THE Fc RECEPTOR ON THYMUS-DERIVED LYMPHOCYTES

IV. Inhibition of Binding of Antigen-Antibody Complexes to Fc Receptor-Positive T Cells by Anti-Ia Sera*

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As our knowledge of the cellular basis for immunity has increased, it has become apparent that the generation of effector lymphocytes does not simply involve reaction of precursors with antigen and subsequent differentiation to effector stage. Thus far, in most models of cellular and humoral immunity studied, the generation of optimal immune responses requires an active collaboration between precursor cells and "accessory" T cells, whose function is to enhance or initiate some stage(s) in the subsequent differentiation of the precursor (1–3). The T cell which collaborates with B cells in the generation of IgG antibody responses is termed a "helper" T cell (1). The T cell which collaborates with progenitors of cytotoxic lymphocytes in the generation of cell-mediated cytolytic responses is termed an "amplifer" T cell (2). These amplifier and helper T cells have their negative counterparts in a population of T cells termed "suppressor" T cells. It is the function of suppressor T cells to actively prevent the generation of effector cells (4–6). It thus appears that the immune system exerts self-control through a network of positive and negative regulator T cells.

Several cytotoxic and fluorescent probes reactive with T-cell membrane components have been prepared in attempts to distinguish these regulator T cells from each other and from the precursors on which they act (1, 2, 7–10). One such probe—fluoresceinated antigen-antibody complexes—defines two distinct subpopulations of peripheral T cells, Fc receptor-positive (FcR^+)[1] and Fc receptor-negative (FcR^-) T cells (10). Functional studies on populations of FcR^- and FcR^+ T cells, purified on the fluorescence-activated cell sorter (FACS), revealed that the FcR^- T-cell subpopulation (*a*) was not responsive to concanavalin A (Con A) in the absence of FcR^+ T cells (11),(*b*) contained the precursors of cytotoxic effectors of cell-mediated lympholytic (CML) responses (12), and (*c*) contained the helper T cells (10) but not the amplifier cells (12). In contrast, the FcR^+ T cells (*a*)

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[1] *Abbreviations used in this paper:* AgAb, antigen-antibody complexes; CML, cell-mediated lympholytic; Con A, concanavalin A; FACS, fluorescence-activated cell sorter; F*AgAb, fluoresceinated AgAb; FcR, Fc receptor; Ia, *I*-region-associated antigens; NMS, normal mouse serum; U, quantitative units of fluorescence intensity.

were responsive to Con A (11), (*b*) contained the differentiated cytotoxic effector cells of CML responses (12), and (*c*) contained the amplifier T cells but not the helper T cells (10, 12). Both FcR^- and FcR^+ T cells were capable of proliferating in mixed lymphocyte cultures upon exposure to allogeneic lymphocytes (12).

Despite the functional distinctions between T cells bearing or lacking the FcR, the functional significance of the FcR remains unclear. Dickler and Sachs (13) have reported evidence for a close association of the FcR on murine B lymphocytes and alloantigens controlled by loci mapping in the *I* region of the *H-2* complex [*I*-region-associated (Ia) antigens]. Binding of aggregated immunoglobulin to FcR of B lymphocytes is specifically inhibited by treatment of the cells with anti-Ia antibody. The *I* region of the *H-2* complex is a genetically complex region that controls the lymphocyte-activating determinants involved in mixed lymphocyte and CML reactivity, as well as the capacity to mount an immune response to a variety of antigens (14).

Recently, Ia antigens have been detected on at least a subpopulation of T cells (15–18). Since the identification of the Ia antigens expressed on subpopulations of T cells would have potential significance for our understanding of the control of immune responses, we examined the ability of anti-Ia antibodies to inhibit the binding of antigen-antibody (AgAb) complexes to the FcR of splenic T lymphocytes. In this report we present evidence that: (*a*) 50–70% of the FcR^+ T cells express Ia antigens; (*b*) only some *I*-region-controlled determinants are expressed on the FcR^+ T cells; and (*c*) subpopulations of T cells within the FcR^+ T-cell population may be distinguishable on the basis of which *I*-region-controlled determinant is expressed on the cell membrane.

Materials and Methods

Methods for preparation of AgAb complexes and cell suspensions, for separation of cells on nylon wool, for fluorescent labeling of cells, and for analysis of cell populations on the FACS (FACS-II; Becton Dickinson Electronics Laboratory, Mountain View, Calif.) have been described in detail in a previous paper (10). Complexes of fluoresceinated egg albumin and the 7S fraction of anti-egg albumin from BAB/14 (Ig^b) mice (F*AgAb) were used throughout this study to label the FcR (10).

Mice. Male and female mice from the inbred strains of CKB, A.TH, A.TL, A.AL, HTI, AKR.M, B10, B10.BR, B10.A, B10.A(3R), B10.A(4R), B10.A(5R), B10.S(9R), B10.HTT, and B10.S(7R) used in these experiments were obtained from our colonies at Stanford. The haplotype origin of the *H-2* regions carried by each of these strains is presented in Table I.

Antisera. A rabbit antiserum prepared against a saline EDTA extract of mouse thymocytes and absorbed with mouse bone marrow cells was shown to be specific for T cells (19). Anti-Thy 1.2 (anti-theta) was prepared by Dr. G. Michael Iverson, Stanford University, from ascites fluid elicited by intraperitoneal injection of sarcoma 180 cells into AKR mice after immunization with CBA thymocytes according to the method of Reif and Allen (20). Anti-*Tla* serum was prepared and kindly provided by Dr. E. A. Boyse (21).

Antiserum specific for mouse IgM was prepared by immunization of goats with MOPC-104E myeloma protein. The serum was sequentially absorbed on columns of Sepharose-4B conjugated with S-4 (γA) and RPC-5 (γ_{2a}) myeloma proteins. Antisera specific for mouse γ_1 and γ_{2a} were prepared by immunizing goats with the Fc fragments of MOPC-21 or RPC-5, respectively. The anti-γ_1 serum was absorbed on Sepharose-4B conjugated with RPC-5 (γ_{2a} myeloma protein). The anti-γ_{2a} serum was sequentially absorbed on columns of Sepharose-4B conjugated with MOPC-21 (γ_1) and MPC-11 (γ_{2b}) myeloma proteins. These class-specific anti-Ig sera were prepared by Mr. Derek Hewgill, Stanford University. The specificity of the above reagents for their designated antigens was confirmed by radioimmune assay. Fluorescein conjugates of the ammonium sulfate-purified Ig of these antisera were prepared according to the method of Cebra and Goldstein (22).

TABLE I
Haplotype Origin of H-2 Regions of Mouse Strains Utilized

Strain	*H-2* haplotype	*H-2* region*								
		K	I					S	G	D
			A	B	J	E	C			
B10	b	b	b	b	b	b	b	b	b	b
CKB, B10.BR	k	k	k	k	k	k	k	k	k	k
B10.A	a	k	k	k	k	k	d	d	d	d
A.AL	a1	k	k	k	k	k	k	k	k	d
B10.A(4R)	h4	k	k	b	b	b	b	b	b	b
HTI	i	b	b	b	b	b	b	b	?	d
B10.A(3R)	i3	b	b	b	b	k	d	d	d	d
B10.A(5R)	i5	b	b	b	k	k	d	d	d	d
AKR.M	m	k	k	k	k	k	k	k	k	q
A.TL	t1	s	k	k	k	k	k	k	k	d
A.TH, B10.S(7R)	t2	s	s	s	s	s	s	s	s	d
B10.HTT	t3	s	s	s	s	k	k	k	k	d
B10.S(9R)	t4	s	s	?	k	k	d	d	d	d

* Adapted from Shreffler and David (14), Shreffler et al. (27), and Murphy et al. (33).

Anti-*H-2* and anti-Ia sera used in this study are listed in Table II. These sera were prepared by immunizing with spleen and lymph node suspension (10–20 × 10^6 cells per mouse) on days 0, 28, 35, and 42. Mice were bled on days 49 and 51. Thereafter, the mice were boosted every 3rd week, and bled accordingly. Pools of antisera examined were from mice that had received 5 to 10 booster injections. (A.AL × B10)F_1 anti-A.TL and (B10 × AKR.M)F_1 anti-B10.A sera were generously provided by Dr. D. C. Shreffler, Washington University, St. Louis, Mo. Titers of these sera were determined in the dye exclusion microcytotoxicity assay using serum donor lymph node target cells (23). All serum preparations were deaggregated by centrifugation at 70,000 *g* for 1 h immediately before use.

*Inhibition of F*AgAb Binding by Anti-H-2 Sera.* Cell pellets containing 5 × 10^6 cells each were resuspended in 50 μl of the test antiserum and incubated for 30 min at 37°C. The cells were then pelleted through a layer of fetal calf serum, resuspended in 50 μl of F*AgAb (10), and incubated for 30 min at 37°C. The cells were then washed and analyzed for proportion of fluorescent cells using the FACS-II.

Removal of Phagocytic Cells from Lymphocyte Suspensions. The phagocytic cells present in the nylon wool-purified splenic T-cell population were removed by the carbonyl-iron technique described by Van Rood et al. (24). Briefly, the cells were suspended to 15 × 10^6/ml RPMI-1640 supplemented with fetal calf serum to 5% vol/vol. Powdered iron (Fisher Scientific Co., Santa Clara, Clalif.) was added to a concentration of approximately 50 mg/ml and the cells were incubated for 20 min at 37°C. The cell suspension was then layered over an equal volume of Ficoll-Paque (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and centrifuged at 450 *g* for 45 min. The cells banding at the RPMI-Ficoll interface were collected with a Pasteur pipette and washed once before use. The extent of contamination with monocytes and/or granulocytes was determined cytochemically by the esterase-staining method of Yam et al. (25).

Results

Inhibition of AgAb Complex Binding to FcR^+ T Cells by Anti-Ia Antibody. Incubation of nylon wool-purified splenic T cells for 30 min at 37°C with F*AgAb complexes results in labeling of 20–40% of the T-cell population (reference 10 and Table III). The proportion of these FcR^+ T cells which are labeled by this technique can be reduced by 50–70% by incubating the T cells, before

TABLE II
Specificity and Titers of Antisera

Anti-serum	Strain combination	*H-2* region incompatibility									Titer*
		K	A	B	J	E	C	S	G	D	
A	(B10.A(5R) × A.TL)F_1 anti-B10.A	k									160
B	(A.AL × B10)F_1 anti-A.TL	s									320
C	(B10 × AKR.M)F_1 anti-B10.A						d	d	d	d	320
D	A.TH anti-A.TL		k	k	k	k	k	k	k		2,560
E	A.TL anti-A.TH		s	s	s	s	s	s	s		1,280
F	(B10.HTT × A.TFR5)F_1 anti-A.TL		k	k	k						160
G	(B10.A × A.TL)F_1 anti-B10.HTT		s	s	s						160
H	B10.A(4R) anti-B10	b	b								640
I	B10.A(3R) anti-B10.A(5R)				k						0‡
J	(B10.T(6R) × B10.D2)F_1 anti-B10.AQR		k	k	k	k					1,280
K	(B10 × HTI)F_1 anti-B10.A(5R)				k	k	d	d	(d?)		120
L	B10.S(7R) anti-B10.HTT					k	k	k	k		40

* Titers listed are the reciprocal dilution of antiserum which gives 50% of maximum cytolysis of donor lymph node targets in the dye exclusion microcytotoxicity assay (23).

‡ Although not significantly cytotoxic for either T or B cells in the dye exclusion assay, antibody in this serum is cytotoxic for suppressor T cells as determined by functional assays (33).

TABLE III
Inhibition of AgAb Binding to T Lymphocytes by Anti-Ia Antisera

Mouse strain	% FcR^+ T	% Decrease in proportion FcR^+ T cells by:*			
		Anti-Thy-1.2‡	Anti-T1a§	Anti-I^s‖	Anti-I^k¶
A.TL	26	–	0	4	54
A.TH	31	–	0	60	5
B10.BR	29	0	–	5	52
B10.S(7R)	40	–	–	63	5
CKB	28	–	–	0	68

* Nylon wool-purified T cells (≥95% Thy-1.2^+ as determined by cytotoxicity assay) were incubated 30 min at 37°C with the designated antisera or NMS and washed through an FCS gradient. The cells were then labeled with F* AgAb complexes and assayed on the FACS for proportion of labeled cells. Fraction of Fc^+ T cells and degree of inhibition were based on analysis of 10,000 viable cells.

‡ AKR anti-C3H Thy-1, used at a 1:10 dilution, has a 100% cytotoxicity end point of >1:50 under conditions used for Fc inhibition. Similar results were obtained with a heterologous rabbit anti-thymocyte serum and rabbit anti-brain-associated T-antigen serum.

§ Anti-*Tla* 1, 2, 3, used at a 1:10 dilution in this study, has a cytotoxicity titer of >1:2,000 for thymocytes.

‖ A.TL anti-A.TH, used at a 1:20 dilution.

¶ A.TH anti-A.TL, used at a 1:20 dilution.

labeling with F*AgAb, for 30 min at 37°C in a 1:20 dilution of antisera directed against products controlled by the *I* region of the *H-2* complex. This inhibition displayed serological specificity in that FcR$^+$ T cells from A.TL, B10.BR, and CKB mice (I^k) were inhibited by A.TH anti-A.TL antiserum (anti-I^k) but not by A.TL anti-A.TH antiserum (anti-I^s), whereas FcR$^+$ T cells from A.TH and B10.S(7R) mice were inhibited by anti-I^s but not by anti-I^k (Table III).

The inhibitory effect of these antisera cannot be ascribed to antibody against determinants controlled by loci mapping in the *Tla* region since treatment of T cells with antisera prepared against *Tla*-region determinants failed to inhibit subsequent binding of F*AgAb complexes (Table III). In addition, strain B10.BR, which appears to have the same *Tla* region as strain A.TH, was not inhibited by the A.TL anti-A.TH serum.

Treatment of T cells with either a T-cell specific alloantiserum (e.g., anti-Thy 1.2) or xenoantiserum (e.g., rabbit anti-mouse brain) did not inhibit subsequent binding of F*AgAb complexes (Table III). Both of the anti-T-cell reagents displayed high cytotoxicity titers (>1:1,000) against T-cell targets. Thus, the blockade of the FcR observed with anti-Ia sera does not appear to be a generalized effect of antibody reacting with T-cell membrane determinants.

*Inhibition of F*AgAb Binding to T Cells by Anti-H-2K or Anti-H-2D Antibodies.* To determine whether antisera directed against determinants controlled by regions of the *H-2* gene complex other than the *I* region could inhibit the binding of F*AgAb complexes to T cells, splenic T cells were incubated for 30 min at 37°C with anti-*H-2D* or anti-*H-2K* sera and subsequently labeled with F*AgAb complexes. Treatment with anti-$H\text{-}2K^k$ (serum A, Table II) reduced the proportion of labeled FcR$^+$ T cells by 39% in populations of strain A splenic T cells but not in populations of strain A.TL splenic T cells (Table IV). Conversely, treatment with anti-$H\text{-}2K^s$ (serum B, Table II) reduced the proportion of labeled FcR$^+$ T cells in strain A.TL but not in strain A splenic T cells. Treatment with a 1:5 dilution of anti-$H\text{-}2D^d$ (serum C, Table II) reduced the proportion of labeled FcR$^+$ T cells in populations of HTI splenic T cells but not in populations of B10 splenic T cells (Table IV).

Comparison of Immunofluorescent Labeling Titer with FcR Inhibition Titer of Anti-H-2 Sera. In light of the ability of anti-Ia sera to inhibit binding of F*AgAb to T cells, an attempt was made to detect binding of anti-Ia antibodies to T cells by indirect immunofluorescence. Spleen cells or nylon wool-purified splenic T cells from A.TL mice were incubated with a 1:10 dilution of A.TH anti-A.TL serum for 30 min at 37°C (the same conditions which result in blockade of the FcR), washed, and incubated for an additional 30 min with fluoresceinated anti-(mouse γ_1 and γ_{2a})Ig. Only 41% of the spleen cells (Fig. 1 *a*) and 2% of the splenic T cells (Fig. 1 *d*) were labeled with a fluorescence intensity greater than the normal mouse serum (NMS) controls (10 units). Similar treatment of HTI lymphocytes with (B10 × AKR.M)F_1 anti-B10.A serum (anti-$H\text{-}2D^d$) or A.TL lymphocytes with (A.AL × B10)F_1 anti-A.TL serum (anti-$H\text{-}2K^s$) followed by fluoresceinated anti-mouse (γ_1 and γ_{2a}) Ig resulted in significant labeling (>10 U) of at least 95% of the lymphocytes (Figs. 1 *b, c, e,* and *f*). The same results were obtained with splenic T cells using a fluoresceinated anti (mouse Ig)-Ig or anti-(mouse IgM)-Ig as the second step. The failure to detect labeling of A.TL T

TABLE IV

Inhibition of AgAb Binding to T Cells by High Concentrations of Anti-H-2 Antisera

Mouse Strain	% Fc^+ T	% Decrease in proportion of Fc^+ T cells by:*		
		Anti-H-$2K^k$‡	Anti-H-$2K^s$§	Anti-H-$2D^d$‖
A.TL	27	0	57	–
A	22	39	0	–
B10	27	–	–	<5
HTI	30	–	–	53

* See Footnote (*), Table III.

‡ Serum A, Table II, used at 1:5 dilution.

§ Serum B, Table II, used at 1:5 dilution.

‖ Serum C, Table II, used at 1:5 dilution.

cells by A.TH anti-A.TL serum was therefore not due to class restriction of the anti-Ia antibodies.

During the course of the above experiments, it was noted that the FcR inhibitory activity of the anti-*H-2* sera did not strictly correlate with the cytotoxicity titers of the antisera. To determine whether the difference in FcR inhibition activity reflected differences in relative amount of antibody binding to the cell surface, the antisera were titrated simultaneously both by indirect immunofluorescence and by FcR inhibition. The titration by indirect immunofluorescence was run on spleen cells rather than splenic T cells since no significant labeling of T cells could be detected with the anti-Ia reagents (Fig. 1 *d*). In the cases of anti-*H-2D* and anti-*H-2K*, the immunofluorescent titer obtained with spleen cells was either the same or somewhat lower than that obtained with nylon wool-purified splenic T cells. In all cases, splenic T cells were used for titration of FcR inhibition activity. The results are presented in Fig. 2.

Dilutions of A.TH anti-A.TL up to 1:80 consistently labeled half of the B10.BR spleen cells with a fluorescence intensity greater than 10 U and reduced the proportion of FcR^+ T cells detected by 50%. Both the proportion of spleen cells labeled and the degree of FcR inhibition decreased as the antiserum was diluted beyond 1:80. A 1:10 dilution of A.TL anti-A.TH did not label B10.BR spleen cells or inhibit B10.BR T-cell FcR. A 1:5 dilution of (B10 × AKR.M)F_1 anti-B10.A (anti-$H\text{-}2D^d$) serum labeled (>10 U) 98% of HTI spleen cells and reduced the proportion of FcR^+ T cells detected by 56%. As the antiserum was diluted beyond 1:5, both the proportion of spleen cells labeled and the degree of FcR inhibition decreased (Fig. 2 *b*). Dilutions of (A.AL × B10)F_1 anti-A.TL serum (anti-$H\text{-}2K^s$) up to 1:40 labeled greater than 90% of A.TL spleen cells. However, the FcR inhibitory activity of this antiserum did not show a plateau but rather decreased as the antiserum was diluted, effecting only a 13% reduction in proportion of FcR^+ T cells at a 1:40 dilution (Fig. 2 *c*).

In none of the experiments described above was a difference in the fluorescence distribution of F*AgAb-labeled T cells discerned after treatment with inhibitory concentrations of anti-*H-2D*, anti-*H-2K*, or anti-Ia sera. For example, the fluorescence distributions of A.TL splenic T cells labeled with F*AgAb after treatment with a 1:5 dilution of NMS, A.TH anti-A.TL, or (A.AL × B10)F_1 anti-A.TL are shown in Fig. 3. Both antisera reduced the proportion of T cells labeled

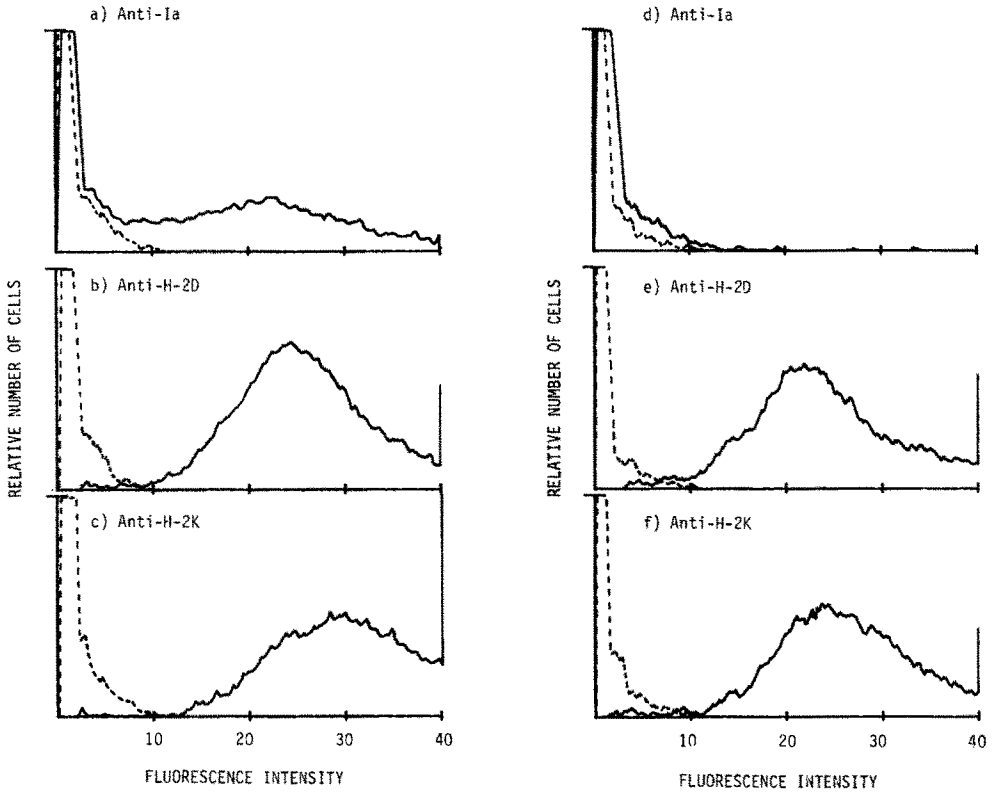

FIG. 1. Fluorescence distribution of spleen cells labeled with anti-H-2 antisera. Spleen cells (*a–c*) or nylon-purified splenic T cells (*d–f*) were incubated for 30 min at 37°C with a 1:10 dilution of the specified reagent, washed, and incubated for an additional 30 min at 37°C with a fluoresceinated rabbit anti-mouse γ_1 and γ_{2a} immunoglobulin reagent. The cells were then washed and analyzed using the FACS. Distributions are based on cumulative analyses of 10,000 viable cells. (*a, d*) A.TL splenic lymphocytes incubated with a 1:10 dilution of NMS (---) or A.TH anti-A.TL (—) (41% of spleen cells and 2% of T cells labeled with a fluorescence intensity >10 U); (*b, e*) HTI splenic lymphocytes incubated with a 1:10 dilution of NMS (---) or (B10 × AKR.M)F_1 anti-B10.A (—) (98% of spleen cells and 96% of T cells labeled with fluorescence intensity >10 U); (*c, f*) A.TL splenic lymphocytes incubated with a 1:10 dilution of NMS (---) or (A.AL × B10)F_1 anti-A.TL (—) (99% of spleen cells and 97% of T cells labeled with fluorescence intensity >10 U).

with a fluorescence intensity greater than 20 U by approximately 50% relative to NMS-treated controls. The median fluorescence intensity of the labeled populations remained within 150-200 U after treatment with either antisera. That is, the inhibition of FcR did not seem to be due to a partial inhibition of binding activity of all FcR^+ T cells resulting in half of the cells decreasing in fluorescence intensity below 20 U.

Identification of I Subregions Controlling Determinants on FcR^+ T Cells. Having established that the majority (>50%) of FcR^+ T cells bear *I*-region determinants, experiments were undertaken to map the locus (loci) controlling these determinants. These studies were conducted with several *I*-region recombinant strains which permit mapping to different *I* subregions.

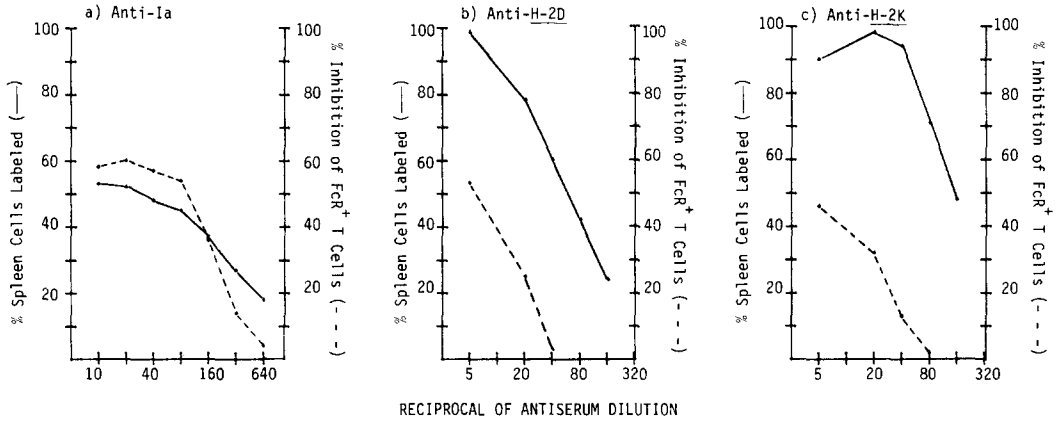

FIG. 2. Titration of anti-*H-2* antisera: correlation of fluorescent-labeling titer with inhibition of AgAb binding to T cells. Spleen cells (—) or nylon wool-purified splenic T cells (---) were incubated for 30 min at 37°C with the specified antisera. The cells were washed and subsequently incubated for 30 min at 37°C with either fluoresceinated rabbit anti-mouse γ_1 and γ_2 immunoglobulin (—) or F*AgAb complexes (---). The cells were washed and assayed on the FACS to determine the proportion of spleen cells labeled (—) or the degree of inhibition of AgAb binding to the splenic T cells (---) relative to a NMS control. Values are based on cumulative analysis of 10,000 viable cells. (*a*) B10.BR spleen or splenic T cells treated with anit-I^k (A.TH anti-A.TL); (*b*) HTI spleen or splenic T cells treated with anti-$H\text{-}2D^d$ [(B10 × AKR.M)F_1 anti-B10.A]; (*c*) A.TL spleen or splenic T cells treated with an anti-$H\text{-}2K^s$ [(A.AL × B10)F_1 anti-A.TL].

The data obtained from studies on FcR inhibitory activity of anti-Ia serum on splenic T cells from B10.HTT mice demonstrate that at least two loci control determinants present on FcR^+ T cells (Table V). The crossover in strain B10.HTT occurred between the *I-J* and *I-E* subregions, such that it carries the *I-A*, *I-B*, and *I-J* subregions derived from the $H\text{-}2^s$ haplotype and the *I-E* and *I-C* subregions derived from the $H\text{-}2^k$ haplotype. Treatment of B10.HTT splenic T cells with A.TL anti-A.TH (anti-I^s) serum resulted in a 50% reduction in FcR^+ T cells, whereas treatment with A.TH anti-A.TL (anti-I^k) serum resulted in a 30% reduction in FcR^+ T cells (Table V). Similar results were obtained using (A.TL × B10.A)F_1 anti-B10.HTT and B10.S(7R) anti-B10.HTT (Table V). It thus appears that loci mapping on opposite sides of the crossover in B10.HTT control determinants found on FcR^+ T cells.

The FcR inhibition data obtained with strains B10.A(4R) and B10 demonstrate that one of the loci discussed above maps in the *I-A* subregion. These two strains differ in the *H-2K* and *I-A* subregions. Serum produced by immunizing B10.A(4R) recipients with B10 donor tissue contains antibody against determinants controlled by both regions. After absorption with EL-4 tumor cells to remove H-2K antibody, this serum inhibited binding of F*AgAb complexes to B10 FcR^+ T cells (Table V). Similar results were obtained with a (B10.HTT × A.TFR5)F_1 anti-A.TL serum, which contains antibody reactive with *I-A* determinants carried by strain B10.A(4R) (Table V).

Determinants controlled by the *I-J* subregion do not appear to be present on FcR^+ T cells, since a B10.A(3R) anti-B10.A(5R) serum, which was known to contain antibody against determinants controlled by the *I-J* subregion of the

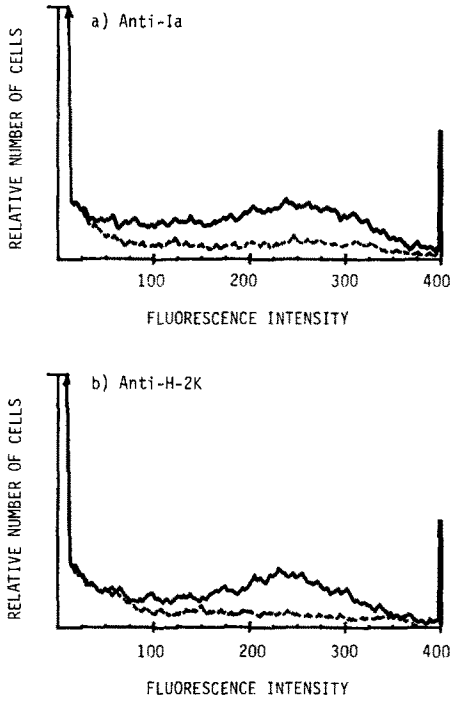

FIG. 3. Fluorescence distribution of splenic T cells labeled with F*AgAb complexes after treatment with anti-H-2 antisera. Nylon wool-purified A.TL splenic T cells were incubated with a 1:5 dilution of NMS (—) or specific anti-H-2 sera (---) for 30 min at 37°C. The cells were subsequently washed and incubated for 30 min at 37°C with F* AgAb complexes. The cells were washed and analyzed on the FACS. Distributions are based on cumulative analysis of 10,000 viable cells of which 27% were FcR^+. (*a*) A.TL splenic T cells treated with NMS (—) or with A.TH anti-A.TL (---); (*b*) A.TL splenic T cells treated with NMS (—) or (A.AL × B10)F_1 anti-A.TL (---). Both antisera reduced the frequency of AgAb-binding T cells by approximately 50% relative to NMS-treated cells.

TABLE V

Inhibition of AgAb Binding to T Cells by Antisera Specific for I-Region-Controlled Determinants

Mouse strain	I-Region					% FcR^+	% Decrease in proportion FcR^+ T cells* after treatment with antisera specific for:								
	A	B	J	E	C		I^k‡	I^s§	$A^kB^kJ^s$‖	$A^kB^kJ^k$¶	$A^kB^kJ^kE^k$**	A^b‡‡	J^k§§	$J^kE^kC^d$‖‖	E^kC^k¶¶
B10.BR	k	k	k	k	k	27	52	5	9	44	64	–	–	–	–
B10.S(7R)	s	s	s	s	s	24	5	63	41	–	–	–	–	4	7
B10	b	b	b	b	b	22	–	–	–	7	9	35	3	0	4
B10.HTT	s	s	s	k	k	26	31	52	40	0	–	–	–	–	30
B10.A(3R)	b	b	b	k	d	24	–	–	–	3	9	–	4	40	5
B10.A(4R)	k	b	b	b	b	21	–	–	–	30	–	0	–	0	–
B10.A(5R)	b	b	k	k	d	33	–	–	–	0	0	–	2	37	3
B10.S(9R)	s	s	k	k	d	20	–	–	31	–	–	–	–	47	0

* See footnote (*), Table III.

† Serum D, Table II, used at 1:20 dilution.

§ Serum E, Table II, used at 1:20 dilution.

‖ Serum G, Table II, used at 1:10 dilution.

¶ Serum F, Table II, used at 1:5 dilution.

** Serum J, Table II, used at 1:10 dilution.

‡‡ Serum H, Table II, used at 1:5 dilution after absorption with EL-4.

§§ Serum I, Table II, used at 1:5 dilution.

‖‖ Serum K, Table II, used at 1:5 dilution. Similar results obtained with 1:20 dilution.

¶¶ Serum L, Table II, used at 1:5 dilution. Similar results obtained with 1:20 dilution.

H-2^k haplotype, failed to inhibit the binding of F*AgAb complexes to B10.A(5R) FcR$^+$ T cells (Table V). This conclusion was supported by the failure of (B10.HTT × A.TFR5)F_1 anti-A.TL serum to inhibit binding of F*AgAb complexes to B10.A(5R) FcR$^+$ T cells.

A second locus controlling determinants on FcR$^+$ T cells appears to map to the right of the *I-E* subregion. Determinants controlled by the *I-E* subregion were initially detected by the cytotoxic reactivity of a (C3H.Q × B10.D2)F_1 anti-B10.AQR serum with B10.A(5R) spleen cell targets (26–27). A serum produced in a similar strain combination [(B10.T(6R) × B10.D2)F_1 anti-B10.AQR] gives negligible inhibition of F*AgAb binding to B10.A(5R) FcR$^+$ T cells (Table V). In addition, a B10.S(7R) anti-B10.HTT serum, which contains antibody against *I-E*-subregion-controlled determinants, failed to inhibit binding of F*AgAb complexes to FcR$^+$ T cells from strains sharing the same *I-E* subregion [B10.A(5R), B10.A(3R), and B10.S(9R)]. These data suggest that determinants controlled by the *I-E* subregion are not present on FcR$^+$ T cells.

In contrast to its effect on FcR$^+$ T cells from strains 3R, 5R, and 9R, the B10.S(7R) anti-B10.HTT serum significantly inhibits the binding of F*AgAb complexes to B10.HTT FcR$^+$ T cells (Table V). This inhibition must be due to determinants controlled by a locus mapping in the *I-C, S,* or *G* regions. Data obtained with a (B10 × HTI)F_1 anti-B10.A(5R) serum would also be consistent with this interpretation (Table V). Experiments are in progress to definitively map this locus.

Evidence for Distinct FcR$^+$ T-Cell Subpopulations Expressing Determinants Controlled by Different I Subregions. The experiments presented above demonstrated that at least two loci mapping on opposite sides of the crossover in B10.HTT mice control determinants that are expressed on FcR$^+$ T cells. Antisera reactive with these two sets of determinants were titrated in the FcR inhibition assay individually or in combination to determine whether both sets of determinants were present on the same subpopulation of FcR$^+$ T cells or on different subpopulations of FcR$^+$ T cells. Treatment of B10.HTT splenic T cells with 1:40 dilution of A.TH anti-A.TL serum resulted in a 30% reduction in the proportion of FcR$^+$ T cells labeled, whereas treatment with a 1:40 dilution of A.TL anti-A.TH serum resulted in a 50% reduction in the proportion of FcR$^+$ T cells (Fig. 4). Increasing the concentration of either serum did not result in increases in FcR inhibition. Treatment of the B10.HTT splenic T cells with both antisera resulted in a 70% reduction in FcR$^+$ T cells (Fig. 4). In several repetitions of this experiment, treatment with a 1:10 to 1:40 dilution of A.TL anti-A.TH serum resulted in a 30–50% reduction in FcR$^+$ T cells, treatment with a 1:10 to 1:40 dilution of A.TH anti-A.TL resulted in a 20–30% inhibition, and treatment with a 1:10 to 1:40 dilution of both antisera resulted in a 50–70% reduction in FcR$^+$ T cells. The partially additive effect of these two antisera indicates that some FcR$^+$ T cells express one, but not both, of the two sets of Ia determinants.

In all of the above experiments, contamination of the splenic T-cell preparation with "non-T" cells was less than 5% as determined by immunofluorescent labeling with anti-Thy 1.2, heterologous anti-T antisera, and/or anti-Ig. To exclude the possibility that a macrophage contaminant might represent one of

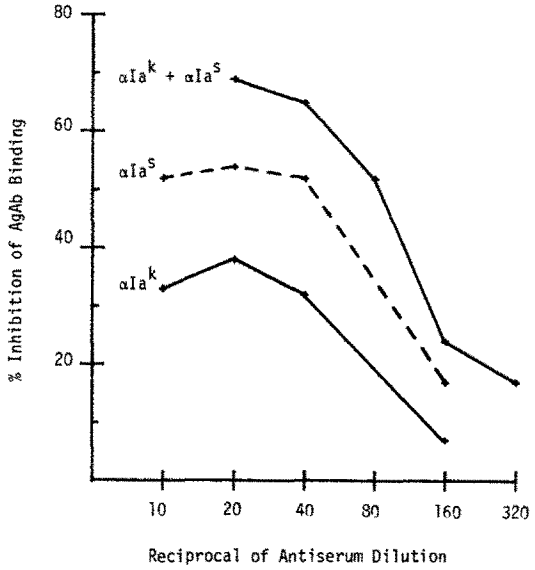

FIG. 4. Inhibition of AgAb binding to B10.HTT splenic T cells by anti-*I*-region antisera. Nylon wool-purified B10.HTT splenic T cells were incubated for 30 min at 37°C with anti-I^k (A.TH anti-A.TL), anti-I^s (A.TL anti-A.TH), or both. The cells were washed and subsequently incubated for 30 min at 37°C with F*AgAb complexes. The cells were washed and assayed on the FACS for proportion of labeled cells. Degree of inhibition relative to NMS/treated controls is based on cumulative analysis of 10,000 viable cells.

TABLE VI

I-Subregion-Specific Inhibition of AgAb Binding to Macrophage-Depleted B10.HTT Splenic T Cells

Cell population	% Esterase$^+$	% FcR$^+$	% Decrease in proportion of Fc$^+$ cells* by:	
			Anti-I^k‡	Anti-I^s§
Nylon T	3	23	25	48
Macrophage-depleted‖ nylon T	<1	20	40	40

* See footnote (*), Table III.

‡ A.TH anti-A.TL, 1:20 dilution.

§ A.TL anti-A.TH, 1:20 dilution.

‖ After nylon wool purification, the splenic T cells were incubated with iron filings for 45 min at 37°C, layered over a cusion of Ficoll-Hypaque, and centrifuged for 1 h. The cells banding above the Ficoll-Hypaque were recovered and used as the macrophage-depleted T cells.

the populations of cells inhibited by one of the antisera, B10.HTT splenic T cells (3% esterase positive) were depleted of phagocytic cells by the carbonyl-iron technique. The T-cell population treated in this manner contained less than 1% esterase-positive cells (Table VI). Treatment of these macrophage-depleted B10.HTT splenic T cells with either A.TH anti-A.TL or A.TL anti-A.TH re-

sulted in a 40% reduction in the proportion of FcR^+ cells subsequently labeled, suggesting that the FcR^+ cells inhibited by either antisera were T cells.

Discussion

It has previously been demonstrated in several laboratories that treatment of B lymphocytes with anti-Ia sera markedly inhibits the binding of AgAb complexes to the B-cell FcR (reviewed in reference 28). In the present report, we have demonstrated that treatment of splenic T cells with anti-Ia sera inhibits the binding of AgAb complexes to the majority of FcR^+ T cells.

Several potential mechanisms have been proposed to explain the inhibition of FcR by anti-Ia antibodies: (*a*) the antibodies, once bound to their membrane antigens, form AgAb complexes and bind to the FcR via their Fc portions (29); (*b*) antibodies that bind to cell membrane determinants sterically obstruct the FcR (29); and (*c*) the Ia antigens are physically associated with, or identical to, the FcR in the cell membrane (13). The first possibility seems unlikely in view of the observation that Fab fragments of anti-Ia antibodies inhibit the FcR, as well as intact molecules (13, 28, 29). The second hypothesis predicts that any antibody which binds to membrane determinants, regardless of whether or not those determinants are associated with the FcR, will inhibit the binding of AgAb complexes to the FcR. This hypothesis was based on the observation that any antibody which binds to the B-cell membrane—including anti-Ig, anti-Ly-4.2, anti-*H-2D*, and anti-*H-2K*—will inhibit rosetting of antibody-coated erythrocytes (29). However, it is possible that the rosette assay may be excessively sensitive to this type of steric inhibition (28, 30). Dickler and Sachs (13), who used aggregated Ig to detect the FcR, have found that inhibition of the B-cell FcR, is not effected by anti-Ig, anti-*H-2K*, or anti-*H-2D*. In the case of the T-cell FcR, we have found that anti-Thy 1.2 or heterologous anti-T-cell sera, which densely label the T-cell membrane, do not produce any detectable inhibition of binding of F*AgAb complexes to the T-cell FcR (Table III).

Contrary to the findings of Dickler and Sachs (13) that anti-*H-2D* or anti-*H-2K* antibodies did not inhibit the B-cell FcR, we have observed that both anti-*H-2D* and anti-*H-2K* sera inhibit, to some degree, the T-cell FcR. This FcR inhibitory effect of the anti-*H-2D* and anti-*H-2K* sera, in contrast to that of the anti-Ia sera, was only observed at relatively high serum concentrations. In the case of the (A.AL × B10)F_1 anti-A.TL serum, the FcR inhibition titer was markedly lower than the fluorescence-labeling titer (Fig. 2 *c*). It is thus possible that the observed inhibition was due to steric effects resulting from saturation of the membrane with anti-*H-2D* or anti-*H-2K* antibodies. Alternatively, the inhibition may have been due to the presence of antibodies against currently unknown determinants controlled by loci mapping close to the *D* or *K* ends of the *H-2* gene complex.

The data presented in this report neither proves nor disproves a physical association between the T-cell FcR and Ia antigens. The arguments for or against such an association have been amply discussed elsewhere (13, 28, 29) and will not be elaborated here. One point of agreement, however, is that in order for an antibody to interfere with the FcR on the cell membrane, that antibody must first bind to its antigen (28, 29). Therefore, the ability of anti-Ia

antibodies to block the T-cell FcR demonstrates the existence of Ia antigens on peripheral FcR^+ T lymphocytes.

Initial serological studies with peripheral T lymphocytes (16, 31, 32), Con A-stimulated blasts (18), and cortisone-resistant thymocytes (15, 17) suggested that at least some thymus-derived lymphocytes express *I*-region determinants. Data presented in this report show that determinants controlled by loci mapping in the *I-A* and *I-C*, *S*, or *G* regions are present on the population of peripheral T lymphocytes which bear an Fc receptor. Whether these determinants are controlled by the *Ia-1* and *Ia-3* loci, which mark the *I-A* and *I-C* subregions, respectively, and whether they are shared with B lymphocytes has not been resolved. In contrast, determinants controlled by loci mapping in the *I-J* and *I-E* subregions are not detectable on the FcR^+ T-cell population, which indicates either that antibodies against these determinants do not interfere with the T-cell FcR or that these determinants are not expressed on the FcR^+ T cells.

Recently, distinct functional subpopulations of T lymphocytes have been shown to carry determinants controlled by separate *Ia* loci. The *Ia-4* locus, which marks the *I-J* subregion, controls determinants present on allotype (Ig-1b) suppressor T lymphocytes but not on helper T or B lymphocytes (33). The same locus, or other loci mapping in *I-J*, control determinants found on carrier (keyhole limpet hemocyanin)-specific suppressor T cells (34).

There is currently conflicting evidence regarding expression of *I*-region loci in helper T lymphocytes. Hämmerling (35) and Press [reviewed by McDevitt et al. (36)] have not been able to detect Ia determinants on this subpopulation. In contrast, Okumura et al. (37) have reported that determinants controlled by an *I*-region locus (subregion unknown) distinct from *Ia-4* distinguishes helper T cells from suppressor T cells. The determinants found on helper T cells appear to be distinct from those found on FcR^+ T cells since (*a*) helper T cells are not included in the FcR^+ T-cell population (10), and (*b*) sera containing antibody against *I-A*-subregion-controlled determinants inhibit binding of AgAb complexes to FcR^+ T cells but do not kill helper T cells (37). Taken together, these data suggest that different *I*-region loci are selectively expressed in three distinct T-cell subpopulations: suppressor T cells, helper T cells, and Fc receptor-bearing T cells.

Evidence for phenotypic and functional heterogeneity within the FcR^+ T-cell population has been accumulating in other systems. The FcR^+ T cells appear heterogeneous with respect to their expression of the Ly determinants defined by Shiku et al. (8). Phenotypic analysis of FcR^+ T cells isolated from spleens and lymph nodes of normal mice suggest that all three Ly phenotypes – Ly-1,2,3, Ly-1, and Ly-2, 3 – exist within the FcR^+ T-cell population.[2] The FcR^+ T cells also appear to be phenotypically heterogeneous with respect to their expression of Ia determinants. Only 50–70% of the splenic FcR^+ T cells are inhibitable by anti-Ia sera. Similar results have been recently obtained using thymocytes (15). It is unlikely that this failure to obtain 100% inhibition by anti-Ia represents a limitation of the assay system since (*a*) 90% inhibition of B-cell FcR can be

[2] Stout, R. D., H. Cantor, F-W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. The Fc Receptor on thymus-derived lymphocytes. V. Distinct subsets within FcR^- and FcR^+ T cells defined by their expression of Thy 1 and Ly antigens. Manuscription in preparation.

obtained by anti-Ia treatment (13, 29), and (*b*) the degree of inhibition of FcR^+ T cells plateaued at 50–70% over a 40-fold dilution range of the anti-Ia sera. It therefore appears that 30% of the FcR^+ T cells do not express Ia determinants detectable by the FcR inhibition assay. [Dickler et al. (38) have reported inhibition of FcR activity with antisera directed against determinants controlled by loci mapping outside of the *H-2* gene complex. It would be of interest to determine if these determinants are expressed on FcR^+ T cells, specifically those that are not inhibited by anti-Ia sera.]

Phenotypic heterogeneity with respect to expression of *Ia* determinants may also exist within the population of FcR^+ T cells which are inhibited by anti-Ia sera. Treatment of B10.HTT splenic T cells with antisera directed against determinants controlled by loci mapping to the right of *I-J* resulted in 30–40% inhibition of AgAb complex binding over a 40-fold dilution range of antiserum, whereas treatment with antiserum directed against determinants controlled by loci mapping to the left of *I-E* resulted in 40–50% inhibition (Fig. 4 and Table V). Treatment with both antisera resulted in 50–70% inhibition. This additive effect of the antisera, taken together with the data presented in Table V and discussed above, suggests the existence of subsets of FcR^+ T cells which exclusively express *I-A*-controlled determinants and/or FcR^+ T cells which exclusively express *I-C*-, *S*-, or *G*-controlled determinants. How much overlap might exist between these two subpopulations is not known.

We have previously demonstrated that the FcR^+ T-cell subpopulation is functionally heterogeneous (10–12). It is considered possible that these functional subsets may be distinguishable by their selective expression of Ia determinants since (*a*) other functional subsets of T cells—e.g., helper and suppressor T cells—have been distinguished on the basis of selective expression of Ia determinants (33, 34, 37), (*b*) selective expression of Ia determinants on FcR^+ T cells has been demonstrated (*vide supra*), and (*c*) functional activities attributable to FcR^+ T cells have been shown to be inhibitable by treatment of the T cells with anti-Ia sera plus complement (33, 39).

For example, we have demonstrated that FcR^+ T cells are responsive to Con A, whereas FcR^- T cells, in the absence of FcR^+ T cells, do not respond to Con A (11). FcR^- T cells do, however, seem to respond to Con A in the presence of FcR^+ T cells. On the basis of responses of mixtures of FcR^+ and FcR^- T cells, we postulated that the Con A-stimulated FcR^+ T cells are capable of either (*a*) initiating or amplifying a mitotic response to Con A in the FcR^- T-cell population, or (*b*) "recruiting" FcR^- T cells into a mitotic response, e.g., by elaboration of a mitogenic factor (11). Recently, Niederhuber et al. (40) have reported that Con A responsiveness of T cells can be abrogated by treatment with anti-Ia serum plus complement before exposure to Con A. It would thus appear that the Con A-responsive FcR^+ T cell falls into the FcR^+ T-cell subset whose FcR are sensitive to inhibition by anti-Ia sera.

Similarly, it has been demonstrated that the FcR^+ T-cell population contains the amplifier T cell (Ly-1, FcR^+) and the differentiated cytotoxic effector T cell (Ly-2, FcR^+) involved in CML reactions (12). It has been recently reported that treatment of the responder T cells with anti-Ia plus complement reduces the generation of cytotoxic lymphocytes (39). However, it was not clear whether the

Ia determinants were expressed on amplifier T cells, the precursor of the cytotoxic T cell, or both. Previous studies have not indicated the presence of Ia determinants on either precursors of cytotoxic lymphocytes or the cytotoxic lymphocyte itself (32). Whether the FcR^+ amplifier or cytotoxic T cells express Ia determinants and, if so, which *I*-region loci control these determinants is presently under investigation.

Summary

Treatment of splenic T lymphocytes with anti-Ia antiserum inhibits the binding of antigen-antibody (AgAb) complexes to the majority (>50%) of Fc receptor-positive (FcR^+) T cells. A similar inhibition was observed with anti-*H-2D* and anti-*H-2K* sera but not with anti-Thy 1.2. Despite the presence of Ia determinants on peripheral T cells, as established by the inhibition of AgAb binding, Ia could not be detected on peripheral T cells by immunofluorescence assays.

Data obtained with the AgAb-binding inhibition assay indicate that determinants controlled by loci mapping in the *I-A* and *I-C*, *S*, or *G* regions are present on the FcR^+ T cells. Evidence is presented that subpopulations of T cells within the FcR^+ T-cell population may be distinguishable on the basis of which *I*-region-controlled determinant is expressed. The data are discussed in terms of phenotypic and functional heterogeneity of T lymphocytes.

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References

1. Greaves, M. F., J. J. T. Owen, and M. C. Raff. 1973. T and B Lymphocytes. American Elsevier Publishing Co., Inc., New York.
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. Häyry, P., and L. C. Andersson. 1974. T cell synergy in mixed lymphocyte culture-induced cytolysis. *Eur. J. Immunol.* 4:145.
4. Gershon, R. K. 1974. T cell control of antibody production. Contemp. Top. Immunobiol. 3:1.
5. Herzenberg, L. A., and L. A. Herzenberg. 1974. Short-term and chronic allotype suppression in mice. Contemp. Top. Immunobiol. 3:41.
6. Gershon, R. K., E. M. Lance, and K. Kondo. 1974. Immuno-regulatory role of spleen localizing thymocytes. *J. Immunol.* 112:546.
7. Cantor, H. 1972. The effects of anti-theta serum upon graft-vs-host activity of spleen and lymph node cells. *Cell. Immunol.* 3:461.
8. Shiku, H., P. Kisielow, M. A. Bean, T. Takahashi, E. A. Boyse, H. F. Oettgen, and L. J. Old. 1975. Expression of T-cell differentiation antigens on effector cells in cell-

mediated cytotoxicity in vitro: evidence for functional heterogeneity related to surface phenotype of T cells. *J. Exp. Med.* **141**:227.

9. Stout, R. D., S. D. Waksal, V. L. Sato, K. Okumura, and L. A. Herzenberg. 1976. Functional studies of lymphoid cells defined and isolated by a fluorescence activated cell sorter (FACS) from normal and athymic mice. *In* Leukocyte Membrane Determinants Regulating Immune Reactivity. V. P. Eijsvogel, D. Roos, and W. P. Zeijlemaker, editors. Academic Press, Inc., New York. 173.
10. 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.
11. 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.
12. 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.
13. Dickler, H. B., and D. H. Sachs. 1974. Evidence for identity or close association of the Fc receptor of B lymphocytes and alloantigens determined by the *Ir* region of the *H-2* complex. *J. Exp. Med.* **140**:779.
14. 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.
15. Dickler, H. B., R. D. Arbeit, P. A. Henkart, and D. H. Sachs. 1976. Association between Ia antigens and the Fc receptors of certain T lymphocytes. *J. Exp. Med.* **144**:282.
16. 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.
17. Goding, J. W., E. White, and J. J. Marchalonis. 1975. Partial characterization of Ia antigens on murine thymocytes. *Nature (Lond.)*. **257**:230.
18. David, C., T. Meo, J. McCormick, and D. Shreffler. 1976. Expression of individual Ia specificities on T and B cells. I. Studies with mitogen-induced blast cells. *J. Exp. Med.* **143**:218.
19. Sarkar, S., R. Hyman, T. Masuda, and L. A. Herzenberg. 1973. A rabbit antiserum to a thymus extract specific for mouse thymus-derived cells. *J. Immunol.* **110**:1222.
20. Reif, A. E., and J. M. Allen. 1966. Mouse thymic alloantigens. *Nature (Lond.)*. **209**:521.
21. Boyse, E. A., E. Stockert, and L. J. Old. 1969. Properties of four antigens specified by the *Tla* locus: similarities and differences. *In* International Convocation on Immunology. N Rose and F. Milgrom, editors. S. Karger AG., Basel, Switzerland. pp. 353–357.
22. Cebra, J. J., and G. Goldstein. 1965. Chromatographic purification of tetra methyl rhodamine-immunoglobulin conjugates and their use in the cellular localization of rabbit γ-globulin polypeptide chain. *J. Immunol.* **95**:230.
23. 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.
24. Van Rood, J. J., A. van Leeuwen, J. J. Keuning, and A. Blussé van Oud Alblas. 1975. The serological recognition of the human MLC determinants using a modified cytotoxicity technique. *Tissue Antigens*. **5**:73.
25. Yam, L. T., C. Y. Li, and W. H. Crosby. 1971. Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.* **55**:283.

26. 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.
27. Shreffler, D. C., C. S. David, S. E. Cullen, J. A. Frelinger, and J. E. Niederhuber. 1976. Serological and functional evidence for further subdivision of the I-region of the H-2 gene complex. *Cold Spring Harbor Symp. Quant. Biol.* 41:in press.
28. Dickler, H. B. 1976. Lymphocyte receptors for immunoglobulin. *Adv. Immunol.* 24:in press.
29. Halloran, P., V. Schirrmacher, and C. S. David. 1975. The specificity and significance of the inhibition of Fc receptor binding by anti-*H-2* sera. *Immunogenetics*. 2:349.
30. Arbeit, R. D., P. A. Henkart, and H. B. Dickler. 1976. Lymphocyte binding of heat-aggregated and antigen-complexed immunoglobulin. *In* In Vitro Methods in Cell Mediated Immunity. B. Bloom and J. David, editors. Academic Press, Inc., New York, 2:in press.
31. Gotze, D. 1975. T(Ia-t)- and B(Ia-b)-cell alloantigens determined by the *H-2* linked *I*-region in mice. *Immunogenetics*. 1:495.
32. 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.
33. 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.
34. 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.
35. Hämmerling, G. I. 1976. Tissue distribution of Ia antigens and their expression on lymphocyte subpopulations. *Transplant. Rev.* 30:864.
36. 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.
37. 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.
38. Dickler, H. B., J. L. Cone, M. T. Kubicek, and D. H. Sachs. 1975. Detection of non-H-2 antigen(s) which, like Ia antigens, are associated with the Fc receptor of B lymphocytes. *J. Exp. Med.* 142:796.
39. Plate, J. M. D. 1976. Cellular responses to murine alloantigens of the major histocompatibility complex. The role of cell subpopulations that express different quantities of *H-2* associated antigenic markers. *Eur. J. Immunol.* 6:180.
40. 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.