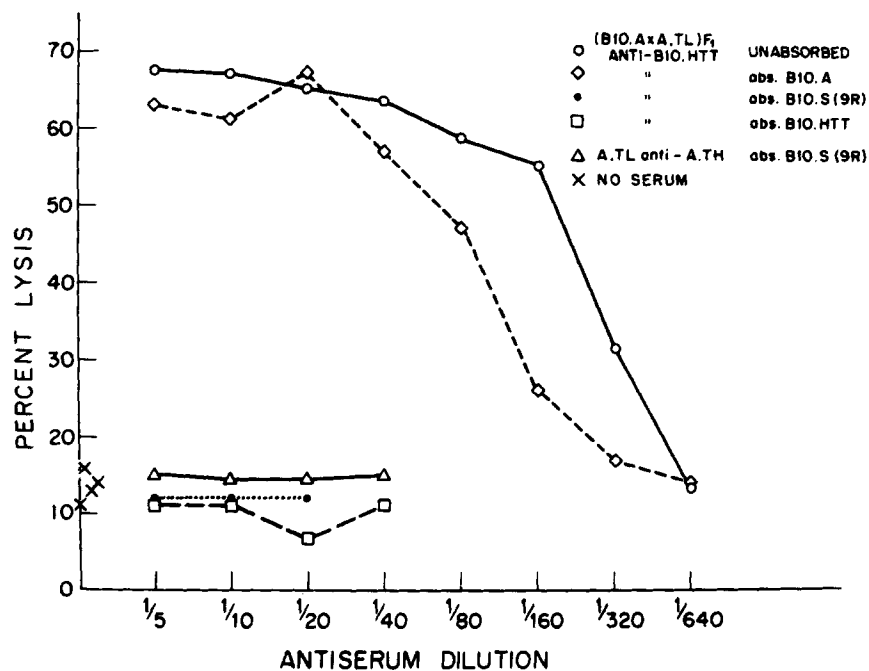


FIG. 2. Failure of anti-I-J serum to kill (BALB/c x SJL)F₁ splenic targets in the dye exclusion assay.

Presence of Ia-4 Determinants on T but not B Cells. Previously recognized *I*-region determinants have been shown to be present predominantly on B cells (2, 4-6). The loci (*Ia-1*, *Ia-3*) controlling these determinants, we have shown above, are genetically separable from *Ia-4*. Data in Table IV show that *Ia-1* and *Ia-3* controlled determinants are also physically separated from those controlled by *Ia-4*, in that *Ia-4* determinants are present on T rather than B lymphocytes.

Absorption studies with antisera rendered specific for I-J (as described above) show that nylon wool-passed splenic T cells of the appropriate haplotype completely absorb anti-Ia-4. An equivalent number of T-depleted B lymphocytes from the same spleen cell preparation, however, did not detectably absorb T_s killing activity (see Table IV). Since the T-cell preparation used to absorb contained less than 1% B cells as judged by fluorescent staining with an anti-immunoglobulin reagent, these studies demonstrate that *Ia-4* determinants are found predominantly (if not exclusively) on T cells.

We have shown elsewhere that only a subset of peripheral T cells carry *Ia-4* determinants, since anti-Ia-4 antibody does not kill helper T cells (17). The evidence presented here that B cells do not carry *Ia-4* antigens indicates that they mark this subset exclusively. The data presented above, showing that anti-I-J kills less than 10% of spleen cells, is consistent with this conclusion, and suggests that the *Ia-4*-bearing subset of T cells is quite small.

Discussion

Allotype T_s carry surface determinants controlled by the *I* region of the *H-2* complex (14, 16). We show here that these determinants are controlled by a new

TABLE IV
T Cells, but not B Cells, Absorb Anti-Ia-4 Antibody

(BALB/c × SJL)F ₁ spleen cells trans- ferred (× 10 ⁶)		Treatment of Ts		IgG _{2b} DNP-PFC/10 ⁶		Conclusion: anti-Ia-4 ^s absorbed
DNP- KLH primed	Ig-1b sup- pressed spleen (Ts)*	Serum‡	Absorbed§ with	Ig-1b	Ig-1a	
7	—	—	—	1,160	1,100	—
"	4	NMS	—	147	1,080	—
"	"	Anti-I-J ^s	—	1,450	1,310	—
"	"	"	"T"	185	1,480	Yes
"	"	"	"B"	1,210	1,190	No

* Number of cells before complement-dependent cytotoxicity. No adjustment in cell number made after this treatment.

‡ Anti-I-J^s reagent (containing anti-Ia-4^s antibody) used was A.TL anti-A.TH absorbed in vivo with B10.S(9R).

§ "T", nylon wool-passed (BALB/c × SJL)F₁ splenic T cells (84% stained with a fluoresceinated T-cell-specific rabbit anti-mouse brain serum (BAT positive); 1% stained with a fluoresceinated anti-Ig reagent which detects B cells). "B", anti-BAT-treated spleen (3% BAT positive, 85% Ig positive).

locus, *Ia-4*, which marks a previously undefined *I*-region chromosomal segment (*I-J*) intercalated between the *I-B* and *I-C* subregions. *Ia-4* determinants are present on a subset of T lymphocytes which includes allotype Ts but not helper T cells [17]. Data presented here also show that B lymphocytes do not carry these determinants.

I-J-controlled determinants have been detected on cells involved in two other T-cell functions: Con A responsiveness (Frelinger, personal communication) and carrier (KLH)-specific suppression of antibody responses (Tada, personal communication). In the latter case, I-J determinants are also found on the soluble factors (KLH-TsF, soluble suppressive factor) which suppress these responses (18). In fact, the evidence that a locus controlling *I*-region determinants exists between the *I-B* and *I-C* subregions was first described in studies with these factors (29).

Tada and Taniguchi (29) demonstrated that *I*-region antisera removed the soluble factors (KLH-TsF) prepared from thymus or spleen that specifically suppress antibody responses to KLH or to haptens coupled to KLH as a carrier. They showed that antibody to the known *I*-region specificities, with the exclusion of those in the *I-E* subregion, which also maps between *I-B* and *I-C* (see below), could not account for the removal of the KLH-TsF. The discovery of crossover pairs described here, which define the *I-J* subregion, allowed definitive mapping of the locus-controlling KLH-TsF determinants to the *I-J* subregion (18). Thus, both allotype and carrier-specific suppression are associated with *I*-region determinants controlled by closely linked (or possibly the same) loci within the *I-J* subregion. This close linkage or identity is of interest since the mechanism of suppression in both cases shows striking similarities. (These similarities are discussed in an accompanying publication [17].)

The detection of I-J determinants on Con A responsive cells suggests another association between I-J and suppression of immune responses. Frelinger (personal communication) has shown that cytotoxic pretreatment of T-cell populations with anti-I-J sera prevents stimulation by Con A, a T-cell mitogen known to induce production of soluble factors which suppress immune responses (30). It is tempting to speculate that the cells removed by cytotoxic anti-I-J treatment are those that produce these factors; however, Stout and Herzenberg (31) have shown that a small T-cell subpopulation contains cells that respond to Con A and that are required to initiate (promote) the response of other T-cell subsets to this mitogen. The initiator (promoter) cells are killed by the I-J antisera (Frelinger, personal communication). Whether they or other T-cell subsets are responsible for production of the suppressive factors has not been determined.

I-region determinants associated with T cells or T-cell products have been described which differ from the I-J determinants discussed here. Some of these have been localized to other *I* subregions, while others show different serological characteristics.

Blocking studies by Stout (personal communication, 14) have shown that antibody against I-A or I-C products inhibits the binding of antigen-antibody complexes to the Fc receptor on the majority of Fc-bearing T lymphocytes. It is interesting to note that anti-I-J antibody does not measurably block complex binding, although the Con A promoter cell described previously is a Fc receptor-positive cell. Either the I-J-positive population is too small to be detected in the blocking studies, or I-J antisera, unlike I-A or I-C antisera, do not inhibit complex binding.

Serological studies have also suggested that *I-A* or *I-C* region-controlled antigens are present on T lymphocytes. David et al. (9) found that specificities Ia.1-10 are present on Con A-stimulated blasts. Although most of the antisera they examined potentially contain anti-I-J activity, it is unlikely that I-J antibody mediates this effect since absorption-elution experiments indicated that the specificities involved are on both T and B cells. Götze (7) defined a T-cell Ia specificity which is controlled by a gene mapping in the *I-B--G* interval. Since B lymphocytes absorb completely for T lymphocytes, and the pattern of reactivity with various recombinant lines is not consistent with that predicted for the I-J polymorphism (Table I), the Ia antigens examined in this study are probably not controlled by I-J.

Functional studies by Tada and Taniguchi (29) have shown that the *I* region codes for receptors on T cells required for KLH T-cell factor-mediated suppression. Loci controlling these determinants most likely map to the left of the *I-C* subregion. In an accompanying publication, we show that *I*-region determinants different from Ia-4 are present on carrier-primed helper populations (17). The locus controlling these determinants has not been localized to a particular *I* subregion.

Soluble T-cell factors which potentiate the immune response also carry *I*-region determinants. Taussig et al. (32) have described a factor that is capable of replacing helper function in generating an IgM antibody response. This factor appears to bear determinants controlled by the *I-A* subregion. Armerding et al. (33) and Delovitch (personal communication, 14) have shown that the allogeneic effect factor, which interacts with B lymphocytes to give an IgG response, also

bears Ia determinants. Finally, Tada et al. (18) have described a soluble factor which enhances in vitro IgG responses. Activity of this factor appears to require *I-A* subregion matching between donor and target populations.

The data discussed above suggest that the *I* region may control at least two different sets of determinants on T lymphocytes. The Ia-4 determinants described here are one unique set which is selectively expressed on allotype suppressor T cells. These determinants are not found on B cells or helper T cells. The *Ia-4* locus maps to the same *I* subregion (*I-J*) as loci that control determinants on carrier-specific suppressor T cells (or factors) and on Con A response promoter T cells. Other determinants found on T cells appear to be a heterogeneous group which map to different subregions and may be shared with B cells.

Several points concerning the definition of the *I-J* subregion warrant consideration. This chromosomal segment is defined by the crossover positions in strain B10.A(5R) (*K*-end boundary) and B10.HTT (*D*-end boundary). The *I-J* subregion appears to be bounded by the *I-B* and *I-C* subregions; however, there are two potential problems with this localization: (a) the *I-B* subregion as currently defined may not exist; (b) another *I* subregion, *I-E*, has been postulated which maps to the left (*K* end) of *I-C*.

The necessity for postulating an *I-B* subregion has recently been questioned (34). Since no cell surface determinants are controlled by this region, its definition depends on *Ir* gene markers which appear to map within it. Mapping of these markers depends, in turn, on a key assumption: that the response to a given antigen is controlled by a single *Ir* gene. The validity of this assumption should perhaps be re-evaluated, considering the complexities known to be associated with control of the immune response (35-38).

The *Ir-1B* locus, which purportedly marks the *I-B* subregion, controls the response to an IgG_{2a} myeloma protein (M173). Lieberman et al. (26) clearly demonstrated that the crossover event in recombinant strain B10.A(4R) (*I-A^k*, *I-B^b*, *I-C^b*) separated this locus from *Ir-1A*. This strain, like parental strain B10 (*I-A^b*, *I-B^b*, *I-C^b*), is a high responder to the antigen, while parental strain A/WySn (*I-A^k*, *I-B^k*, *I-C^d*) is a low responder. Since a second recombinant strain, B10.A(5R) (*I-A^b*, *I-B^b*, *I-C^d*), is also a high responder, the crossover positions in the two recombinant lines appeared to define a region, designated *I-B*. However, if a gene in either the *I-A^b* or *I-C^b* subregions is independently sufficient to give a high response, then there is no reason to define an *I-B* subregion, i.e., both B10.A(4R) (*I-C^b*) and B10.A(5R) (*I-A^b*) would respond. Similar considerations pertain to the genetic control of the response to other antigens that map in this region.

Supportive evidence for this concept comes from studies that show serological cross-reactivity between the products of *I-A* and *I-C* subregion genes (14, 22, 39). Such cross-reactivity suggests structural homology and implies that this chromosomal segment may have been derived by a process of tandem duplication of a common ancestral gene. The localization of two or more *Ir* genes with similar functions in different *I* subregions would not be unreasonable under these circumstances. Should the *I-B* subregion prove to be an artifact, then the *I-J* subregion would be bounded on the left (*K* end) by *I-A*.

The righthand (*D* end) boundary of the *I-J* subregion is defined by the crossover in strain B10.HTT. According to current *I*-region maps, this recombi-

natory event occurred to the left (*K* end) of the *I-C* subregion (2). Recently, a new *I* subregion, designated *I-E*, has been postulated which also maps to the left (*K* end) of *I-C* (David, personal communication). This new subregion lies between *I-J* and *I-C*.

The existence of the *I-E* subregion was proposed to explain the cytotoxic reaction of a (C3H.Q × B10.D2)_F₁ anti-B10.AQR (anti-*I*^k) serum with B10.A(5R) target cells in a dye exclusion assay (40). Since this serum does not react with B10 (*H-2*^b) target cells, and since strain B10.D2 (*H-2*^d) is present in the serum producer, it would not be expected to kill B10.A(5R) cells unless this strain carried a portion of the *I*-region chromosome derived from the *H-2*^k haplotype. (B10.A(5R) was thought to have *I* subregions derived only from the *H-2*^b and *H-2*^d haplotypes.) Similar observations have been made in other laboratories (10, 41, Sachs personal communication; Murphy, unpublished data). These data are consistent with the evidence presented in this publication that B10.A(5R) carries *I-J*^k determinants.

The determinants controlled by the postulated *I-E* subregion, however, are different from those controlled by *I-J* for two reasons. First, *I-E*-controlled determinants were studied utilizing standard serotyping procedures. Antibody against *I-J* determinants cannot be detected in conventional dye exclusion assays. Second, in the dye exclusion assay, strains B10.S(9R), B10.HTT, B10.A(3R), and B10.A(5R) all completely absorb antibody activity for B10.A(5R) targets from the (B10.T(6R) × B10.D2)_F₁ anti-B10.AQR serum used in this study (Murphy, unpublished data). These data suggest that the four strains used for absorption share the same *I-E* subregion. Since the *I-J* subregion is defined on the basis of differences between these strains, the two regions must be distinct. Furthermore, this evidence indicates that the *I-J* subregion must map to the left (*K* end) of *I-E*.

The crossover positions in strains B10.A(5R) and B10.HTT thus define the *I-J* subregion. Considering the reservations stated above, this subregion clearly maps between *I-A* and *I-C*. If both the *I-B* and *I-E* subregions are validly defined, the map order would be *I-A, I-B, I-J, I-E, I-C*.

During the past few years, it has become apparent that one mechanism by which *I*-region genes may regulate the immune response is by suppression. This possibility was first suggested by Gershon et al. (42), who found that the synthetic polypeptide GAT (L-glutamic acid-L-alanine-L-tyrosine) readily induces tolerance in nonresponder animals to GAT coupled to methylated bovine serum albumin (MBSA). Later studies with this antigen by Kapp et al. (43) showed that GAT-specific suppressor T cells could be generated in nonresponder strains. The response to GAT is controlled by *I**r* genes which map between the *K* and *I-C* regions (35). Recently, Debré et al. (44) have shown that some, but not all strains develop GT (L-glutamic acid-L-tyrosine)-induced suppression of GT-MBSA responses. Gene(s) that govern this trait have been designated as immune suppression (*I**s*) genes and are linked to the *H-2* complex.

The observation that *I*-region-controlled products are selectively expressed on allotype suppressor T lymphocytes and antigen-specific soluble suppressor factors (18, 29) further argues for suppressor-type mechanisms as one means by which *I*-region genes regulate the immune response. Other control mechanisms may be based on the expression of different *I*-region determinants selectively on

helper T cells (14, 17), helper T-cell factors (14, 18, 32, 33), B cells [14], or perhaps even on antigen-committed subpopulations of these cells. The demonstration that products of *I*-region loci are selectively expressed on functionally different lymphocytes offers a new framework for consideration of the complex processes which regulate immune responses.

Summary

In an accompanying publication we show that a subpopulation of T lymphocytes, which includes allotype suppressor T cells, selectively expresses *I*-region determinants. In this report, we show that these determinants are controlled by a new locus, *Ia-4*. Unlike the classically defined *Ia* antigens, they are not found on B lymphocytes. Antibody against *Ia-4* determinants cannot be detected by conventional dye exclusion cytotoxicity assays, suggesting that they are present on a small subpopulation (less than 10%) of peripheral T lymphocytes.

The *Ia-4* locus marks a new *I* subregion, provisionally designated *I-J*. This chromosomal segment is defined by the crossover positions in strains B10.A(5R) (*K*-end boundary) and B10.HTT (*D*-end boundary), and maps between the *I-B* and *I-C* subregions.

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References

1. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science*. (Wash. D. C.). 175:273.
2. Shreffler, D. C., and C. S. David. 1975. The *H-2* major histocompatibility complex and the *I* immune response region: genetic variation, function, and organization. *Adv. Immunol.* 20:125.
3. Klein, J. 1975. *Biology of the Mouse Histocompatibility-2 Complex* Springer-Verlag New York Inc., New York.
4. Sachs, D. H., and J. L. Cone. 1973. A mouse B-cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *J. Exp. Med.* 138:1289.
5. Hämmerling, G. J., B. D. Deak, G. Mauve, U. Hämmerling, and H. O. McDevitt. 1974. B lymphocyte alloantigens controlled by the *I* region of the major histocompatibility complex in mice. *Immunogenetics.* 1:68.
6. Frelinger, J. A., J. E. Neiderhuber, C. S. David, and D. C. Shreffler. 1974. Evidence for the expression of *Ia* (*H-2* associated) antigens on thymus-derived lymphocytes. *J. Exp. Med.* 140:1273.
7. Götze, D. 1975. T(*Iat*)-and B(*Iab*)-cell alloantigens determined by the *H-2* linked *I* region in mice. *Immunogenetics.* 1:495.
8. Neiderhuber, J. E., J. A. Frelinger, M. S. Dire, P. Shoffner, P. Dugan, and D. C. Shreffler. 1976. Effects of anti-*Ia* sera on mitogenic responses. II. Differential expression of the *Ia* marker on phytohemagglutinin – and concanavalin A – reactive T cells. *J. Exp. Med.* 143:372.
9. David, C. S., T. Meo, J. McCormick, and D. C. Shreffler. 1975. Expression of individual *Ia* specificities on T and B cells. I. Studies with mitogen-induced blast cells. *J. Exp. Med.* 143:218.

10. Götze, D. 1976. Serological characterization of Ia antigens on the *H-2^k*, *H-2^s*, and *H-2^g* haplotypes by antisera produced against skin, lymphocytes, and lymphoblasts. Strain distribution pattern of Ia antigens and their relationship to *Ir* genes. *Immunogenetics*. 3:139.
11. Lonai, P. 1975. Genetic control of the stimulator and effector function in allogeneic lymphocyte interaction: the expression of *I* region gene products on T and B lymphocytes. *Proc. 9th Leucocyte Cult. Conf.* 683.
12. Fathman, C. G., J. L. Cone, S. O. Sharrow, H. Tyrer, and D. H. Sachs. 1975. Ia alloantigens detected on thymocytes by use of a fluorescence-activated cell sorter. *J. Immunol.* 115:584.
13. 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.* In press.
14. McDevitt, H. O., T. L. Delovitch, J. L. Press, and D. B. Murphy. 1976. Genetic and functional analysis of the Ia antigens: their possible role in regulating the immune response. *Transplant. Rev.* 30: in press.
15. Hämmerling, G. J., S. J. Black, S. Segal, and K. Eichmann. 1975. Cellular expression of Ia antigens and their possible role in immune reactions. In *Leukocyte Membrane Determinants Regulating Immune Reactivity*. V. P. Eijssvoogel, D. Roos, and W. P. Zeijlemaker, editors. Academic Press, Inc., New York. 367.
16. McDevitt, H. O. 1976. Functional analysis of Ia antigens in relation to genetic control of the immune response. In *The Role of the Products of the Histocompatibility Gene Complex in Immune Responses*. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc. New York. 257
17. Okumura, K., L. A. Herzenberg, D. B. Murphy, H. O. McDevitt, and L. A. Herzenberg. 1976. Selective expression of *H-2* (*I* region) loci controlling determinants on helper and suppressor T lymphocytes. *J. Exp. Med.* 144:685.
18. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response in the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the *H-2* histocompatibility complex. *J. Exp. Med.* 144:713.
19. Klein, J., V. Hauptfeld, and M. Hauptfeld. 1975. Evidence for a fifth (G) region in the *H-2* complex of the mouse. *Immunogenetics*. 2:141.
20. David, C. S., J. H. Stimpfling, and D. C. Shreffler. 1975. Identification of specificity *H-2.7* as an erythrocyte antigen: control by an independent locus, *H-2G*, between the *S* and *D* regions. *Immunogenetics*. 2:131.
21. Frelinger, J. A., D. B. Murphy, and J. F. McCormick. 1974. Tla types of *H-2* congenic and recombinant mice. *Transplantation (Baltimore)*. 18:292.
22. 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.
23. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
24. Sato, V. L., S. D. Waksal, and L. A. Herzenberg. 1976. Identification and separation of pre-T-cells from nu/nu mice: differentiation by preculture with thymic reticuloepithelial cells. *Cell Immunol.* 24:173.
25. Herzenberg, L. A., K. Okumura, and C. M. Metzler. 1975. Regulation of immunoglobulin and antibody production by allotype suppressor T cells in mice. *Transplant. Rev.* 27:57.
26. Lieberman, R., W. E. Paul, W. Humphrey, Jr., and J. H. Stimpfling. 1972. *H-2*-linked immune response (*Ir*) genes. Independent loci for *Ir-IgG* and *Ir-IgA* genes. *J. Exp. Med.* 136:1231.

27. David, C. S. 1976. Serologic and genetic aspects of murine Ia antigens. *Transplant. Rev.* 30: in press.
28. David, C. S., and S. E. Cullen. 1976. Murine Ia antigens: mapping studies on Ia.3 and 15. *Fed. Proc.* 35:2787. (Abstr.)
29. Tada, T., and J. Taniguchi. 1976. Characterization of the antigen-specific suppressive T cell factor with special reference to the expression of I region genes. In *The Role of the Products of the Histocompatibility Gene Complex in Immune Responses*. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 513.
30. Rich, R. R., and C. W. Pierce. 1973. Biological expressions of lymphocyte activation. I. Effects of phyto mitogens on antibody synthesis in vitro. *J. Exp. Med.* 137:205.
31. Stout, R. D., and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived lymphocytes II. Mitogen responsiveness of T lymphocytes bearing the Fc receptor. *J. Exp. Med.* 142:1041.
32. Taussig, M. J., A. J. Munro, R. Campbell, C. S. David, and N. A. Staines. 1975. Antigen-specific T-cell factor in cell cooperation. Mapping within the I region of the H-2 complex and ability to cooperate across allogeneic barriers. *J. Exp. Med.* 142:694.
33. Armerding, D., D. H. Sachs, and D. H. Katz. 1974. Activation of T and B lymphocytes in vitro. III. Presence of Ia determinants on allogeneic effect factor. *J. Exp. Med.* 140:1717.
34. Shreffler, D. C., T. Meo, and C. S. David. 1976. Genetic resolution of the products and functions of I and S region genes of the mouse H-2 complex. In *The Role of the Products of the Histocompatibility Gene Complex in Immune Responses*. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 3.
35. Merryman, C. F., P. H. Maurer, and J. H. Stimpfling. 1975. Unigenic and multigenic I region control of the immune responses of mice to GAT¹⁰ and GL ϕ -GLT terpolymers. *Immunogenetics.* 2:441.
36. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1975. Requirement for two H-2 complex Ir genes for the immune response to the L-Glu,L-Lys,L-Phe terpolymer. *J. Exp. Med.* 141:1459.
37. Melchers, I., and K. Rajewsky. 1975. Specific control of responsiveness by two complementing Ir loci in the H-2 complex. *Eur. J. Immunol.* 5:753.
38. Waltenbaugh, C. R., P. Debre, M. Dorf, and B. Benacerraf. 1976. Two H-2 gene control of GT specific immune suppression. *Fed. Proc.* 35:2796.
39. Murphy, D. 1975. Serological cross-reactivity between the products of discrete Ia genes. *Fed. Proc.* 34:1016. (Abstr.)
40. Colombani, J., M. Colombani, D. C. Shreffler, and C. S. David. 1976. Separation of anti-Ia (I region associated antigens) from anti-H-2 antibodies in complex sera by absorption on blood platelets. Description of three new specificities. *Tissue Antigens.* 7:74.
41. Lindahl, K. 1975. Specificity of xenograft reactions *in vitro*. Ph.D. Thesis. University of Wisconsin, Madison, Wis.
42. Gershon, R. K., P. H. Maurer, and C. F. Merryman. 1973. A cellular basis for genetically controlled immunologic unresponsiveness in mice: tolerance induction in T-cells. *Proc. Natl. Acad. Sci. U. S. A.* 70:250.
43. Kapp, J. A., C. W. Pierce, S. Schlossman, and B. Benacerraf. 1974. Genetic control of immune responses *in vitro*. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT). *J. Exp. Med.* 140:648.
44. Debré, P., J. A. Kapp, M. E. Dorf, and B. Benacerraf. 1975. Genetic control of specific immune suppression. II. H-2-linked determinant control of immune suppression by the random copolymer L-glutamic acid-L-tyrosine (GT). *J. Exp. Med.* 142:1447.