FUNCTIONAL ANALYSIS OF T CELLS EXPRESSING Ia ANTIGENS

I. Demonstration of Helper T-Cell Heterogeneity*

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The expression of *I*-region gene products on immunocompetent cells is intimately involved with cellular function. Ia molecules, both on macrophages (MØ)[1] and on B cells, participate in the presentation of antigen necessary for helper T-cell activation (1–6). They also have been shown to stimulate mixed lymphocyte (7) and graft-versus-host type reactions (8). In addition, *I*-region determinants have served to delineate functional subpopulations of B cells (9), MØ (10), and at least one class of T lymphocytes, the *I-J*-bearing T suppressor cell (11).

However, the presence of *I*-region products on T cells with other functions has been widely debated. Evidence for and against the expression of Ia on T cells involved in concanavalin A (Con A) mitogenesis (12, 13), antigen-induced proliferation (14), delayed hypersensitivity, and helper and cytotoxic activities (11, 15–19) have all been reported. These discrepancies have been blamed on the fact that with available anti-Ia antisera, only a paucity of Ia molecules could be detected on a small fraction of T cells. This is especially disconcerting in view of the overwhelming theoretical importance that Ia molecules may have for potential T cell-T-cell interactions.

Experiments showing that the interaction of helper and cytotoxic T cells apparently depended upon the ability of helper cells to recognize *I-A* region products on the cytotoxic T-cell precursors, predicted that Ia antigens should be found on these precursors, though they have been difficult to demonstrate (20, 21). Similarly the demonstration that *I*-region-controlled immune response genes could be expressed sometimes in T cells themselves, rather than in virally infected target cells or antigen presenting B cells and MØ, also predicted that Ia antigens should be demonstrable in T cells (22).

For some years we have been interested in the problem of T-cell functional heterogeneity. To pursue this interest we wished to use anti-Ia sera of different specificities, in experiments analogous to those already done so successfully with anti-Ly antisera (23). We have however, been rather unsuccessful in such studies when

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[1] *Abbreviations used in this paper:* B10, C57BL/10; BSS, balanced salt solution; CFA, complete Freund's adjuvant; Con A, concanavalin A; FCS, fetal calf serum; KLH, keyhole limpet hemocyanin; LNC, lymph node cells; LPS, lipopolysaccharide; MØ, macrophage, MLC, mixed lymphocyte culture; PFC, plaque-forming cells; TNP, trinitrophenyl(ated).

using anti-Ia hybridoma antibodies or antisera raised conventionally against normal spleen cells. An alternate approach to the study of T-cell Ia antigens has been the production of anti-Ia sera against mitogen-activated T-cell blasts. Such antisera have been shown by David et al. (24), Götze (25), and most recently by Hayes and Bach (26) to have higher cytotoxic titers than those raised against unstimulated lymphocytes, and to kill higher percentages of T cells.

We have therefore repeated our functional studies using an anti-Ia serum raised against Con A-stimulated blast cells. In this communication we investigated the expression of Ia on T cells of various functions with one such antiserum, an A.TH anti-A.TL (anti-I^k, S^k, G^k) made against thymic and peripheral T-cell blasts. Our results indicated that this antiserum plus complement killed a high percentage of peripheral T lymphocytes. Moreover, we found unequivocal evidence that Ia antigens were expressed differentially by T-cell subsets with different functions. One type of helper T-cell necessary for B-cell responses to antigen, and the helper T cell required to generate alloreactive cytotoxic responses were found to be Ia^+. The antiserum did not kill cytotoxic T cells themselves, or their precursors, and also did not kill a second type of helper T cell required for B-cell responses to protein antigens. Finally, absorption experiments revealed at least two specificities in the antiserum, one, mapping to the right of *I-J*, and a second mapping in *I-A*, which in the presence of complement caused >95% killing of the Ia^+ helper T cell.

Materials and Methods

Mice. C57BL/10.Sn (B10), B10.A, and C57BL/6 (B6) mice were purchased from The Jackson Laboratories, Bar Harbor, Maine. B10.S, B10.A (4R), A.TH, and A.TL animals were bred in our facilities from breeding pairs kindly given to us by Dr. Chella David (Mayo Clinic, Rochester, Minn.). B10.HTT mice were derived from breeding pairs kindly sent by Dr. David Sachs (National Institutes of Health, Bethesda, Md.).

Antigens. Keyhole limpet hemocyanin (KLH) was purchased from CalBiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. and *Escherichia coli* lipopolysaccharide (LPS) from Difco Laboratories, Detroit, Mich. Horse erythrocytes (HRBC) were obtained from the Colorado Serum Co., Denver, Colo. These antigens were prepared and used in their unsubstituted and trinitrophenylated (TNP) forms as previously described (27).

Immunizations. To obtain carrier-specific helper T cells, mice were primed with KLH in Complete Freund's Adjuvant (CFA) intraperitoneally 7 d before use (27). B cells were primed for anti-hapten (TNP) responses by immunization with TNP-LPS 1 wk before use (28). Alloreactive cytotoxic T cells were generated in vitro in a primary one-way mixed lymphocyte reaction. Equal numbers of splenic responder and irradiated (1,000 rads from a ^{60}Co source) splenic stimulator cells were cultured for 5 d at 10^7 cells/ml in 0.5-ml cultures. After induction, the cells were harvested and assayed for cytotoxicity by ^{51}Cr release as described below.

Preparation of Cells. Purified T cells from primed or normal mice were prepared by passage of spleen cells over nylon fiber columns (as described in detail elsewhere [29]). Splenic B cells and MØ were prepared by treating spleen cell suspensions with anti-T cell serum, or anti-Thy 1 hybridoma antibody, and guinea pig complement (27). The anti-Thy 1 hybridoma antibody was the culture supernate of a hybridoma, T24/40.7, which was very kindly given to us by Dr. Ian Trowbridge, Salk Institute. Peritoneal washings, for use as a source of MØ, were prepared from normal animals.

Culture Conditions. KLH-specific helper T cells were assayed in a culture medium modified from that of Mishell and Dutton (29, 30) and hereafter referred to as complete medium. Mixed lymphocyte cultures (MLC) were performed in identical medium, however the cytotoxic T cells generated were then assayed in RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) with 5% fetal calf serum (FCS), 10 mM Hepes, and 5×10^{-5} M 2-mercaptoethanol.

Assay for Helper T-Cell Activity in B-Cell Responses. KLH-primed T cells were assayed for

helper activity by titration into triplicate cultures containing constant numbers of splenic, TNP-primed B cells and MØ and 1 μg/ml TNP-KLH. After 4 d, cultures were harvested and assayed for anti-TNP plaque-forming cells (PFC). The PFC response was plotted versus the number of primed T cells added, and a straight line fit to the initial linear portion of the curve. The slope of this line was taken as a measure of the helper activity of the T-cell preparation. Helper T-cell activities were therefore expressed in terms of these slopes, as anti-TNP PFC per culture per 10^6 T cells ± their standard errors (4, 6, 27).

Assay for Cytotoxic T-Cell Activity. Cytotoxic T-cell activities after induction were measured on ^{51}Cr-labeled EL-4 (H-2^b) target cells by modifications of the method of Cerrotini and Brunner (31). Briefly, cells to be assayed were titrated in triplicate into microcultures containing 1×10^4 ^{51}Cr-labeled EL-4 targets. After 4 h, cell death was measured by chromium release. EL-4 cells incubated alone and 1.0 N HCl-lysed EL-4 cells served as controls for background release and maximum release of ^{51}Cr, respectively. The percent specific release was calculated and displayed on log-log plots versus the number of effector cells added. Nonspecific release was usually between 6 and 8%. A modified linear regression analysis was then performed on the linear portions of these curves using a program which fit the best set of parallel lines to the data from a single experiment. The number of effector cells required to kill 50% of the target cells was arbitrarily chosen to be a unit of cytolytic activity. Results were therefore expressed as units of cytolytic activity per 10^6 cells ± the 95% confidence limits.

Antisera. Conventional A.TH anti-A.TL antisera were raised by immunization of recipients with A.TL spleen cells on a weekly, followed by a biweekly basis (32). A.TH anti-A.TL T cell blast sera were raised by modifications of the methods of David et al. (24) and Hayes and Bach (26) using Con A-stimulated thymocytes together with Con A-stimulated splenic and lymph node T cells. A.TL thymus cells were incubated in complete medium at 1.5×10^7/ml with 5 μg/ml Con A. A.TL lymph node and splenic T cells were prepared by passage over nylon fiber columns (29) and then incubated at 5×10^6/ml in the presence of 5×10^5/ml A.TL peritoneal cells, added as a source of MØ and 5 μg/ml Con A. After 2 d culture at 37°C, the cells were harvested, extensively washed in serum-free balanced salt solution (BSS) and used as immunogens. Recipient A.TH mice were immunized i.p. with 3×10^7 cells emulsified in Freund's complete adjuvant (Difco Laboratories) followed by 6 weekly, then biweekly injections of 1–3 $\times 10^7$ cells i.p. in BSS. Animals were bled every other week beginning after the sixth injection. Anti-*H-2*^{s}-specific antisera were raised by immunizing (B10.A × D1.LP)F_1 animals with B10.S spleen cells as previously described (32).

Antisera Absorption. A.TH anti-A.TL blast serum was absorbed five to six times with B10 (*H-2*^{b}) cells as follows: 0.1 ml packed cells was resuspended in 1.0 ml serum at 0°C for 30 min per absorption. The B10 cells were a mixture of normal and Con A-stimulated thymus, spleen, and lymph node cells.

Antisera Killing of Cells. Before use in functional assays, cell preparations were treated with antisera as follows. Cells were killed with anti-*H-2*^{s} antiserum and complement by resuspending them at 10^7/ml in 1:5 anti-*H-2*^{s} in BSS for 30 min at 0°C. Controls were similarly incubated with 1:5 FCS in BSS. The suspensions were then thoroughly washed with BSS and resuspended in 1:15 rabbit complement previously tested for low cytotoxicity on mouse lymphocytes. The suspensions were then incubated at 37°C for 30 min before thorough washing.

A similar procedure was followed when killing with anti-Ia antisera and complement, except that the anti-Ia sera were used at concentrations ranging from 1:5 to 1:20 without appreciable difference in effect. In addition, when purified T cells were to be treated, they were incubated overnight in complete medium after nylon fiber passage before anti-Ia + complement treatment. We found, as previously shown by Hayes and Bach (26), that this procedure increased their susceptibility to killing with anti-Ia. Presumably, it allowed the regeneration of Ia molecules stripped from the T-cell surface during nylon fiber fractionation. T-cell activities are reported based on their original cell number before antisera treatment.

Assays for cytotoxic titers of various antisera were performed in microtitre plates, using procedures similar to those described above except that cell preparations were spun over Ficoll-Hypaque (Winthrop Laboratories, N. Y.) to remove dead cells before antisera treatment. After killing, dead and live cells were scored by ethidium bromide staining, followed by counting on a multiparameter, fluorescence-activated cell sorter (33). The percent specific kill was calculated according to the formula:

TABLE I
*Haplotype Origin of Mice Used in this Paper**

Strain	Haplotype origin of region									
	K	I-A	I-B	I-J	I-E	I-C	S	G	D	Tla
A.TH	s	s	s	s	s	s	s	s	d	a
A.TL	s	k	k	k	k	k	k	k	d	c
B10.A	k	k	k	k	k	d	d	d	d	a
B10.A(4R)	k	k	b	b	b	b	b	b	b	(b)
B10.HTT	s	s	s	s	k	k	k	k	d	c
B10.S	s	s	s	s	s	s	s	s	s	b
B10	b	b	b	b	b	b	b	b	b	b

* Data from Klein et. al. 1978. (34).

$$\% \text{ specific lysis} = 100 \times \frac{\% \text{ dead, experimental} - \% \text{ dead, C' control}}{100\% - \% \text{ dead, C' control}}$$

Results

Cytotoxicity of Anti-Ia Antisera on Lymphocytes. Our initial experiments sought to determine whether the expression of Ia on T cells could more readily be detected using an anti-Ia antiserum raised by immunization with Con A blast cells. For this purpose we chose to make a broad *I*-region reactive antiserum using the congenic strains A.TH and A.TL, which share the K and D regions of *H-2*. A.TH anti-A.TL antisera should detect only those antigens coded for by the *I*-region ($I\text{-}A^k$, B^k, J^k, E^k, C^k). In addition, the S^k, G^k regions and certain antigens encoded by genes to the right of *H-2*, notably *Qa-1* and *Tla* might also be detected (Table I). In all experiments therefore, B10.A ($H\text{-}2^a$) mice were used as the positive prototype strain because they share *Qa* and *Tla* genes with A.TH and should be reactive solely on the basis of I, S, G region disparity. Lymphocytes from B10.S ($H\text{-}2^s$) mice served as controls for nonspecific or autoreactive activities in the antisera. The *H-2* haplotypes for all strains used are shown in Table I.

A.TH anti-A.TL antiserum raised by conventional immunization, and A.TH anti-A.TL blast antiserum were titrated for cytotoxicity on purified splenic T and B lymphocytes from B10.A or B10.S animals. In a representative experiment, neither the conventional antiserum (Fig. 1 A) nor the anti-blast antiserum (Fig. 1 B) had appreciable activity on B10.S B or T cells. The conventionally raised antiserum killed B10.A B cells at high titer, but as expected, showed a minimal activity on B10.A T cells. In contrast, the antiserum raised against Con A blasts killed a high percentage of both B10.A B and T cells though the T-cell titer declined more rapidly (Fig. 1 B). This antiserum was also cytotoxic for unfractionated spleen cells and, to a much greater degree, for lymph node cells (LNC). Although this experiment does not test whether the same antigens are present on both T and B cells, it suggests that Ia antigens must be present at least in different proportions on the two cell types because the ratio of cytotoxic titers of the two anti-Ia sera on T and B cells was different. Similar results have been reported by others in the past (35, 36).

Effect of Anti-Ia Blast Serum on Helper T Cells for B-Cell Responses. With an antiserum having the capacity to detect Ia antigens on a high percentage of T lymphocytes, we could now ask whether expression of these determinants was related to T-cell function.

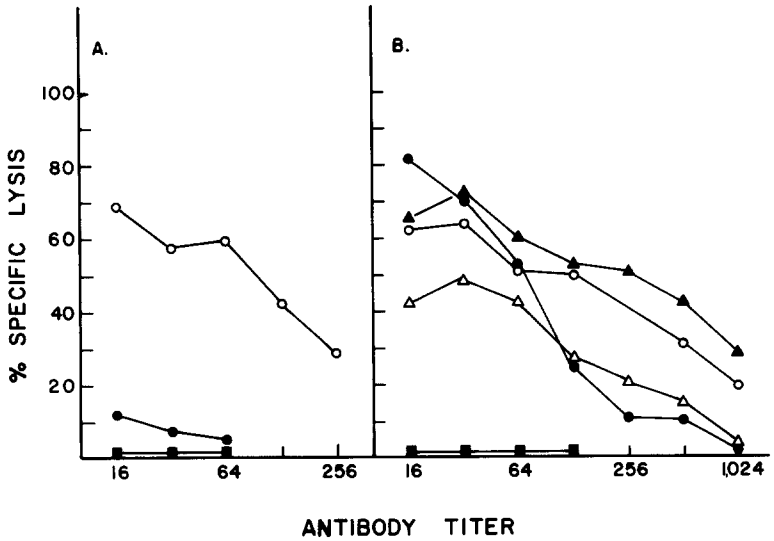

FIG. 1. Cytotoxic activity of anti-Ia serum on mouse lymphocytes. A.TH anti-A.TL antiserum made by conventional methods (A) or by Con A blast immunization (B) was titrated for cytotoxicity on B10.S B cell (■), B10.S T cell (■), B10.A B cell (○), B10.A T cell (●), and B10.A spleen (△) and lymph node (▲) populations. B cells were prepared from spleen cell suspensions treated with anti-Thy 1 + C′. T cells were obtained after passage of spleen cells over nylon wool followed by overnight incubation in medium. All cells were spun over Ficoll-Hypaque and added to serial dilutions of antiserum in BSS. A microtiter plate technique for determining complement-mediated cytotoxicity was then performed as described in the Materials and Methods.

To examine the effect on T-helper cells involved in in vitro antibody responses, mice of various strains were primed with KLH. 7 d later their splenic T cells were isolated by nylon wool passage. After an overnight incubation they were treated with A.TH anti-A.TL blast antiserum and complement before being titrated for helper activity in anti-TNP-KLH responses of syngeneic B cells and MØ. Control T cells were treated with FCS plus complement. In early studies (not shown) we had tested both conventional anti-Ia and hybridoma produced anti-Ia antibody for their effects on helper function. The results varied considerably and at best we were able to inhibit helper activity by only 30–60% at very high serum concentrations. As shown in Table II, however, treatment with the Ia blast antiserum and complement eliminated the helper activity of B10.A and B10.A(4R) T cells (96% inhibition). Such treatment also inhibited the helper activity of T cells that had not been previously cultured overnight, although to a lesser degree (81% inhibition in data not shown). As mentioned in the Materials and Methods this is consistent with a decreased expression of Ia on freshly nylon-passaged T cells. Therefore we routinely precultured purified T cells before antiserum treatment. Although there was no significant effect on B10.S helper T cells, there was a partial killing of B10.HTT helper T cells resulting in a 64% inhibition of helper activity. These results imply that A.TH anti-A.TL blast antiserum detected at least two different specificities on helper T cells. One, by reactivity on B10.HTT, mapped to the right of *I-J*, and the other, detected on B10.A(4R) appeared to map in the *I-A* subregion (Table I). However, in a subsequent experiment the antiserum was found to be reactive with B10 helper cells as well (Table II, bottom) indicating the presence of a cross-reacting determinant(s) in the $H\text{-}2^b$ haplotype.

TABLE II

Effect of A.TH Anti-A.TL Blast Serum on Helper T-Cell Activity

Exp.	Source of KLH-primed helper T cells	Regions shared with A.TL*	Treatment of T cells	Helper activity‡ (anti-TNP PFC/culture/ 10^6 T cells ± SE)	Percent inhibition of T-cell help
					%
I	B10.S	—	C′ only	364 ± 39	
			anti-Ia + C′	295 ± 39	19§
	B10.A	IA^k-E^k	C′ only	539 ± 21	
			anti-Ia + C′	22 ± 21	96
	B10.A(4R)	IA^k	C′ only	112 ± 5	
			anti-Ia + C′	3 ± 5	97
	B10.HTT	IE^k-G^k, Tla^c	C′ only	1,255 ± 54	
			anti-Ia + C′	453 ± 54	64
II	B10.A	IA^k-E^k	C′ only	1,959 ± 110	
			anti-Ia + C′	102 ± 110	95
	B10	—	C′ only	1,507 ± 96	
			anti-Ia + C′	605 ± 96	60

* In addition, cross-reactivities with other regions are also possible.

‡ Tested in vitro with TNP-primed syngeneic B cells and MØ and TNP-KLH antigen.

§ Not significant.

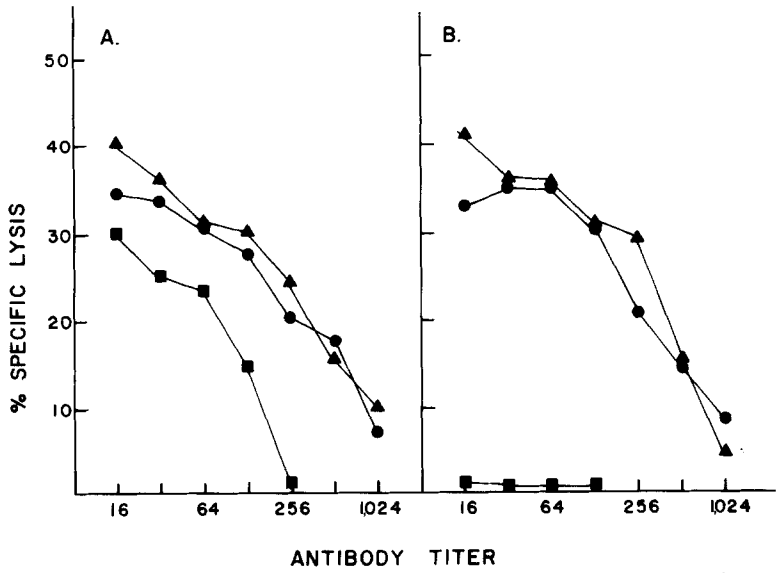

FIG. 2. Cytotoxic activity of anti-Ia blast serum after removal of cross-reactive *H-2*^{b} activity. A portion of A.TH anti-A.TL blast serum was exhaustively absorbed with B10 cells as described in the Materials and Methods. Both the unabsorbed (A) and the absorbed (B) antisera were then titrated for cytotoxicity on lymph node cells from B10.A (●), B10.A(4R) (▲), or B10 (■) mice.

Helper T Cells Bear I-A Region Antigens. To determine whether the reactivity on B10.A(4R) T cells was the result of antibody directed at the *I-A*^{k} subregion or antibody to a cross-reacting *H-2*^{b} determinant located elsewhere in the *I*-region, we tested anti-Ia blast serum that had been exhaustively absorbed with B10 cells. As shown in Fig. 2A, the unabsorbed serum killed LNC from B10.A, B10.A(4R), and also B10 mice. Absorption with B10 removed the reactivity against *H-2*^{b} but did not remove cytotoxicity for B10.A or B10.A(4R) (Fig. 2B). In confirmation of the cytotoxic

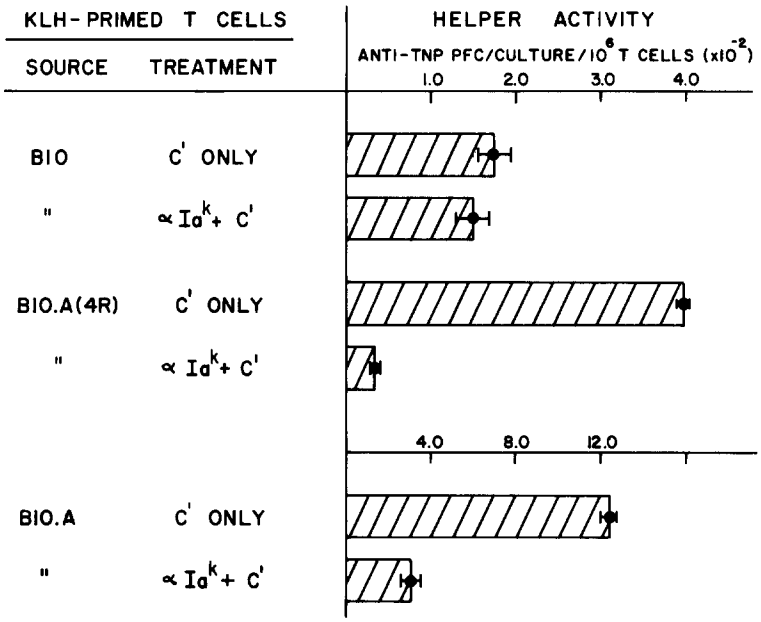

FIG. 3. Effect of B10-absorbed anti-Ia blast serum on helper T-cell activity. T cells from KLH-primed B10, B10.A, and B10.A(4R) mice were obtained by passage of spleen cells over nylon fiber columns. After overnight incubation, the T cells were then treated with B10-absorbed anti-Ia blast serum + C′ or C′ alone, and titrated for helper activity into cultures of syngeneic TNP-primed B cells + MØ. After 4 d, the anti-TNP PFC response was determined and plotted versus the number of T cells added. The initial slope of this titration was taken as a measure of helper activity and is expressed as anti-TNP PFC per culture per 10^6 T cells ± SE.

result, the absorbed antisera also failed to inhibit B10 helper T-cell function (Fig. 3). Nevertheless it fully retained its ability to eliminate the helper activity of B10.A(4R) T cells. These data indicate that a determinant(s) which maps to the *I-A* subregion is detected by A.TH anti-A.TL blast antiserum on the surface of a helper T-cell population.

Anti-Ia is not Passively Carried Over into Culture. The above results could alternatively be explained if Ia antibody, bound specifically or nonspecifically (via Fc) to the T cells, were carried over into culture to act directly on B cells and/or MØ. To eliminate this possibility we measured the helper activity of KLH-primed T cells treated only with anti-Ia (no complement), then washed and added to B cells and MØ. In addition, we tested whether anti-Ia + C′-treated T cells could affect the helper function of untreated KLH-primed T cells when both populations were added to responding B-cell cultures. As shown in Table III, no inhibition of the PFC response was observed in either instance.

A.TH Anti-A.TL Blast Antiserum Defines a Subpopulation of Helper T Cells. Recently we described experiments showing that two different types of helper T cells are required for B-cell responses to protein-bound antigens (37–39). The activity of one of these T cells, which is required relatively late in the response, can be replaced by a factor(s) present in the supernate of Con A-activated spleen cells (Con A SN) (37–39). We therefore set out to determine whether both types of T cells were killed with A.TH anti-A.TL blast serum plus complement, by testing whether addition of Con A SN to the treated cells would restore their activity. KLH-primed T cells from B10.A or

TABLE III

Failure to Detect Anti-Ia Carryover into B Cell/MØ Cultures

KLH-primed B10.A T cells		Helper activity (anti-TNP PFC/culture/10^6 T cells ± SE)	
C′ treated only		605 ± 55	
Anti-Ia treated only		590 ± 55	
Anti-Ia + C′ treated		33 ± 4	
C′ treated cells plus anti-Ia + C′ treated	*	340 ± 42	observed
		319	expected

* C′-treated T cells were diluted 1:1 with anti-Ia + C′-treated T cells and the mixture titrated for helper activity on syngeneic TNP-primed B cells and MØ. The expected PFC response represents the average of the two individual responses.

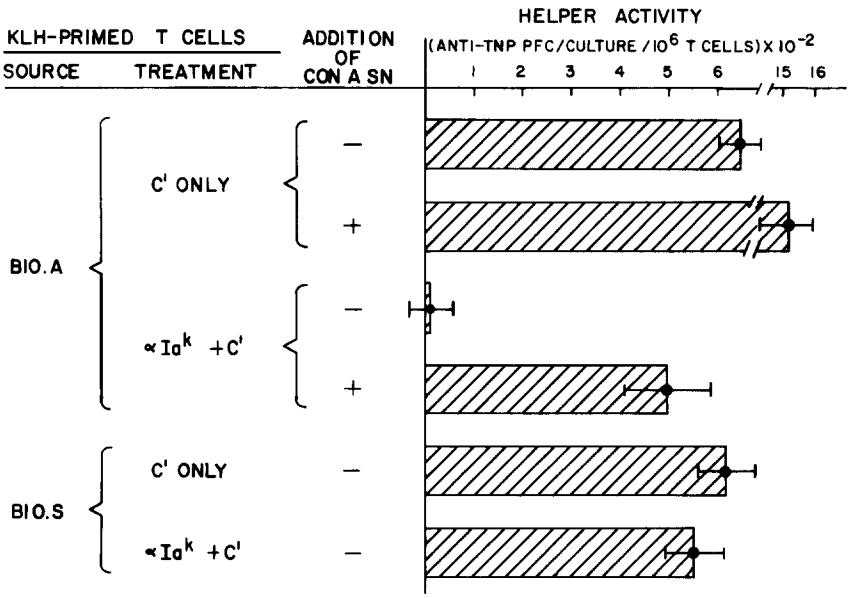

FIG. 4. Restoration of helper activity with Con A supernate. KLH-primed T cells from B10.A or B10.S mice were treated with A.TH anti-A.TL blast serum + C′ or C′ alone and titrated for helper activity on syngeneic TNP-primed B cells and MØ in the presence or absence of Con A SN. Con A SN was added 24 h after the initiation of cultures at a final concentration of 30%.

B10.S strains were treated with the anti-Ia blast serum and complement, and titrated for helper activity on syngeneic B cells and MØ in the presence or absence of Con A SN. Con A SN was prepared by culturing spleen cells (1×10^7/ml) with 4 μg/ml Con A in complete medium for 24 h. The supernate was absorbed with Sephadex G-200 to remove residual Con A before use. One of four similar experiments (Fig. 4) shows that although there was again no reactivity against B10.S, the helper activity of B10.A T cells was eliminated by anti-blast serum treatment. This activity was restored by addition of Con A SN to the cultures. In our hands, although Con A SN augments the response of untreated cells (Fig. 4), it cannot replace the function of both helper T cells (37–39). These results therefore suggest that only one type of helper cell was killed by the anti-Ia blast serum. This Ia^+ T cell apparently functions by releasing a nonspecific signal(s), replaceable by Con A SN, necessary for B-cell activation (38,

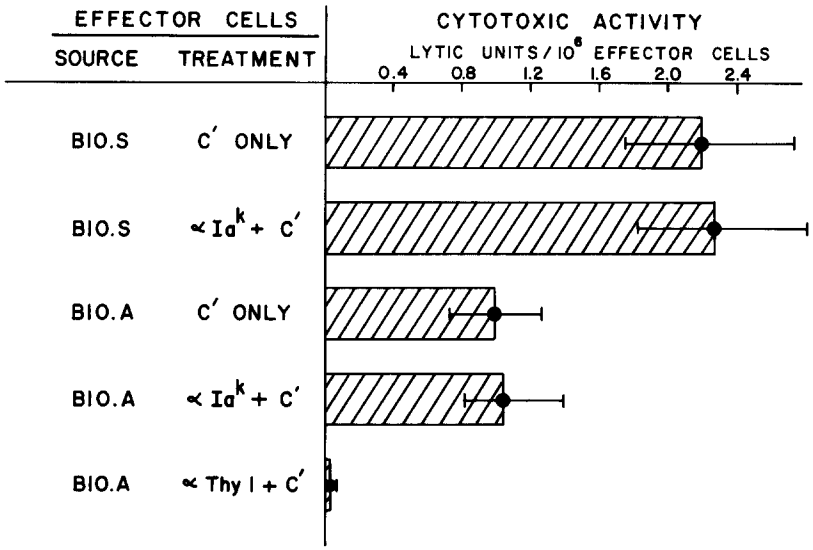

FIG. 5. Effect of anti-Ia blast serum treatment on cytotoxic effector cells. B10.S or B10.A spleen cells were cultured in a one-way MLC with irradiated C57BL/6 stimulator cells. After 5 d, the cells were harvested, treated with B10-absorbed A.TH anti-A.TL blast serum + C′, anti-Thy 1 + C′, or C′ alone, and titrated for cytotoxicity on ^{51}Cr-labeled EL-4(*H-2*^{b}) targets. The percent specific release was calculated and plotted versus the number of effector cells added. A linear regression analysis was performed on these curves and the number of effector cells required to kill 50% of the targets was chosen as a unit of cytolytic activity. Results are expressed as lytic units per 10^6 effector cells ± the 95% confidence limits.

39). Identical results were obtained with both B10-absorbed and nonabsorbed antiserum.

Effect of Anti-Ia Blast Serum on Cytotoxic T Cells. B10.A and B10.S T-cells cytotoxic for *H-2*^{b} antigens were raised by culturing B10.A or B10.S spleen cells with irradiated C57BL/6 stimulator cells for 5 d. After induction, the cells were treated with B10-absorbed A.TH anti-A.TL blast serum and complement, or complement alone, and titrated for cytotoxic activity on ^{51}Cr-labeled EL-4(*H-2*^{b}) cells. We had previously shown that splenic lymphocytes were susceptible to killing with anti-Ia blast serum and complement (Fig. 1 B). However, as shown in Fig. 5, the cytolytic activity of both strains was unaffected by treatment with this antiserum plus complement. Control cells treated with anti-Thy 1 plus complement after induction, indicated that the activity being measured was indeed T-cell mediated and susceptible to antibody plus complement killing.

Effect of A.TH Anti-A.TL Blast Antiserum on Cytotoxic Precursor T Cells and Their Helper T Cells. Zinkernagel et al. (20) and von Boehmer et al. (21) have published experiments suggesting that the interaction of helper T cells and cytotoxic precursors is controlled by *I*-region-encoded antigens expressed by the cytotoxic precursor cell. We therefore wished to test directly whether these precursors or the helper cells themselves bore Ia antigens. This was accomplished by treating the MLC-responder populations with anti-Ia blast serum and complement before the induction of cytotoxic cells. We reasoned that if the helper T cells alone were Ia$^+$ and killed by anti-Ia, we could still generate cytotoxic effector cells by the addition of a bystander source of helper cells to the cultures. If however, the cytotoxic precursor was Ia$^+$, and killed,

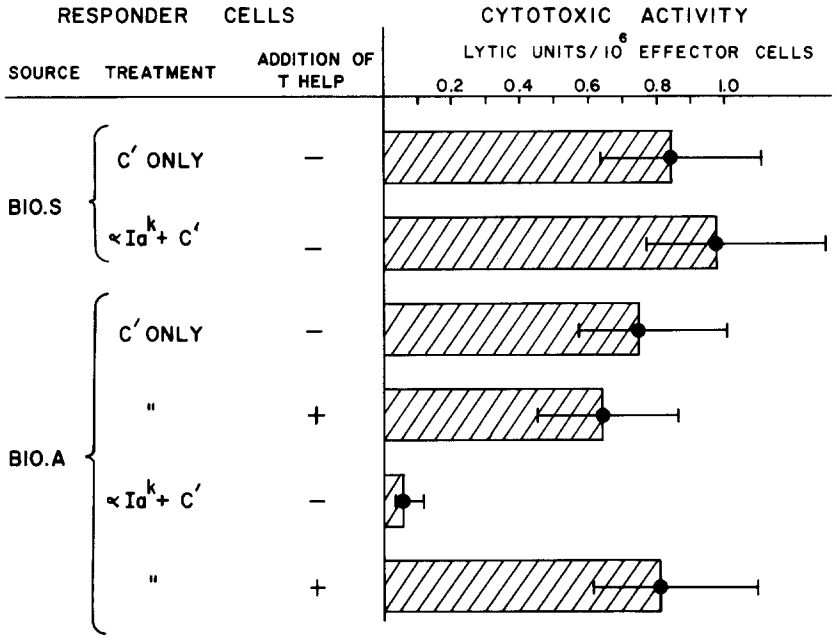

FIG. 6. Effect of anti-Ia blast serum on cytotoxic precursor cells and their helper T cells. B10.S or B10.A responder spleen cells were treated with A.TH anti-A.TL blast serum + C′ or C′ alone. They were then incubated with irradiated C57BL/6 stimulators with or without 1×10^6 (B10.A × B10.S)F_1 splenic T cells added as a source of help. After 5 d the cells were harvested and treated with anti-*H-2*^{s} serum + C′ to kill remaining F_1 cells before measuring cytotoxicity on labeled EL-4 targets. Control experiments (not shown) indicated that the cytotoxic activity of F_1 effector cells (1.05 lytic units) could be effectively eliminated (0.02 U) by treatment with anti-*H-2*^{s} + C′ after induction.

then no amount of additional help would generate a cytotoxic response. For bystander helper cells we used purified T-cells semi-syngeneic with the responders to prevent unwanted allogeneic effects.

In the experiment shown in Fig. 6, B10.A or B10.S spleen cells were treated with A.TH anti-A.TL blast serum and complement, or complement alone and were then incubated with irradiated C57BL/6 stimulators with or without nylon-purified (B10.A × B10.S)F_1 splenic T cells as a source of help. After 5 d the cells were harvested and the F_1 helper cells killed with anti-*H-2*^{s} antiserum plus complement. Controls were included to assure that this treatment completely eliminated the cytotoxic potential of these F_1 cells (Fig. 6).

As shown in Fig. 6, the anti-blast serum had no effect on the B10.S cytotoxic response. However, B10.A cytotoxic T-cell induction was inhibited 90% by prior treatment with anti-Ia blast serum and complement. The induction of B10.A cytotoxic T cells was restored if bystander, F_1 helper T cells were present in the cultures during the 5-d priming. From these experiments we concluded that the helper cell needed to induce cytotoxic T cells was Ia^+ but that cytotoxic precursors themselves were Ia^- at least as detected by our antiserum. Although we have not yet formally mapped the I-region involved, both the B10-absorbed and the non-absorbed antiserum gave similar results in experiments of this type.

Discussion

Two major points emerge from these studies. First, in agreement with other laboratories (24–26), we have found that *I*-region gene products on the surface of T lymphocytes can more easily be detected with an anti-Ia antiserum raised against mitogen-stimulated T cells. This is consistent with the proposal that activation of T cells may result in a quantitative increase in Ia expression, or an increase in a particular population bearing Ia determinants (24, 26, 48). In either case, immunization with such cells would increase the probability of raising antibody to T-cell-associated Ia antigens. Second, and more importantly, we have shown that these antisera can now be used as a probe to delineate functional subsets of T cells which differ in their expression of Ia. Not only did this allow us to discriminate between T cells with widely differing activities (i.e., helper vs. cytotoxic) but enabled us to confirm our earlier observation that a functional heterogeneity exists within the helper T-cell population itself.

The A.TH anti-A.TL blast serum described here had properties similar to the anti-blast antibodies studied by Götze (25). Both sera reacted with a very high percentage of peripheral T cells and both were cytotoxic for B cells as well as T. In this respect they differed from an anti-Ia serum recently reported by Hayes and Bach (26) to be specific for T lymphocytes only. These investigators however, employed only thymic and not peripheral T lymphoblasts for immunization and studied an antiserum with a specificity restricted to the *I-J, E* subregions which may account for the differences. We have not as yet performed the necessary absorption experiments to formally determine whether any of the specificities in our antiserum are directed against T-cell-specific Ia determinants. The differences in cytotoxic activities between our antiblast and conventional A.TH anti-A.TL serum however, would suggest that this may be so. In any case the antiserum is clearly cytotoxic for a population of primed helper T cells.

There is a good deal of controversy over the presence of Ia determinants on helper T cells. Although some investigators have been unable to demonstrate any inhibition of helper function with anti-Ia sera (14, 17, 18, 40), others have been successful both in murine systems (11, 15, 41) and recently in humans (42). Yet in many of these latter studies the inhibitory effects on T cells were neither as striking nor as consistent as they were on B cells. Our results offer a possible resolution to this problem. The profound effect on helper function that we observed is most likely related to the greater sensitivity of anti-Ia blast serum in detecting T-cell Ia antigens. Helper activity over a wide range of doses was eliminated by antibody directed against the *I-A* subregion in our experiments. This was unequivocally demonstrated both by cytotoxicity and by inhibition of B10.A and B10.A(4R) helper activity once cross-reactivity for the $H\text{-}2^b$ haplotype had been removed. Cross-reactivities in anti-Ia blast serum have been reported by others (25, 26) and must be taken into account when determining the specificity of these antisera. The region(s) controlling the antigens on B.10 which cross-reacted with I^k in our preparation has not yet been mapped. In addition, a new locus, *H-2*T, recently described by Klein (43) and definable by A.TH anti-A.TL cytotoxic lymphocytes, should be considered. This particular specificity, however, has not been detected in anti-Ia antisera made in these strains, and furthermore, A.TH has the same allele of *H-2*T as B10.A (43). Thus we feel confident

that a major specificity detected on the surface of helper T cells by our antiserum maps to the *I-A* subregion.

The anti-Ia blast serum also caused partial killing of B10.HTT helper T cells suggesting additional reactivity for antigens to the right of *I-J*. Eardley et al. (44) have recently shown that one type of helper T cell is Qa^+. Because B10.HTT and A.TL are identical at, at least, *Tla*, it is possible that this reactivity on B10.HTT is due to antibodies against *Qa*-controlled antigens, even though such antibodies have not previously been demonstrated in conventionally raised A.TH anti-A.TL sera (45). Our future experiments will determine the target region on B10.HTT helper cells of the antiserum.

Recently we have described experiments showing that two different helper T cells are required for anti-protein bound responses of B cells (37–39). One of these is required relatively late in the response and appears responsible for *H-2* restricted T-B collaboration. Because its activity, and hence, *H-2* restriction, can be replaced by a factor(s) found in Con A supernate, we have proposed that this cell functions in the delivery of a nonspecific signal to the B lymphocyte, during direct cell-cell contact. In the experiments reported here, Con A SN was also able to restore the activity of anti-Ia treated helper T cells, although Con A SN alone was not sufficient for the response (38, 39). This lends additional weight to our hypothesis and suggests that the helper cell involved in direct T-B interaction is Ia^+, presumably $I\text{-}A^+$. The other type of helper T cell, the activity of which is not replaceable by Con A SN (37–39) did not appear to be Ia^+ with our antisera. This differential expression of Ia may be another reason for the discrepancies in the literature concerning *I*-region products on helper T cells. Depending on the balance of activities of one T cell or the other in a given experimental system (i.e. which one is limiting for the antibody response being measured), an effect of anti-Ia treatment may or may not be observed. We have not yet examined the question of *I-J* expression on helper T cells for B-cell responses, though it would be of interest to do so, because Tada et al. (46) have described two types of helper T cells which synergize in anti-TNP-protein responses. They have shown that one of these T cells, which acts in a nonspecific way to stimulate B-cell responses, is $I\text{-}J^+$, and is probably analogous to the $I\text{-}A^+$ helper cell in our experiments.

Our results also showed that helper T cells for cytotoxic lymphocytes generated against allogeneic *H-2*, were Ia^+, though we have not yet defined the region of *I* expressed. Because the studies of Plate (47, 48) have also suggested that such helpers bear Ia antigens and are replaceable by a nonspecific factor(s), perhaps this is evidence that one of the helper T cells for B-cell responses is of the same lineage as the helper T cell for cytotoxic induction. These results are in contrast with those of Lonai (49) who found that treatment of responder cells with conventional A.TH anti-A.TL serum had no effect on the development of cytotoxic lymphocytes. This is most likely a result of differences between antisera as already mentioned.

In agreement with other investigators (17, 19, 48, 49), we could find no evidence for Ia expression on alloreactive cytotoxic effector cells. Moreover, using techniques that enabled us to distinguish helper cells from cytotoxic precursors, we determined that this precursor is Ia^- as well. In light of this result, the experiments of Zinkernagel et al. (29) and von Boehmer et al. (21) suggesting *I*-region-controlled interaction between helper T cells and cytotoxic T cells are difficult to interpret. Perhaps the *I*-region control detected in their experiments reflected the presence of *I*-region products on

the helper T cells concerned, rather than the cytotoxic T cells themselves. If so, perhaps the interaction under *I*-region control was between helper T cells and some inducer T cell (50). However, the situation is far from clear since certain tumor-specific murine cytotoxic T cells (51) as well as human cytotoxic cells activated by a cell-free mediator (52) have recently been shown to express Ia or Ia-like antigens. Thus, both the manner of activation and the type of target recognized may influence the ultimate expression of Ia. It may thus be worthwhile to comment on the recent report of Perry et al. (53), who were able to inhibit syngeneic tumor rejection by in vivo administration of anti-Ia antibodies directed against the *I-A* region of the host. Our results would suggest that an additional possibility for the interference in effector T-cell function which they observed, may be related to direct inhibition at the level of the helper T cell. Because anti-*I-J* antiserum has similarly been used in vivo to eliminate T-suppressor cells (54), it may eventually be possible, using highly specific anti-Ia sera, to selectively modulate the immune response at any given level (MØ, B, T suppressor, T helper, and effector).

Although our results indicate that functional T cells bear Ia antigens, we have not addressed the question of whether or not these antigens are endogenous to the cell or acquired during interactions with other cell types. T cells have been shown to acquire Ia from MØ in culture (55), or from stimulator cells during MLC (56) reactions. However, we have detected Ia on purified T cells even after extended culture in the absence of other cell types. Additionally, if Ia were acquired, then this acquisition would have to depend on the function of the T cell because certain helper cells are Ia^+, whereas others, and cytotoxic cells, are Ia^-. These possibilities are currently under study.

Taken together, our results suggest that T-cell Ia antigens, whether endogenous to the cell or acquired by previous contact, play an important role in cell regulation. Both the helper T cell involved in *H-2*-restricted interactions with B cells and the helper T cell involved in similar interactions with cytotoxic precursors were shown to express *I*-region determinants. Both T cells are also known to elaborate nonspecific signals responsible for cell activation. Ia molecules on the helper cell may serve as a means of specifically delivering those signals to the appropriate cell type, or they may be responsible for the recognition of the helper cell itself by amplifier (inducer) T cells. Only further experimentation will help to clarify these points.

Summary

We have examined the expression of *I*-region antigens on functional subpopulations of murine T cells. A.TH anti-A.TL (anti-I^k, S^k, G^k) alloantiserum was raised by immunization of recipients with concanavalin A (Con A) stimulated thymic and peripheral T-cell blasts. In contrast to similar antisera made by conventional methods, the anti-Ia blast serum was highly cytotoxic for purified T lymphocytes. Moreover, it reacted in a specific fashion with T cells having particular functions.

Treatment of keyhole limpet hemocyanin (KLH)-primed B10.A ($H\text{-}2^a$) T cells with this antiserum plus complement resulted in the elimination of helper activity for B-cell responses to trinitrophenyl-KLH. Inhibition was shown to be a result of the selective killing of one type of helper T cell whose activity could be replaced by a factor(s) found in the supernate of Con A-activated spleen cells. A second type of helper cell required for responses to protein-bound antigens appeared to be Ia^-. By

absorption and analysis on *H-2* recombinants, at least two specificities were detectable on helper T cells; one mapping in the *I-A* subregion and a second in a region(s) to the right of *I-J*. In addition, the helper T cell(s) involved in the generation of alloreactive cytotoxic lymphocytes was shown to be Ia^+, whereas cytotoxic effector cells and their precursors were Ia^- with this antiserum.

These results provide strong evidence for the selective expression of *I*-region determinants on T-cell subsets and suggest that T-cell-associated Ia antigens may play an important role in T-lymphocyte function.

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