

Selective Expression of Separate *I*-region Loci in Functionally Different Lymphocyte Subpopulations

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The selective expression of different loci in the genome allows genetically identical cells to perform different functions. One group of such loci, clustered in the *I* region of the *H-2* gene complex, controls Ia cell-surface determinants (Klein 1975; Shreffler and David 1975; McDevitt et al. 1976). As we will show here, functionally different lymphocyte subpopulations, i.e., B cells, suppressor T cells, and helper T cells, selectively express distinct Ia determinants which may be involved in lymphocyte interactions.

Genetic and functional studies of the *I* region have been facilitated by the development of *H-2* congenic recombinant strains of mice (Snell 1948; Stimpfling and Richardson 1965; Klein 1975; Shreffler and David 1975). These strains have permitted subdivision of the *I* region into discrete chromosomal segments, designated *subregions*. Each subregion is defined by crossovers that have occurred on opposite sides of distinct marker loci (Fig. 1). Originally, three subregions, *I-A*, *I-B*, and *I-C*, were defined (Klein 1975; Shreffler and David 1975). Recently the *I-C* subregion has been provisionally divided into *I-E* and *I-C* (Shreffler et al., this volume). This subdivision is based on serological and genetic studies (Colombani et al. 1976; Shreffler et al., this volume) and is supported by immunochemical studies (McDevitt et al. 1976; T. Delovitch, pers. comm.). Data presented here define and characterize the *I-J* subregion and show that *I-J* is intercalated between *I-B* and *I-E*.

The data presented here also show that distinct *I*-region loci are selectively expressed in cells which play different roles in generating humoral responses. Determinants controlled by the *Ia-4* locus, which marks the *I-J* subregion, control cell-surface determinants found on allotype suppressor T lymphocytes. These determinants are not expressed on helper T lymphocytes or B lymphocytes. In contrast, determinants controlled by the *Ia-1* locus, which maps in the *I-A* subregion, are found on B lymphocytes but not on suppressor T or helper T lymphocytes. Another locus (subregion not known) controls determinants which distinguish helper T lymphocytes from suppressor T lymphocytes. Such selective expression suggests that *I*-region determinants may be involved in the regulation of antibody responses.

THE *I-J* SUBREGION

Definition

Ia-4 determinants¹ are detected by *I*-region antisera which kill allotype suppressor T cells (T_s) in the presence of complement. T_s killing is determined by the removal of T_s activity, which is measured in an adoptive cotransfer assay. Absorptions of these antisera with cells from intra-*I*-region recombinant strains demonstrate that the *Ia-4* locus is genetically distinct from other known marker loci. The crossover positions in strains used for absorption define the *I-J* subregion.

T_s activity is assayed in an adoptive secondary transfer system in which unprimed, allotype-suppressed (BALB/c \times SJL) F_1 spleen cells (containing T_s) are cotransferred with DNP (dinitrophenyl)-KLH (keyhole limpet hemocyanin)-primed F_1 hybrid spleen (containing helper T [T_H] and primed B [^{10}B] lymphocytes) into irradiated BALB/c recipients (Herzenberg et al. 1975). Nonsuppressed DNP-KLH-primed spleen cells transferred alone give rise to roughly equal numbers of Ig-1a and Ig-1b DNP-PFC (plaque-forming cells) 7 days after challenge with DNP-KLH. T_s specifically suppress Ig-1b antibody production, and therefore cotransfer of T_s reduces the Ig-1b responses without affecting the Ig-1a or other IgG responses. In the experiments presented below, the number of T_s transferred is slightly greater than the number required to completely suppress the Ig-1b response by the primed, nonsuppressed cells. The strains of mice used in this study and the *H-2* haplotypes they carry are listed in Table 1.

Data in Tables 2 and 3 show that cytotoxic treatment of T_s with antisera containing antibody against *I*-region determinants removes T_s activity. This activity cannot be restored by cotransferring anti-*Ia*-plus-complement-treated, nylon-passed, suppressed spleen with congenic anti-Thy-1-plus-com-

¹ The notation "*Ia-4* determinants" refers to determinants controlled by the *Ia-4* locus. The same notation is used in reference to determinants controlled by the *Ia-1*, *Ia-3*, and *Ia-5* loci. This notation should not be confused with that used for denoting individual Ia antigenic determinants (specificities) controlled by these loci (e.g., Ia.1, Ia.2, etc.).

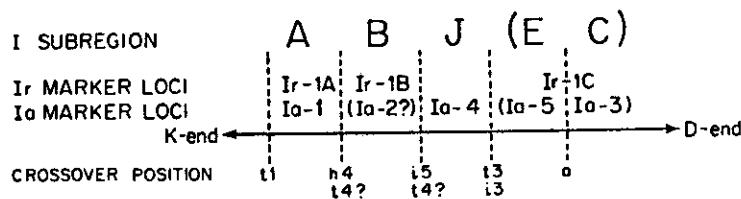


Figure 1. Genetic fine structure of the *I* region. Broken lines indicate crossover positions detected in different recombinant haplotypes. Since studies of the *I-E* subregion are in progress (Shreffler et al., this volume), we have placed *I-E* and *I-C* in parentheses. For this discussion, we will assume the order shown.

Table 1. Haplotype Origin of *H-2* Regions

Strain	Haplotype	Region ^a					
		<i>I</i>					<i>S G D</i>
		<i>K</i>	<i>A</i>	<i>B</i>	<i>J</i>	<i>E</i>	<i>C</i>
B10/Sn	<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>
SJL/JHz	<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>
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>
HTI/BoySf	<i>i</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>k</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>
A.TL/Sf	<i>t1</i>	<i>s</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</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>
B10.HTT/Ph	<i>t3</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>k</i>	<i>k</i>	<i>k</i>
B10.S(9R)/Sg	<i>t4</i>	<i>s</i>	<i>s</i>	<i>?</i>	<i>k</i>	<i>k</i>	<i>d</i>
B10.AQR(N4)/K1j	<i>y1</i>	<i>q</i>	<i>k</i>	<i>k</i>	<i>k</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>q</i>

^a Haplotype origin of regions according to David (1976), Shreffler et al. (this volume), and Murphy et al. (1976). Each region is marked by one or more loci that control distinct products or functions (see Fig. 1). The haplotype origin of a region is determined by the allele at the marker locus.

plement-treated suppressed spleen (Okumura et al. 1976). Thus a Thy-1-positive T cell (i.e., T_s) is the target of anti-Ia-mediated cytotoxicity.

In order to map the locus (*Ia-4*) which controls the determinants on T_s, the Ia antisera were absorbed with intra-*I*-region recombinant strains and then tested for residual T_s killing activity. Two pairs of recombinant strains were used for these absorptions. The first pair, B10.A(5R) and B10.A(3R), carries independently derived recombinant chromosomes which appeared to be identical prior to these studies (David 1976). The crossover in each occurred between the *I-B* and *I-E* subregions (see Table 1). The antiserum used was a (B10.T(6R) × B10.D2)F₁ anti-B10.AQR serum, which contains anti-Ia-4^k antibody cross-reactive with Ia-4^s determinants carried by T_s. Strain B10.A(5R) absorbed antibody activity for T_s, whereas strain B10.A(3R) did not (Table 2).

The difference in the ability of these strains to absorb Ia-4^k antibody indicates that the crossovers occurred on opposite sides of the *Ia-4* locus, i.e., B10.A(5R) carries the Ia-4^k allele, whereas B10.A(3R) carries the other parental allele (*Ia-4*^s) (see Table 1). The crossover in B10.A(5R) separates *Ia-4* from *Ir-1B* (marker locus for the *I-B* subregion), and the crossover in strain B10.A(3R) separates

Table 2. Mapping the *Ia-4* Locus to a New *I* Subregion, *I-J*: Recombinant Strains B10.A(5R) and B10.A(3R)

(BALB/c × SJL)F ₁ spleen cells transferred (× 10 ⁶) ^a	T _h + 1 ^b B ^b	Ig-1b T _s ^c	Treatment of T _s		IgG2a DNP-PFC/10 ⁶		Conclusion: T _s killed	
			serum	absorbed with	<i>I</i> subregion detectable ^d	Ig-1b		Ig-1a
					A B J E			
8	—	—	—	—	—	152	129	—
8	4	NMS	—	—	—	0	122	no
8	4	(B10.T(6R) × B10.D2)F ₁ anti-B10.AQR ^e	—	—		134	131	yes
8	4	(B10.T(6R) × B10.D2)F ₁ anti-B10.AQR ^e	B10.S(7R)	—	-----	23	164	no
8	4	(B10.T(6R) × B10.D2)F ₁ anti-B10.AQR ^e	B10.T(6R)	—		133	151	yes
8	4	(B10.T(6R) × B10.D2)F ₁ anti-B10.AQR ^e	B10.A(3R)	—		118	123	yes
8	4	(B10.T(6R) × B10.D2)F ₁ anti-B10.AQR ^e	B10.A(5R)	—		17	174	no

^a (BALB/c × SJL)F₁ (Ig-1^a/Ig-1^b, H-2^d/H-2^s) donor spleen transferred into irradiated (600 rad) BALB/c recipients.

^b DNP-KLH-primed spleen (helper T [T_h] and primed B [1^bB] source).

^c Ig-1b suppressed spleen (allotype suppressor T-cell [T_s] source).

^d Solid line indicates presence of antibody; broken line indicates absence of antibody.

^e (B10.T(6R) × B10.D2)F₁ anti-B10.AQR (anti-I-A^k, I-B^k, I-J^k, I-E^k). This serum contains anti-Ia-4^k antibody which cross-reacts with Ia-4^s determinants on T_s.

Table 3. Mapping the *Ia-4* Locus to a New *I* Subregion, *I-J*: Recombinant Strains B10.HTT and B10.S(9R)

BALB/c × SJL _{F1} spleen cells transferred (× 10 ⁶) ^a	Treatment of T _S					Ig-1b DNP-PFC/10 ⁶	Conclusion: T _S killed
	T _H + 1 ⁰ B ^b	Ig-1b T _S ^c	serum	absorbed with	<i>I</i> subregion detectable ^d A B J E		
7	—	—	—	—		1161	—
7	4	NMS	—	—		147	no
7	4	(B10.A × A.TL) _{F1} anti-B10.HTT ^e	—	—		1121	yes
7	4	(B10.A × A.TL) _{F1} anti-B10.HTT ^e	B10.A	—		1110	yes
7	4	(B10.A × A.TL) _{F1} anti-B10.HTT ^e	B10.HTT	-----		127	no
7	4	(B10.A × A.TL) _{F1} anti-B10.HTT ^e	B10.S(9R)	---	? ---	1181	yes

^a (BALB/c × SJL)_{F1} (*Ig-1^a/Ig-1^b, H-2^a/H-2^b*) donor spleen transferred into irradiated (600 rad) BALB/c recipients.

^b DNP-KLH-primed spleen (helper T [T_H] and primed B [1⁰B] source).

^c Ig-1b suppressed spleen (allotype suppressor T-cell [T_S] source).

^d Solid line indicates presence of antibody; broken line indicates absence of antibody.

^e (B10.A × A.TL)_{F1} anti-B10.HTT (anti-I-A^a, I-B^a, I-J^a). This serum contains anti-Ia-4^a antibody which reacts with Ia-4^a determinants on T_S.

Ia-4 from *Ia-5* (marker locus for the *I-E* subregion) (see Fig. 1). These crossovers localize the *Ia-4* locus to a previously uncharted segment of chromosome (i.e., a new subregion). Similar results were obtained with a (B10.A × A.TL)_{F1} anti-B10.HTT serum and recombinant strains B10.HTT and B10.S(9R) (Table 3). The crossover positions in strains B10.A(5R) and B10.HTT have been designated as the boundaries of the *I-J* subregion (Murphy et al. 1976).

Other Phenotypic Traits Mapping to the *I-J* Subregion

Several other phenotypic traits have been mapped to the *I-J* subregion. *I-J* determinants have been detected on T lymphocytes (Tada and Taniguchi, this volume) and soluble factors (Tada et al. 1976) which specifically suppress antibody responses to haptens coupled to KLH as a carrier. They are also found on at least one of the T-cell subpopulations which promote (initiate) stimulation to Concanavalin A (Con A) (Stout and Herzenberg 1975; J. Frelinger, pers. comm.; Shreffler et al., this volume). Whether these traits are controlled by *Ia-4* or by other loci mapping in the *I-J* subregion remains to be determined.

Cellular Distribution

Ia cell-surface determinants have generally been detected by the ability of Ia antibody plus complement to kill lymph node or spleen target cells in dye exclusion assays (Klein 1975; Shreffler and David 1975). Cells killed by this method are predominantly B lymphocytes. Reagents specific for determinants controlled by the *I-J* subregion have consistently failed to give detectable killing in this assay, although the same reagents are

capable of killing T_S in functional assays (Murphy et al. 1976). 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) and raise the possibility that *I-J* determinants are not present on B lymphocytes.

Studies with an antiserum rendered specific for *I-J* determinants show that nylon-passed splenic T cells (1% Ig-positive) completely absorb Ia-4 antibody reactive with T_S (Table 4). This antibody activity is not detectably absorbed by an equivalent number of T-depleted splenic lymphocytes (85% Ig-positive) from the same spleen cell preparation. These studies demonstrate that Ia-4 determinants are found on T but not on B lymphocytes. The data also suggest that macrophages do not carry these determinants, since both the "T"- and "B"-cell preparations used should contain this cell population.

The absorption studies described above were performed with fractionated spleen cell populations from normal (nonsuppressed) (BALB/c × SJL)_{F1} mice. Some normal T cells therefore share the same Ia-4 determinants found on allotype suppressor T cells. Whether these cells are suppressor T cells generated in response to environmental self antigens, precursors of allotype (or other types of) suppressor T cells, or other functional T-cell subpopulations remains to be determined.

SELECTIVE EXPRESSION OF *I*-REGION DETERMINANTS ON FUNCTIONALLY DIFFERENT LYMPHOCYTE SUBPOPULATIONS

In retrospect, early serologic studies showing that Ia determinants are found on the majority of B cells and on a fraction of T cells provided the first

Table 4. T Cells, but Not B Cells, Absorb Anti-Ia-4 Antibody

$T_H + 1^0B^b$	Ig-1b T_S^c	Treatment of T_S		Ig-1b DNP-PFC/10 ⁶	Conclusion: T_S killed
		serum	absorbed with		
7	—	—	—	1160	—
7	4	NMS	—	147	no
7	4	anti-I-J ^d	—	1450	yes
7	4	anti-I-J ^d	"T" ^e	185	no
7	4	anti-I-J ^d	"B" ^f	1190	yes

^a (BALB/c × SJL)_{F₁} (Ig-1^a/Ig-1^b, H-2^d/H-2^e) donor spleen transferred into irradiated (600 rad) BALB/c recipients.

^b DNP-KLH-primed spleen (helper T [T_H] and primed B [1^0B] source).

^c Ig-1b suppressed spleen (allotype suppressor T-cell [T_S] source).

^d A.TL anti-A.TH absorbed with B10.S(9R) (contains anti-Ia-4^g antibody).

^e "T" — 200 × 10⁶ nylon-passed (BALB/c × SJL)_{F₁} spleen: 84% stained with a fluoresceinated T-cell-specific rabbit anti-mouse brain serum (BAT) (Sato et al. 1976); 1% stained with a fluoresceinated anti-Ig reagent which detects B cells.

^f "B" — 200 × 10⁶ T-depleted (BALB/c × SJL)_{F₁} spleen (anti-BAT-plus-complement-treated); 3% BAT-positive, 85% Ig-positive.

clue that loci in the *I* region are selectively expressed on subpopulations of lymphocytes (David et al. 1973; Götze et al. 1973; Hauptfeld et al. 1973; Frelinger et al. 1974; Hämmerling et al. 1974; Sachs and Cone 1973). Subsequent functional studies by Hämmerling et al. (1976), demonstrating that determinants controlled by a locus mapping in the *I* region are present on idiotype suppressor T cells but not helper T cells, and by Niederhuber et al. (1976), showing that Con-A- but not phytohemagglutinin (PHA)-reactive T cells bear Ia determinants, confirmed that I-region determinants are expressed on some but not all T lymphocytes. Recent studies (McDevitt et al. 1976; R. Stout pers. comm.) also show that I-region determinants are present on a subpopulation of Fc-receptor-bearing T lymphocytes. We have shown here that allotype suppressor T cells, but not B cells, express Ia-4 determinants. These studies demonstrate restrictive but not selective expression of Ia

loci since they do not address the question of whether different *Ia* loci are expressed in each of the subpopulations tested. In the sections which follow, however, we demonstrate selective expression by showing that suppressor T, helper T, and B-lymphocyte subpopulations carry different Ia determinants controlled by separate loci.

B Cells, T_S , and T_H All Carry I-region Determinants

As indicated above, B cells and T_S bear determinants controlled by loci mapping in the *I* region. Data in Table 5 show that helper T cells also carry I-region determinants. Treatment of T_H with either a (B10.T(6R) × B10.D2)_{F₁} anti-B10.AQR or a (B10 × HTD)_{F₁} anti-B10.A(5R) serum drastically reduces helper activity. Both sera potentially contain antibody against the same T_H determinant controlled by a single locus map-

Table 5. Selective Expression of I-region Determinants on Helper and Suppressor T Lymphocytes

(BALB/c × SJL) _{F₁} spleen cells transferred (× 10 ⁶) ^a	Treatment of T_H		region detected	Total indirect PFC/10 ⁶	Conclusion: T_H killed	Compar- T_S killed		
	DNP-KLH 1 ^b T-depleted ^b (1 ⁰ B)	KLH 1 ⁰ (T_H)					serum	<i>I</i>
								A B J E C S G
4	—	—		219	—	—		
4	4	NMS		1610	no	—		
4	4	(B10.A × A.TL) _{F₁} anti-B10.HTT ^c		1770	no	yes		
4	4	(B10.T(6R) × B10.D2) _{F₁} anti-B10.AQR ^d		332	yes	yes		
4	4	(B10 × HTD) _{F₁} anti-B10.A(5R) ^e	?	415	yes	no		

^a (BALB/c × SJL)_{F₁} (Ig-1^a/Ig-1^b, H-2^d/H-2^e) donor spleen transferred into irradiated (600 rad) BALB/c recipients.

^b Congenic anti-Thy-1-plus-complement-treated.

^c (B10.A × A.TL)_{F₁} anti-B10.HTT (anti-I-A^k, I-B^k, I-J^k).

^d (B10.T(6R) × B10.D2)_{F₁} anti-B10.AQR (anti-I-A^k, I-B^k, I-J^k, I-E^k).

^e (B10 × HTD)_{F₁} anti-B10.A(5R) (anti-I-J^k, I-E^k, I-C^d, S^d, [G^h?]).

ping in the *I-J* or *I-E* subregion. Alternatively, the two antisera may detect different T_H determinants controlled by loci mapping in separate subregions. Absorption studies are currently underway to map the locus (loci) that control(s) determinants on this lymphocyte subpopulation.

The presence of *I*-region determinants on helper T lymphocytes was assayed in an adoptive secondary transfer in which limiting numbers of (BALB/c \times SJL) F_1 KLH 1^0 spleen cells (T_H source) were cotransferred with hybrid T-depleted (congenic anti-Thy-1-treated) DNP-KLH-primed spleen (1^0 B source) into irradiated (600 rad) BALB/c recipients (Okumura et al. 1976). Investigators using other assay systems have seen no effect of anti-Ia treatment on helper-T-cell function (Hämmerling et al. 1976; J. Press, pers. comm.). One major difference between these assay systems and the system we use is that we transfer F_1 cells into an irradiated parental recipient. There is certainly the potential for an allogenic effect or back-stimulation in such a transfer. However, the target of anti-Ia-mediated lysis appears to be a carrier-primed helper T lymphocyte because (1) the DNP-KLH-primed spleen cells treated with congenic anti-Thy-1 (i.e., B-cell source) fail to respond when transferred alone (Table 5), and (2) only KLH-primed T cells will help the B cells respond to DNP-KLH (Herzenberg and Herzenberg 1974).

Determinants Expressed on T_S Are Not Present on T_H or B Cells

We have shown above that B cells do not absorb antibody reactive with the Ia-4 determinants present on T_S . Treatment of T_H with the same antiserum [(B10.A \times A.TL) F_1 anti-B10.HTT] that kills T_S had no effect on helper activity (Table 5). These data demonstrate that Ia-4 determinants are not found on B cells or T_H .

Determinants Expressed on T_H Are Not Present on T_S

T_H , but not T_S , are killed by treatment with a (B10 \times HTI) F_1 anti-B10.A(5R) serum (Table 5). This antiserum contains antibody against *I*, and possibly *S*- or *G*-region-controlled determinants. Preliminary absorption studies indicate that the locus controlling the determinants on T_H maps in the *I* region (D. Murphy, unpubl.). Studies are in progress to determine whether these T_H determinants are present on B lymphocytes.

Determinants Expressed on B Lymphocytes Are Not Present on T_S or T_H

Two loci (*Ia-1* and *Ia-3*) that control determinants on B cells have been identified (Klein 1975; Shreffler and David 1975). A third locus, provi-

sionally designated *Ia-5*, also appears to control determinants on this lymphocyte population (Shreffler et al., this volume). Cytotoxic treatment of T_S with sera that detect either Ia-1 determinants [(B10.T(6R) \times B10.D2) F_1 anti-B10.AQR absorbed with B10.A(5R)] (Table 2) or Ia-3 and Ia-5 determinants [(B10 \times HTI) F_1 anti-B10.A(5R)] (Table 5) (Okumura et al. 1976) fails to eliminate T_S activity. Thus, Ia-1, Ia-3, and Ia-5 determinants are not present on T_S .

Ia-1 determinants also are not present on T_H . Data in Table 5 show that a (B10.A \times A.TL) F_1 anti-B10.HTT serum, which contains antibody reactive with Ia-1 determinants, does not kill T_H . Studies are in progress to determine whether T_H carry Ia-3 and/or Ia-5 determinants.

DISCUSSION

Recognition that the *I* region of the *H-2* complex plays a role in regulation of immune responses dates from the discovery that loci within this region control the ability to respond to a synthetic polypeptide antigen, (H,G)-A-L (McDevitt et al. 1972). Since that time, responses to a wide variety of antigens have been shown to be under the control of different *I*-region (*Ir*) loci (Klein 1975; Shreffler and David 1975), and a number of instances have been found where two loci mapping to different *I* subregions act in concert to control the response to a given antigen (Dorf et al. 1975; Merryman et al. 1975; Melchers and Rajewsky 1975). Precisely how the *Ir* loci exert their regulatory function is unknown; however, T-cell help appears to be missing in some cases (McDevitt 1968; Grumet 1972), whereas T-cell suppression appears to operate in others (Kapp et al. 1974; Debre et al. 1975).

The discovery that *I*-region loci control surface determinants on cells involved in immune responses raised the possibility that the *Ir* loci might function via such surface determinants. Studies indicating that these determinants were found on B rather than T cells (Sachs and Cone 1973; Hämmerling et al. 1974) added some degree of confusion to the picture since the evidence available at the time appeared to favor T cells as the effectors of *Ir* gene control (Benacerraf and McDevitt 1972). Subsequent studies did show that T cells also carry *I*-region-controlled determinants (Götze et al. 1973; Frelinger et al. 1974; Götze 1975; David et al. 1975). To date, however, there is still no direct evidence implicating Ia determinants found on either B or T cells in the mechanism(s) of *Ir* gene control.

The selective expression of different *I*-region loci in functionally distinct subpopulations of lymphocytes (suppressor T cells, helper T cells, B lymphocytes) introduces a new perspective which could be relevant to the mechanism(s) of *Ir* gene control. In addition, selective expression expands the scope of *I*-region involvement in the immune response con-

siderably beyond those responses known to be governed by *Ir* genes. Since interacting lymphocytes carry different Ia determinants, these determinants could serve as part of the language through which these cells recognize and regulate one another.

For example, the allotype suppressor T cells studied here suppress antibody production by inactivating (or killing) helper T cells required to facilitate antibody production by B cells (Herzenberg et al. 1976). This process appears to be mediated by a soluble suppressive factor. The I-J determinants on the suppressor cells could be associated with the surface-bound suppressive factor and be part of the language through which the factor recognizes the helper; or these determinants could be part of the language used by another cell to recognize (and regulate) the suppressor. Similarly, the I-region determinants detected on helper cells could represent surface receptors for suppressive factors or surface-bound effector molecules destined for use in interactions with B cells.

Such a hypothesis would be consistent with studies which show that different soluble T-cell factors bear unique Ia determinants and bind to distinct I-region-controlled acceptors. One factor, which suppresses antibody responses, carries determinants controlled by a locus mapping in the I-J subregion (Tada et al. 1976) and binds to a T-cell acceptor controlled by a locus mapping to the left of the I-C subregion (Tada and Taniguchi, this volume). A second factor, which potentiates IgG antibody responses, carries determinants controlled by a locus mapping in the I-A subregion (Tada and

Taniguchi 1976). The T-cell acceptor for this factor is also controlled by a locus mapping in the I-A subregion. A third factor, which can replace T-cell help in generating IgM antibody responses, bears I-A-subregion determinants and binds to a B-cell acceptor controlled by a locus mapping in the I-A subregion (Taussig et al. 1976). Thus I-region loci control factors and acceptors which appear to be part of the communication system through which signals are conveyed among different immunocompetent cells.

A number of functionally different lymphocyte subpopulations and factors have now been shown to carry I-region determinants. These are listed in Table 6 along with I-subregion mapping data where available. In most cases, selective expression of the marker determinants has not been fully explored; however, none of the cells or factors listed express all of the known I-region markers. Selective expression of I-region loci, therefore, may be the rule rather than the exception.

At least two Ia loci, mapping in different subregions, can be selectively expressed in a single lymphocyte. For example, the majority of B cells express both Ia-1 and Ia-3 determinants. These data do not exclude selective expression of either of these determinants on small subpopulations of B cells or the existence of a subpopulation of these cells carrying neither determinant (Press et al. 1976). Whether individual T cells also express more than one locus is unknown.

Some of the I-region loci appear to be clustered according to function, assuming the phenotypic

Table 6. I-subregion Localization of Loci Controlling Determinants Present on Different Lymphocyte Subpopulations and Immune Factors

Lymphocytes and factors carrying Ia determinants	I subregion					References
	A	B	J	E	C	
Suppressor T						
allotype (Ig-Ib)			—			Murphy et al. (1976)
idiotype (A5A) ^a	-----					Hämmerling et al. (1976)
carrier (KLH)			—			Tada and Taniguchi (1976)
Suppressor factor						
carrier (KLH)			—			Tada et al. (1976)
acceptor for KLH suppressor factor ^a	-----					Tada and Taniguchi (this volume)
Con-A promoter T			—			Shreffler et al. (this volume)
Fc-receptor-bearing T	—				-----	R. Stout (pers. comm.); McDevitt et al. (1976)
Helper T (IgG resp.) ^a	-----					Okumura et al. (1976)
Helper factors						
(T,G)-A--L (IgM resp.)	—					Taussig et al. (1976)
acceptor for (T,G)-A--L helper factor	—					Taussig et al. (1976)
KLH (IgG resp.)	—					Tada and Taniguchi (1976)
acceptor for KLH helper factor	—					Tada and Taniguchi (1976)
allogenic (IgG resp.) ^a	-----					Armerding et al. (1974); McDevitt et al. (1976)
B lymphocytes	—		—	—		Sachs and Cone (1973); Hämmerling et al. (1974); Götze (1975); David et al. (1975); Shreffler et al. (this volume)

^a Not mapped to a subregion.

traits mapping to the same subregion are controlled by different loci. Loci controlling determinants on suppressor T cells and factors map to the *I-J* subregion (see Table 6). In contrast, loci controlling determinants on factors which potentiate immune responses and loci that control acceptors for these factors both map to the *I-A* subregion. The locus controlling T_H determinants has not been localized. If this locus maps to the *I-J* subregion, it will be interesting to determine whether these determinants are involved in T_H-T_S or T_H-B interactions.

A view of the *I* region as a series of subregions (clusters of loci) expressed on functionally related cell types and factors may be an oversimplification of the genetic fine structure of this chromosomal segment. Several examples of *Ir* and *Is* gene control of immune responses have been described where two loci, mapping in different subregions, are both required to regulate the response (Dorf et al. 1975; Merryman et al. 1975; Melchers and Rajewsky 1975; Benacerraf et al., this volume). Nevertheless, the correlations between suppressive activity and expression of *I-J*-subregion determinants and between helper factors and expression of *I-A*-subregion determinants suggests some organization of *I*-region loci along functional lines.

SUMMARY

Determinants controlled by separate loci mapping in the *I* region of the *H-2* gene complex are selectively expressed on subpopulations of lymphocytes which play different roles in generating humoral responses. The *Ia-4* locus, which marks a new *I* subregion, designated *I-J*, controls determinants found on allotype suppressor and normal (nonsuppressed) T lymphocytes. These determinants are not present on helper T lymphocytes or B lymphocytes. In contrast, the *Ia-1* locus, which marks the *I-A* subregion, controls determinants present on B lymphocytes but not on suppressor or helper T lymphocytes. Another locus (subregion not known) controls determinants which distinguish helper T lymphocytes from suppressor T lymphocytes. Such selective expression suggests that the products of these loci may play an integral role in lymphocyte interactions.

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REFERENCES

- 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.
- BENACERRAF, B. and H. O. McDEVITT. 1972. Histocompatibility-linked immune response genes. *Science* 175: 273.
- 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.
- DAVID, C. S. 1976. Serologic and genetic aspects of murine Ia antigens. *Transplant. Rev.* 30: 299.
- DAVID, C. S., D. C. SHREFFLER and J. A. FRELINGER. 1973. New lymphocyte antigen system (Lna) controlled by the *Ir* region of the mouse *H-2* complex. *Proc. Nat. Acad. Sci.* 70: 2509.
- 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.
- DEBRÉ, P., J. A. KAPP, M. E. DORF and B. BENACERRAF. 1975. Genetic control of specific immune suppression. II. *H-2* linked dominant control of immune suppression by the random copolymer L-glutamic acid-L-tyrosine (GT). *J. Exp. Med.* 142: 1447.
- DORF, M. E., J. H. STIMPLING 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.
- FRELINGER, J. A., J. E. NIEDERHUBER, 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.
- GÖTZE, D. 1975. T(lat)- and B(lab)-cell alloantigens determined by the *H-2* linked *I* region in mice. *Immunogenetics* 1: 495.
- GÖTZE, D., R. A. REISFELD and J. KLEIN. 1973. Serologic evidence for antigens controlled by the *Ir* region in mice. *J. Exp. Med.* 138: 1003.
- GRUMET, F. C. 1972. Genetic control of the immune response. A selective defect in immunologic (IgG) memory in nonresponder mice. *J. Exp. Med.* 135: 110.
- HÄMMERLING, G. J., S. J. BLACK, S. SEGAL and K. EICHMANN. 1976. Cellular expression of Ia antigens and their possible role in immune reactions. In *Leukocyte membrane determinants regulating immune reactivity* (ed. V. P. Eijssvoogel et al.), p. 367. Academic Press, New York.
- 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.
- HAUPTFELD, V., D. KLEIN and J. KLEIN. 1973. Serologic identification of an *Ir* region product. *Science* 181: 167.
- HERZENBERG, L. A. and L. A. HERZENBERG. 1974. Allotype suppression and production(?) by thymocytes and thymus-derived cells. In *Progress in immunology II* (ed. L. Brent and J. Holborow), vol. 2, p. 111. North-Holland, Amsterdam.
- 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.

- HERZENBERG, L. A., K. OKUMURA, H. CANTOR, V. L. SATO, F. W. SHEN, E. A. BOYSE and L. A. HERZENBERG. 1976. T cell regulation of antibody responses: Demonstration of allotype specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* 144: 330.
- 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 non-responder mice by the terpolymer L-glutamic acid⁵⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT). *J. Exp. Med.* 140: 648.
- KLEIN, J. 1975. *Biology of the mouse histocompatibility-2 complex*. Springer-Verlag, New York.
- MCDEVITT, H. O. 1968. Genetic control of the antibody response. III. Quantitative and qualitative characteristics of the antibody response to (T,G)-A-L in CBA and C57 mice. *J. Immunol.* 100: 485.
- 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: 197.
- MCDEVITT, H. O., D. B. DEAK, D. C. SHREFFLER, J. KLEIN, J. H. STIMPFLING and G. D. SNELL. 1972. Genetic control of the immune response. Mapping of the Ir-1 locus. *J. Exp. Med.* 135: 1259.
- 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.
- 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 GLPhe-GLT terpolymers. *Immunogenetics* 2: 441.
- MURPHY, D. B., L. A. HERZENBERG, K. OKUMURA, L. A. HERZENBERG and H. O. MCDEVITT. 1976. A new I subregion (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. *J. Exp. Med.* 144: 699.
- NIEDERHUBER, J. E., J. A. FRELINGER, M. S. DINE, P. SHOFFNER, E. 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.
- 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.
- PRESS, J. L., N. R. KLINMAN and H. O. MCDEVITT. 1976. Expression of Ia antigens on hapten-specific B cells. 1. Delineation of B cell subpopulations. *J. Exp. Med.* 144: 414.
- SACHS, D. H. and J. L. CONE. 1973. A mouse B-cell allo-antigen determined by gene(s) linked to the major histocompatibility complex. *J. Exp. Med.* 138: 1289.
- 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.
- 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.
- SNELL, G. D. 1948. Methods for the study of histocompatibility genes. *J. Genet.* 49: 7.
- STIMPFLING, J. H. and A. RICHARDSON. 1965. Recombination within the histocompatibility-2 locus of the mouse. *Genetics* 51: 831.
- 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.
- TADA, T. and M. 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 products of the histocompatibility gene complex in immune responses* (ed. D. H. Katz and B. Benacerraf), p. 513. Academic Press, New York.
- 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) which codes for the suppressive T cell factor in the H-2 histocompatibility complex. *J. Exp. Med.* 144: 713.
- TAUSSIG, M., A. J. MUNRO and A. L. LUZZATI. 1976. I region gene products in cell cooperation. In *The role of products of the histocompatibility gene complex in immune responses* (ed. D. H. Katz and B. Benacerraf), p. 553. Academic Press, New York.