SELECTIVE EXPRESSION OF *H-2* (*I*-REGION) LOCI CONTROLLING DETERMINANTS ON HELPER AND SUPPRESSOR T LYMPHOCYTES*

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

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

Downloaded from www.jem.org on May 25, 2005

T cells, defined as the class of peripheral lymphocytes which carry Thy-1 cell surface antigens, are involved in virtually all immune responses. In addition to serving as precursors and effectors in cellular immunity, these cells play key regulatory roles in both humoral and cellular immunity. A series of different T cells, however, are responsible for these various functions. These belong to different subclasses identifiable by cell surface antigenic characteristics (1–5).

Surface markers used to identify T-cell subclasses include the presence of Ly-1 or Ly-2,3 antigens (1–4) and the presence of Fc receptors (FcR)[1] which bind antigen-antibody complexes (6–8). The Ly-1^+,2^-,3^- (Ly-1) subclass contains amplifier cells for cytotoxic responses, helper cells for humoral responses (Th), and cells that initiate delayed-type hypersensitivity (1, 2). The presence of FcR further subdivides this subclass. Ly-1 FcR^+ cells amplify cytotoxic responses; Ly-1 FcR^- cells help secondary IgG humoral responses (6, 8).

The Ly-1^-,2^+,3^+ (Ly-2) subclass contains the precursors and effector cells for cellular immunity (2). It also contains the allotype suppressor T cells (Ts) used in the studies reported here (5), which suppress production of IgG_{2a} antibodies carrying the Ig-1b allotype in (BALB/c × SJL)F_1 mice (9). The Ly-2 subclass is also subdivided according to presence of Fc receptors. The precursor for cytotoxic responses is Ly-2 FcR^-; however, the cytotoxic effector cell is Ly-2 FcR^+ (2, 8).

B cells also carry identifying cell surface markers, some of which functionally subdivide B-cell populations. All B cells, by definition, carry surface immunoglobulin (10) which indicates the antibody-combining site commitment of the cell and its progeny antibody-forming cells (afc) (11). Memory B cells carry surface IgG which indicates class and allotype commitment as well (11).

Most B cells also carry easily detectable Ia determinants (12–14), although a B-cell subset which carries little or no Ia has been identified and shown to give rise only to IgM

* This research supported, in part, by grants from the National Institute of Child Health & Human Development (No. HD-01287); the National Cancer Institute (No. CA-04681); the National Institute of Allergy and Infectious Diseases (Nos. AI-07757 and AI-08917).

‡ Recipient of National Institutes of Health postdoctoral fellowship, 1974–1976.

[1] *Abbreviations usedin this paper:* afc, antibody-forming cell(s); Con A, concanavalin A; DNP, 2,4-dinitrophenol; FcR, Fc receptor; KLH, keyhole limpet hemocyanin; Ly-1, Ly-1^+,2^-,3^-; Ly-2, Ly-1^-,2^+,3^+; PFC, plaque-forming cells; Th, helper T cell; Ts, suppressor T cell; TsF, soluble suppressive factor.

afc (Press, personal communication [15]). These Ia determinants are controlled by genes in the *I* region of the major histocompatibility complex (*H-2*) (16). Loci that control immune responses (*Ir*) to a variety of antigens also map in the *I* region and thus are closely linked (or identical) with the loci that control B-cell Ia determinants (16, 17).

The T-cell surface markers have been used both to isolate T cells with different functions and to identify the roles that these cells play in the immune response (1–8). For example, in studies on the mechanism of allotype suppression, the difference in Ly phenotypes between Ts and Th was crucial for showing that Ts are different from Th, and that Ts suppress production of antibodies carrying the Ig-1b allotype by removing Th activity capable of helping Ig-1b memory B cells (5).

Using allotype Ts, we have now characterized a new set of T-cell surface determinants (15, 18) which, like Ly, distinguish between Th and Ts populations. Unlike Ly, however, these new T-cell surface determinants are coded for by loci in the *I* region of the *H-2* complex. Their selective expression on Ts or Th suggests they may be directly related to the immunoregulatory functions associated with this chromosomal region.

The presence of *I*-region determinants on mouse peripheral T cells was first detected by serologic studies (14, 19). Later, functional studies showed that anti-*I*-region antisera blocked antigenic stimulation of T cells (20), and that cytotoxic treatment with *I*-region antisera kills the T cells which suppress idiotype (21) or allotype (15, 18) production. In addition, *I*-region incompatible T cells have been shown to stimulate mixed lymphocyte reactions (22). *I*-region antisera have also been shown to both block the binding of antigen-antibody complexes to the Fc receptors on a substantial proportion of FcR^+ T cells (15) and to kill the T cells which initiate (promote) the overall T-cell response to concanavalin A (Con A) (23). Soluble T-cell factors with helper (24, 25) or suppressor (26, 27) activities have also been shown to carry *I*-region determinants.

In this publication, we will show that Ts and Th carry *I*-region determinants. We will also show that different antisera kill Th or Ts, and thus, that each of these cell types selectively express determinants that identify functional T-cell subpopulations. In an accompanying publication, we will show that the Ts determinants are different from the B-cell *I*-region (Ia) determinants and are controlled by a new locus, *Ia-4*, which maps in a previously undefined segment of the *I*-region provisionally designated the *I-J* subregion (28).

Materials and Methods

Mice. (BALB/cNHZ × SJL/JHz)F_1 hybrids (Ig^a/Ig^b, $H\text{-}2^d/H\text{-}2^s$) and BALB/cNHz mice were used for the suppression assay and helper assay experiments reported here. Strains used to prepare anti-Ia sera and for absorption studies were B10/Sn($H\text{-}2^b$), B10.D2/nSn($H\text{-}2^d$), B10.A/Sn($H\text{-}2^a$), HTI/BoySf($H\text{-}2^i$), B10.A(5R)/Sg($H\text{-}2^{i5}$), A.TL/Sf($H\text{-}2^{t1}$), A.TH/Sf and B10.S(7R)/Sg($H\text{-}2^{t2}$), B10.HTT/Ph($H\text{-}2^{t3}$), B10.AQR(N4)/Klj, ($H\text{-}2^{y1}$), B10.T(6R)/Sg($H\text{-}2^{y2}$).

Media. Dulbecco's phosphate-buffered saline, pH 7.5 (29), supplemented with 5% heat-inactivated fetal calf serum was used for cell transfers. Spleen cells to be tested for the presence of plaque-forming cells (PFC) were suspended in modified Eagle's medium with Hanks' balanced salt solution (Grand Island Biological Company, Grand Island, New York).

Antigens. Keyhole limpet hemocyanin (KLH) (Pacific Biomarine Supply Company, Venice, Calif. was dialyzed 24 h against 0.15 M NaCl and then centrifuged at 10,000 rpm to remove precipitated protein. The resulting supernate was then centrifuged at 30,000 rpm in a fixed-angle rotor for 2 h to pellet the protein, and the pellet was resuspended in 0.01 M cacodylate buffer, pH 6.8.

DNP_8-KLH (per 10^5 daltons) was prepared by reacting dinitrofluorobenzene in 40-fold molar excess with the carrier protein for 3 h at pH 8.2 in 0.5 M $NaHCO_3$ buffer (30). Free 2,4-dinitrophenol (DNP) was removed by extensively dialyzing the conjugates against phosphate-buffered saline (0.01 M phosphate, pH 7.1, in 0.15 M NaCl).

Downloaded from www.jem.org on May 25, 2005

Donors for Cell Transfer Experiments. Nonsuppressed donors in adoptive cell transfers were obtained from matings between SJL/JHz males and BALB/cNHz females. Suppressed donors were obtained from matings between SJL/JHz males and BALB/cNHz females immunized against the paternal Ig-1b allotype (9). Suppressed mice were 6-12-mo old when used and had no detectable serum Ig-1b at time of transfer. To purify T cells from these spleen cells, the nylon wool column method was used (31).

Priming. DNP-KLH-primed mice received 100 μg DNP-KLH on alum plus 2×10^9 heat-killed *Bordetella pertussis* (kindly supplied by Lederle Laboratories, Division of American Cyanamid Company, Pearl River, N. Y.) at least 8 wk before use as donors in adoptive transfer. Spleen cells from these mice were used as a combined source of hapten-primed B cells and carrier-primed T cells or (after depletion of T cells as described below) as a source of hapten-primed B cells.

KLH (carrier)-primed mice received 100 μg KLH on alum plus 2×10^9 *Bordetella pertussis* 7 days before use as donors in adoptive transfer. Nylon wool column purified spleen cells were transferred to provide a source of carrier-primed T cells when required.

Depletion of T Cells from Spleen Cell Suspensions. T cells were depleted from spleen cell suspensions by killing with anti-Thy-1 plus complement in a "two-step" killing protocol. Cells were incubated with congenic anti-Thy-1.2 (kindly supplied by Doctors E. A. Boyse and F-W Shen at Sloan Kettering Institute for Cancer Research, New York) for 30 min at 37°C. After the first step, cells were resuspended and incubated with rabbit complement for 30 min at 37°C. After the second step, cells were resuspended and washed once before transfer.

Adoptive Secondary Response to DNP-KLH. Donor cells were injected i.v. into semisyngeneic BALB/c recipients irradiated (600 R) 18–24 h before transfer.

Recipients were challenged i.v. with 10 μg aqueous DNP-KLH immediately after transfer. 7 days later, recipients were killed, their spleens removed, and DNP-PFC estimated as described below. Since very little (<30%) variability in spleen size, cell yield, or response is usually found between individuals in the same group, 3–5 recipients were used per group and their spleen cells pooled before plaquing (5, 11, 32).

Estimation of DNP-PFC in Recipient Spleen. The PFC assay described by Cunningham and Szenberg was used to estimate the number of cells secreting anti-DNP antibody (33).

Samples of individual or pooled spleen cell suspensions to be tested were mixed with trinitrophenyl-coated burro erythrocytes (34), frozen and reconstituted guinea pig serum (complement), and developing antisera (when desired). Chambers were incubated at 37°C for 1 h and the number of DNP-PFC counted with the aid of a dissecting microscope. Counts from a minimum of two chambers were averaged for each determination. The response is expressed as PFC/10^6 recipient spleen cells. The number of indirect PFC was determined by subtracting direct PFC/10^6 (no developing antiserum) from the total PFC/10^6 found when developing antiserum was included in the chamber. In general, the direct PFC response is less than 20% of the total IgG response.

Antisera for PFC Assay. Total IgG PFC were developed with a rabbit antiserum reactive with all IgG classes. Specific anti-allotype antisera were used to develop Ig-1a and Ig-1b PFC. Heteroantisera detecting Ig-1a and Ig-1b were rendered specific by appropriate absorptions with insoluble immunoabsorbents (5, 11). The starting serum for anti-Ig-1a was produced by immunizing a goat with the Ig-1a myeloma protein 5563. The starting serum for the anti-Ig-1b was produced by immunizing a rabbit with C75BL/6 (Ig^b) globulins. (This serum was kindly supplied by Dr. John Coe, Rocky Mountain National Laboratory.) All sera were tested for specificity by radioimmune assays and plaque development.

Preparation and Absorption of Anti-Ia Sera. Mice were immunized i.p. with a suspension of spleen and lymph node cells (approximately 1.5×10^7 cells/mouse). The mice were boosted on days 28, 35, and 42, and bled on days 49 and 51. Thereafter, animals were reimmunized every 3rd wk and bled 7 and 9 days after each booster injection.

In vitro absorption of antiserum was performed as follows: 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. In vivo absorption was done by injecting 0.3 ml of undiluted antiserum i.p. and bleeding 3–4 h later. Table I shows the specificity of antisera used in this study.

Cytotoxic Treatment by Anti-Ia. Cytotoxic treatment was done in a "two-step" killing protocol. Cell suspensions were incubated with appropriately diluted anti-Ia for 30 min at 37°C ($1-2 \times 10^7$ cells/ml). Cells were centrifuged and resuspended in media containing rabbit complement diluted

Downloaded from www.jem.org on May 25, 2005

TABLE I
Antisera Used in These Studies

Antisera	H-2 region incompatibility								Tla incompatibility
		I							
	K	*A*	*B*	*J**	*C*	*S*	*G*	*D*	
A.TL anti-A.TH		*s*‡	*s*	*s*	*s*	*s*	*s*		*a*§
(B10.A × A.TL)F_1 anti-B10.HTT		*s*	*s*	*s*					
(B10.T(6R) × B10.D2)F_1 anti-B10.AQR		*k*	*k*	*k*					
(B10 × HTI)F_1 anti-B10.A(5R)				*k*	*d*	*d*	*d*(?)		

* Evidence defining the *I-J* subregion is presented in an accompanying publication (28).
‡ Haplotype origin of incompatible *H-2* regions carried by immunizing strain (16).
§ Incompatible *Tla* allele of immunizing strain (35).

1:12 and incubated for 40 min at 37°C. After passing through a fetal calf serum gradient, the cells were used for transfer experiments.

Results

Ts activity is assayed by the ability to suppress specifically an adoptive secondary Ig-1b allotype DNP-PFC response by cotransferred DNP-KLH-primed, syngeneic, nonsuppressed spleen cells. Nonsuppressed prime DNP-KLH spleen cells from the allotype heterozygous (Ig^a/Ig^b) (BALB/c × SJL)F_1 hybrid mice used in these studies give rise to approximately equal numbers of Ig-1a and Ig-1b DNP-PFC when transferred without Ts in an adoptive secondary assay. Ts reduce the Ig-1b response without affecting the Ig-1a or other IgG responses. Therefore, suppression is usually evaluated by comparison of the Ig-1b and Ig-1a responses in a given group of adoptive secondary recipients. In the experiments presented below, the Ts dose (number of suppressed spleen cells) used is just slightly greater than the number required to completely suppress the Ig-1b response by the primed nonsuppressed cells (32).

Suppressor T Cells Carry Surface I-Region Determinants. Data in Table II show that allotype Ts carry surface determinants controlled by genetic loci within the *I* region. Three *I*-region sera which kill Ts are shown in this table. One of these antisera, A.TL anti-A.TH, is produced in strains that differ in the *I*, *S*, and G regions of the *H-2* complex as well as for a segment of the 17th chromosome which lies outside the *H-2* complex and includes the *Tla* locus (Table I). The other two antisera, (B10.A × A.TL)F_1 anti-B10.HTT and (B10.T(6R) × B10.D2)F_1 anti-B10.AQR, are made between congenic strains which differ only for determinants controlled by a portion of the *I* region. The ability of these latter sera to kill Ts places the control of a set of Ts surface determinants clearly within the *I* region.

Evidence that a fourth *I*-region antiserum (B10 × HTI)F_1 antiB10.A(5R) does not kill Ts is also presented in Table II. This serum (see below) kills helper T cells required for an IgG adoptive secondary response. *I*-region haplotype differ-

Downloaded from www.jem.org on May 25, 2005

TABLE II

Removal of Suppressor T-Cell Activity by Anti-I-Region Sera

(BALB/c × SJL)F1 spleen cells transferred (× 10⁶)		Treatment of Ts*	Indirect DNP-PFC/10⁶‡			Result: Ts gone
DNP-KLH primed	Ig-1b suppressed spleen (Ts)	Antiserum	Ig-1b	Ig-1a	Total IgG	
8	–	–	215	301	2,540	
"	5	NMS	<10	266	2,010	No
"	"	A.TL anti-A.TH	191	231	1,760	Yes
"	"	(B10.A × A.TL)F1 anti-B10.HTT	201	271	2,810	Yes
"	"	(B10.T(6R) × B10.D2)F1 anti-B10.AQR	186	216	2,000	Yes
"	"	(B10 × HTI)F1 anti-B10.A(5R)	<10	313	2,820	No

* For *I*-region specificity: see Table I. Treatment was done with antiserum plus complement. Final dilution of anti-Ia sera was 1:5. Number of treated cells transferred equals remainder after treatment of indicated cell number. For details of treatment and adoptive transfer, see Materials and Methods section.

‡ Indirect DNP-PFC/10⁶ recipient spleen cells. Direct DNP-PFC (<30) subtracted.

ences between the strains used to prepare this serum (see Table I) indicate that it should also contain antibody against the determinants present on Ts. Its failure to kill Ts apparently reflects biological variability in the response to the immunizing Ia antigens. (We have seen similar variability with respect to Th kill, where one pool of A.TL anti-A.TH kills Th and another does not, although both pools kill Ts.)

Data in Table III show that complement is required for removal of Ts activity. This demonstrates that the *I*-region antisera are killing rather than inactivating (blocking) a cell required for Ts activity. Data in this table also demonstrate that Ts are the direct targets of the *I*-region antisera cytotoxicity. The ability of *I*-region antisera to kill nylon column purified spleen Ts cells rules out indirect killing as the result of massive B-cell death; and the failure of anti-Thy-1-treated (T-depleted) spleen cells from suppressed mice to restore the Ts activity removed by cytotoxic treatment of the nylon-purified cells with *I*-region antisera demonstrates that these antisera are killing the Thy-1 positive cells responsible for suppression, i.e., Ts.

Normal Lymphoid Cells Carry The I-Region Determinants Detected on Allotype Suppressor T Cells. The cytotoxic activity of *I*-region antisera against (BALB/c × SJL)F_1 allotype suppressor T cells (Ts) provides clear evidence that the determinants detected are not restricted to these cells, since (*a*) the lymphoid cells used to raise the antisera came from donor strains which would not be expected to show allotype suppression of the type seen in (BAlB/c × SJL)F_1 hybrids, and (*b*) the donors were not given the exposure to anti-allotype antisera required for the induction of allotype Ts. One of the immunizing donor strains (A.TH) does not even carry the *Ig-1b* allele whose product is suppressed by allotype Ts. Thus, unless allotype Ts are a constituent population in normal lymphoid tissues, the *I*-region determinants found on allotype Ts are carried by other lymphoid cells as well.

Downloaded from www.jem.org on May 25, 2005

TABLE III

Removal of Suppressor T-Cell Activity by Anti-I-Region Antisera: Complement Dependence, Failure of T-Depleted Suppressor Populations to Restore Activity

(BALB/c × SJL)F1 spleen cells transferred (× 10^6)		Treatment of Ts		Indirect DNP-PFC/10^6§		Result: Ts gone
DNP-KLH primed	Ig-1b suppressed Ts*	Antiserum‡	C′	Ig-1b	Ig-1a	
8	–	–		112	149	
"	1.5	NMS	+	18	141	No
"	"	A.TL anti-A.TH	+	87	102	Yes
"	"	"	–	<10	131	No
"	"	(B10.A × A.TL)F1 anti-B10.HTT	+	96	147	Yes
"	"	"	–	<10	103	No
8	1.5	ATL anti-A.TH plus 10^7 T-depleted‖ (B10.A × A.TL)F1	+	99	121	Yes
"	"	Anti-HTT plus 10^7 T depleted	+	101	138	Yes

*Nylon wool column purified T cells were used as a source of Ts.

‡Final dilution of anti-Ia antisera used in this experiment was 1:5. Number of cells transferred equals remainder after treatment of indicated cell number. For details of treatment and adoptive transfer, see Materials and Methods section.

§Indirect DNP-PFC/10^6 recipient spleen cells. Direct DNP-PFC (<20) subtracted.

‖T depleted: suppressed spleen cells added after treatment as a source of potential "non-T" cells required for suppression. T cells were depleted by anti-Thy 1.2 complement using siliconized glassware to prevent depletion of glass adherent cell populations.

The same point is demonstrated by in vivo and in vitro absorption studies with normal nonsuppressed animals. Cytotoxic activity for Ts was completely removed from the three *I*-region antisera described above by absorption with the immunizing strain or strains carrying the same *I*-region haplotypes. No activity was removed by absorption with the serum donor strain or strains carrying the same *I*-region haplotype, thereby demonstrating the specificity of these sera for *I*-region determinants (18, 28). In the representative absorption experiment shown in Table IV, (B10.A × A.TL)F1 anti-B10.HTT was absorbed in vitro with spleen cells from B10.A and B10.HTT. B10.A, one of the parental strains in the serum producer, absorbed no cytotoxic activity, while B10.HTT, the immunizing strain, was able to absorb all the activity. The absorptions demonstrate that mouse strains of the appropriate haplotype have lymphoid cells carrying the same determinants found on allotype Ts.

Data presented elsewhere (28) show that the Ts *I*-region determinants are present on T but not B cells; and data presented here show that at least some of the *I*-region determinants detected on Ts are not present on helper T cells. Thus, these determinants mark a subset T lymphocytes which, in allotype-suppressed

Downloaded from www.jem.org on May 25, 2005

TABLE IV
Absorption of Anti-Suppressor T-Cell Activity with Normal Lymphoid Cells

(BALB/c × SJL)F1 spleen cells transferred (× 10^6)		Treatment of Ts*		Indirect DNP-PFC/10^6‡		Result: Ts gone
DNP-KLH primed	Ig-1b suppressed spleen (Ts)	Antiserum	In vitro absorption	Ig-1b	Ig-1a	
7	–	–	–	1,160	1,100	
"	4	NMS	–	147	1,080	No
"	"	(B10.A × A.TL)F1 anti-B10.HTT	–	1,120	1,220	Yes
"	"	"	B10.A	1,110	1,200	Yes
"	"	"	B10.HTT	127	1,080	No

*For *I*-region specificity: see Table I. Treatment was done with antiserum plus complement. Absorption of antisera was performed in vitro with spleen and lymph node cells. Final dilution of anti-Ia sera was 1:5. Number of treated cells transferred equals remainder after treatment of indicated cell number. For details of treatment and adoptive transfer, see Materials and Methods section.

‡Indirect DNP-PFC/10^6 recipient spleen cells. Direct DNP-PFC (<200) subtracted.

mice, contains the allotype suppressor T cell. Whether the T cells in normal mice which bear the Ts *I*-region determinants are also suppressor T cells, perhaps generated in response to environmental or self-antigens, remains to be determined.

Helper T Cells Carry I-Region Determinants. Th activity in these studies is measured in a "homologous" adoptive secondary antibody response to DNP. DNP-KLH-primed spleen cells treated with anti-Thy-1.2 plus complement are used as a source of DNP-primed B cells. These cells are cotransferred with Th (KLH-primed spleen cells) untreated or pretreated with *I*-region antisera plus complement. The Th dose is chosen such that the splenic IgG DNP-PFC response in recipients of untreated Th is proportional to the amount of Th activity injected (i.e., the Th dose limits the response). Donors are (BALB/c × SJL)F_1 hybrids. Recipients are irradiated (600 R) BALB/c.

Three sera containing *I*-region antibody were found which kill Th (see Table V). One of these sera, (B10.T(6R) × B10.D2)F_1 anti-B10.AQR, was produced between congenic strains differing only within the *I* region, indicating that surface determinants detected on Th are controlled by loci within this segment. Furthermore, since the strains used to prepare this serum differ only in the *I-A*, *I-B*, and *I-J* (Table I) subregions, the loci controlling the Th determinants must map to one of these subregions.

Th activity is also removed from carrier-primed nylon-passed T cells by treatment with anti-*I*-region sera. This suggests that Th killing by these antisera (like Ts killing) is not due to the concomitant death of large numbers of Ia positive B cells (see Table VI). Data in the same table also show the titration of A.TL anti-A.TH cytotoxic activity for Th, and that removal of Th activity is complement dependent. This Th killing activity is removed from the antisera by in vivo absorption with normal (unprimed) animals of the appropriate haplotype (18).

In both of these experiments, as in all other Th removal experiments, DNP-KLH-primed B cells treated with congenic anti-Thy-1 to remove endogenous Thy-1 positive helper cells were used as the source of hapten-primed B cells. These Thy-1-depleted spleen cell populations should supply all other types of

Downloaded from www.jem.org on May 25, 2005

TABLE V
Removal of Helper Activity by Anti-I-Region Sera

(BALB/c × SJL)F_1 spleen cells transferred (× 10^6)			Treatment of Th‡	
Exp. no.	DNP-KLH primed T-depleted (B)*	KLH-primed spleen (Th)	Antiserum	Total indirect DNP-PFC/10^6§
1	5	–	–	125
	"	8	NMS	3,960
	"	4	NMS	2,290
	"	"	A.TL anti-A.TH	417
2	4	–	–	219
	"	4	NMS	1,610
	"	"	A.TL anti-A.TH	318
	"	"	(B10.A × A.TL)F_1 anti-B10.HTT	1,770
	"	"	(B10.T(6R) × B10.D2)F_1 anti-B10.AQR	322
	"	"	(B10 × HTI)F_1 anti-B10.A(5R)	415

*T cells were depleted by treatment with anti-Thy-1.2 plus complement.
‡Indirect DNP-PFC/10^6 recipient spleen cells. Direct (<40) subtracted.
§For *I*-region specificity: see Table I. Treatment was done with antiserum plus complement. Final dilution of anti-Ia sera used was 1:5. Number of treated cells transferred equals remainder after treatment of indicated cell number. For details of treatment and adoptive transfer, see Materials and Methods section.

TABLE VI
Titration of I-Region Sera for Removal of Helper T-Cell Activity

(BALB/c × SJL)F_1 spleen cells transferred (× 10^6)		Treatment of Th§		
DNP-KLH primed T-depleted (B)*	KLH primed Th‡	Antiserum	Dilution	Total indirect DNP-PFC/10^6‖
5	–	–	–	146
"	2	NMS	1:5	2,210
"	"	A.TL anti-A.TH	1:5	547
"	"	"	1:10	988
"	"	"	1:20	2,360
"	"	"¶	1:5	1,950

*T cells were depleted by treatment with anti-Thy-1.2 plus complement.
‡Nylon wool column purified T cells were used a source of Th.
§For *I*-region specificity: see Table I. Treatment was done with antiserum plus complement. Number of treated cells transferred equals remainder after treatment of indicated cell number. For details of treatment and adoptive transfer Materials and Methods section.
‖Indirect DNP-PFC/10^6 recipient spleen cells. Direct (<40) subtracted.
¶Cells were treated without complement.

Downloaded from www.jem.org on May 25, 2005

cells required for the adoptive secondary response. Therefore, the removal of Th activity by *I*-region antisera demonstrates that these sera kill the Thy-1 positive cells which help IgG responses, i.e., helper T cells.

The removal of Th activity by the *I*-region antisera used here is considerably less effective than the removal of Ts. To detect a loss of Th activity, the Th assay had to be optimally sensitive, i.e., the Th dose used had to limit the response. We have shown in other studies (unpublished) that even a twofold excess of Th activity virtually obscures the measurable reduction of Th activity due to cytotoxic treatment of the Th with *I*-region antisera. This is consistent with evidence that shows that Th killing by these antisera is incomplete under the conditions we use (see Table V).

Reports by Hämmerling et al. (21) and Press (in reference 15) have appeared indicating that *I*-region antisera do not kill Th. Differences in antisera, mouse strains, or assay conditions could account for the discrepancy between these results and ours. In particular, we measure helper activity by transfer of F_1 hybrid cells into one of the parental strains (BALB/c). Although the recipient is sufficiently irradiated (600 R) so that the graft survives indefinitely in over 95% of recipients, there appears to be an incompatibility ("back-stimulation") reaction which increases the PFC response approximately two to threefold over that obtained by transfer in syngeneic hybrid recipients (Herzenberg, unpublished observation). This augmentation is not due to T-cell replacement due to an allogeneic effect since primed helper cells are required to obtain a DNP-PFC response in this transfer system. The augmentation may, however, be important in allowing the demonstration of Th removal by *I*-region antisera.

The reduction in Th activity reported here could in fact be due to interference with the augmentation mechanism rather than to direct removal of Th, i.e., the residual Th activity we observe after treatment with *I*-region sera could actually be the Th activity measurable under nonaugmentation conditions. The consistent removal of 80% or more of the Th activity observed after antiserum treatment argues against this possibility, since the residual Th activity appears to be significantly less than would be expected in the absence of augmentation. Work is in progress to conclusively resolve this problem. At present, it is not clear whether the type of Th activity removed is demonstrable only in the adoptive transfer assay used in our studies or also can be identified in the usual syngeneic transfer system.

Selective Expression of Determinants on Helper and Suppressor T Cells. We have shown in the preceding sections that one of the antisera which kills Ts, (B10.A × A.TL)F_1 anti-B10.HTT, does not kill Th (see Tables III and V). This demonstrates that Ts carry *I*-region determinants not found on Th.

Our studies also show that Th carry determinants not found on Ts, since one of the antisera which kills Th, (B10 × HTI)F_1 anti-B10.A(5R), does not kill Ts. This antiserum, however, is produced in strains that differ not only in the *I* region, but also in the *S* and possibly *G* regions of the *H-2* complex (see Table I). Thus, we cannot conclude that the Th determinants it detects are definitely controlled within the *I* region. Data with another antiserum, (B10.T(6R) × B10.D2)F_1 anti-B10.AQR, demonstrate clearly that Th do carry *I*-region determinants (see Table V). This antiserum, however, also kills Ts, and therefore, could be detecting the same *I*-region determinants on Ts and Th. Nevertheless,

Downloaded from www.jem.org on May 25, 2005

the most economical interpretation of the data obtained with these two antisera suggests that Th carry *I*-region determinants which are not present on Ts.

Discussion

The detection of *I*-region determinants on Th and Ts provides a new tool for studying the cellular interactions which regulate immune responses. It also provides some interesting new insights into the role of the *I*-region itself in immunoregulation.

We have shown elsewhere that the *I*-region determinants on Ts are controlled by a locus, *Ia-4*, separable by crossing over from the loci which control *I*-region (Ia) determinants on B cells (28). This locus marks a new subregion, *I-J*, located between the *I-B* and *I-C* subregions. It also contains loci (possibly the same as *Ia-4*) which code for determinants on the T cells which initiate (promote) Con A responsiveness (Frelinger, personal communication) and for determinants on the T cells and soluble factors which suppress antibody responses dependent on KLH for carrier function (27).

Ia-4 determinants do not appear to be restricted to allotype Ts, since spleen cells from nonsuppressed mice were used both to generate and to absorb the anti-*Ia-4* antibody. Nevertheless, these determinants are restricted to a subpopulation of T lymphocytes. Absorption studies presented elsewhere (28) show that B cells do not carry Ia-4 determinants although T cells do; and data presented here show that Ts, but not Th, carry *Ia-4*. (If Ts and Th carry shared *I*-region determinants these would have to be controlled by a separate locus, assuming one locus per gene product.) Thus Ia-4 determinants are carried on a subset of T lymphocytes, including allotype Ts.

Th also carry *H-2*-controlled determinants restricted to a subset of T lymphocytes. As we have shown here, an antiserum that kills Th does not kill Ts; therefore, the Th determinants define a T-cell subset which does not include Ts. Whether these determinants on Th are controlled by the *I, S,* or G subregions and whether they are different from the Ia determinants on B cells remains to be determined. The evidence that Th definitely carry *I*-region determinants suggests that the locus that controls the unique Th determinants are in the *I*-region.

The Ly surface antigens also identify distinct T-cell subsets containing Ts (Ly-2) or Th (Ly-1); however, the Ly-2 subset apparently contains more functionally identifiable cells than those contained within the Ia-4-bearing subset. This subdivision of Ly-2 cells into Ia-4 positive or negative bears on the question of whether Ts are cytotoxic T cells. Cytotoxic precursor and effector cells, like allotype Ts, are Ly-2 (2); however, in contrast to allotype Ts, cytotoxic precursors and effector cells do not appear to carry *I*-region surface determinants (21). If the same pools of *I-J* antisera used here to kill Ts fail to kill (BALB/c × SJL)F_1 cytotoxic cells, then evidence for a subdivision of Ly-2 cells will be complete. As a corollary, this would demonstrate that the mechanism of allotype suppression, i.e., allotype Ts removal of Th activity (Ig-1b Th) which helps Ig-1b memory B cells (5, 32), does not involve killing by cytotoxic T cells such as those responsible for cellular immunity (36).

Other evidence also suggests that removal of Ig-1b Th activity by Ts is not due to cytotoxic killer cells. Cultured spleen cells from allotype-suppressed mice

Downloaded from www.jem.org on May 25, 2005

produce a soluble suppressive factor (TsF) which specifically removes Ig-1b helper activity. Carrier-primed spleen cells cultured for 24 h in the presence of TsF lose virtually all Ig-1b Th activity detectable in the adoptive transfer assay although other Th activity is unaffected (5). Serum from suppressed mice also contains TsF activity (unpublished observation). Thus allotype suppression appears to be mediated, at least in part, by TsF. Cytotoxic killer cells, in contrast, appear to kill by direct cell-to-cell contact (26).

The presence of *I*-region determinants on Th and Ts provides a different framework for consideration of the mechanism of suppression. The location of the loci coding for these determinants in close proximity to the *Ir* loci which control immune responsiveness to a variety of antigens suggests that the Ts and Th determinants may in general play a role in immunoregulation. This thesis is considerably strengthened by the demonstration that soluble factors, which mediate suppression (26) or help (15, 24, 25), carry *I*-region determinants and by evidence that specific immune suppression (*Is*) genes map in the *I*-region (37, 38). Although the evidence is still too fragmentary to allow clear definition of the role of these determinants, the existing data are consistent with their being part of the regulatory language used for communication among responding lymphocytes.

For example, in allotype suppression three cells make up a basic network: Ig-1b memory cells which give rise to Ig-1b afc, Ig-1b Th which are required for the differentiation and expansion of Ig-1b memory cells to afc, and Ig-1b Ts which remove Ig-1b Th activity (5). How Ig-1b allotype Ts recognize the Ig-1b Th is unknown; however, as indicated above, Ig-1b Th activity can be removed from carrier-primed spleen cells by pretreatment with a TsF produced by Ts containing spleen cells. At least one of the cells in this network (Ts) is marked by unique *I*-region determinants. The other two cells (Th and B) carry *I*-region determinants which may (or may not) be shared; however, Th carry *H-2*-controlled determinants (probably *I*-region) which are not present on Ts.

Tada has described what may be a similar network regulating antibody responses to KLH and haptens coupled to KLH. These studies directly implicate *I-J* subregion determinants (not necessarily Ia-4) in what appears to be a TsF-mediated interaction between Ts and Th. Tada and co-workers have shown (*a*) that KLH-specific Ts carry surface *I-J* determinants (personal communication); (*b*) that soluble suppressive factors (KLH-TsF) extracted from Ts-containing thymus or spleen also carry *I-J* determinants (27); and (*c*) that effective suppression with KLH-TsF requires the presence of *I*-region-encoded receptors for KLH-TsF on the responding (target) strain T (but not B) cells (39).

Tada has not yet directly determined whether KLH-TsF removes Th activity. We have not yet determined whether allotype TsF carries *I-J* determinants. But the similarities between the two systems suggest that at this point the information gathered should be integrated for consideration of overall mechanisms of regulation of antibody production. The results of such "integration" suggest to us that Ts produce soluble factors which kill or inactivate Th; and that *I-J* determinants on the soluble factors are involved in the recognition (removal) of the target Th. Thus the selective expression of *I*-region determinants on cells and factors involved in antibody production appears to be an integral part of the mechanism for regulation of this process.

Downloaded from www.jem.org on May 25, 2005

Summary

Data presented here show that loci in the *I*-region of the *H-2* gene complex are selectively expressed in different functional T-cell subpopulations. These loci are closely linked (or possibly identical) to loci that control immune responses. They control surface determinants which identify helper and suppressor T lymphocytes.

Determinants described here on allotype suppressor T cells (Ts) are found on normal (nonsuppressed) lymphoid cells, but are not found on helper T cells (Th). These determinants are controlled by a locus mapping in the *I* region of the *H-2* complex. In an accompanying publication we show that this locus (*Ia-4*) marks a new *I* subregion (*I-J*) and is expressed only on T cells. Thus *Ia-4* determinants identify a T-cell subpopulation which includes Ts but not Th.

Th also carry identifying surface determinants controlled by loci that map to the *H-2* complex, probably within the *I* region. These determinants are not found on Ts. Data presented also establish that loci in the *I* region control determinants on Th, but do not conclusively demonstrate that these are the determinants that distinguish Th from Ts.

The selective expression of *H-2*-controlled determinants on Ts and Th suggests that these determinants are directly involved in immunoregulation.

The authors wish to thank Dr. Donald Shreffler for constructive criticism and excellent advice; F. T. Gadus, Theta Tsu, and Ken Odom for technical assistance; and Jean Anderson, Jon Marx, and Shayne Frankel for patient preparation of this manuscript.

Received for publication 16 May 1976.

References

1. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. Exp. Med.* 141:1376.
2. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of killer activity. *J. Exp. Med.* 141:1390.
3. Cantor, H., F-W. Shen, and E. A. Boyse. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: after immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. *J. Exp. Med.* 143:1391.
4. Jandinski, J., H. Cantor, T. Tadakuma, D. L. Peavy, and C. W. Pierce. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: suppressor and helper activities are inherent properties of distinct T-cell subclasses. *J. Exp. Med.* 143:1382.
5. 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.
6. Stout, R. D., and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived lymphocytes. I. Detection of a subpopulation of murine T lymphocytes bearing the Fc receptor. *J. Exp. Med.* 142:611.
7. Stout, R. D., and L. A. Herzenberg. 1975. The Fc receptor on thymus-derived

Downloaded from www.jem.org on May 25, 2005

lymphocytes. II. Mitogen responsiveness of T lymphocytes bearing the Fc receptor. *J. Exp. Med.* 142:1041.

8. Stout, R. D., S. D. Waksal, and L. A. Herzenberg. 1976. The Fc receptor on thymus-derived lymphocytes. III. Mixed lymphocyte reactivity and cell-mediated lympholytic activity of Fc^- and Fc^+ T lymphocytes. *J. Exp. Med.* 144:54.
9. Herzenberg, Leonore, and Leonard A. Herzenberg. 1974. Short-term and chronic allotype suppression in mice. *In* Contemporary Topics in Immunobiology. M. D. Cooper, and N. L. Warner, editors. Plenum Publishing Corporation, New York. 3:41–75.
10. Greaves, M. F., J. J. T. Owen, and M. C. Raff. 1973. T and B Lymphocytes. Excerpta Medica, American Elsevier Publishing Co., New York.
11. Okumura, K., M. H. Julius, T. Tsu, L. A. Herzenberg, and L. A. Herzenberg. 1976. Demonstration that IgG memory is carried by IgG bearing cells. *Eur. J. Immunol.* In press
12. 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.
13. Hämmerling, G. J., B. D. Deak, C. 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.
14. 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.* 104:1273.
15. 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.* In press
16. 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.
17. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D.C.).* 175:273.
18. 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 Response. D. H. Katz, and B. Benacerraf, editors. Academic Press, Inc., New York. 257.
19. Gotze, D. 1975. T(Iat)-and B(Iab)-cell alloantigens determined by the *H-2* linked *I* region in mice. *Immunogenetics.* 1:495.
20. Schwartz, R. H., C. S. David, D. H. Sachs, and W. E. Paul. 1976. T lymphocyte-enriched murine peritoneal exudate cells. III. Inhibition of antigen-induced T lymphocyte proliferation with anti-Ia antisera. *J. Immunol.* In press
21. 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* Leucocyte Membrane Determinants Regulating Immune Reactivity. V. P. Eijsvoogel, D. Roos, and W. P. Zeylemaker, editors. Academic Press, Inc., New York. In press
22. 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. *In* Immune Recognition. A. S. Rosenthal, editor. Academic Press, Inc., New York. 683.
23. 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.

Downloaded from www.jem.org on May 25, 2005

24. Taussig, M. J., A. J. Munro, R. Campbell, C. S. David, and W. 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.
25. 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.
26. 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 the Products of the Histocompatibility Gene Complex in Immune Responses. D. H. Katz, and B. Benacerraf, editors. Academic Press, Inc., New York. 513.
27. 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.
28. 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.
29. Dulbecco, R., and M. Vogt. 1954. Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med.* 99:167.
30. Iverson, G. M. 1973. Assay methods for antigen-mediated cell co-operation. *In* Handbook of Experimental Immunology. D. M. Weir, editor. Blackwell Scientific Publications Ltd. Oxford, England. 2nd edition. Chap. 29.
31. 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.
32. 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.* 27:56.
33. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* 14:599.
34. Rittenberg, M. B., and K. L. Pratt. 1968. Anti-trinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med.* 132:575.
35. Frelinger, J. A., D. B. Murphy, and J. F. McCormick. 1974. *Tla* types of *H-2* congenic and recombinant mice. *Transplantation (Baltimore).* 18:292.
36. Cerottini, J-C., and K. T. Brunner. 1974. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. *In* Advances in Immunology. F. J. Dixon, and H. G. Kunkel, editors. Academic Press, Inc., New York. 18:67.
37. 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.
38. Walterbaugh, C. R., P. Debré, M. Dorf, and B. Bennacerraf. 1976. Two *H-2* gene control of GT specific immune suppression. *Fed. Proc.* 35 (3):713. (Abstr. 2796).
39. Taniguchi, M., T. Tada, and T. Tokuhisa. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response in the mouse. III. Dual gene control of the T cell-mediated suppression of the antibody response. *J. Exp. Med.* 144:20.

Downloaded from www.jem.org on May 25, 2005