Brief Definitive Report

EXPRESSION OF INDIVIDUAL Ia SPECIFICITIES ON T AND B CELLS

I. Studies with Mitogen-Induced Blast Cells*

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The *Ir* genes were originally postulated to have a role in antigen recognition by the T lymphocyte (1). Antisera were produced in *Ir*-incompatible recombinant strain combinations in an attempt to identify the *Ir* gene product serologically (2, 3). The antisera reacted weakly or not at all with normal thymocytes, and their reaction with normal spleen and lymph node cells was found to be predominantly against B cells (4, 5). This result was unexpected, and the association of these antigens (later designated Ia, reference 6) with the *Ir* gene products was questioned. Further data suggested that at least some Ia antigens are also expressed on T cells (7). More recently, it seems evident that *Ir* gene expression occurs in both T and B cells (8) and in some cases two *Ir* genes appear to be involved in the control of a specific response (9, 10). It has been suggested that one of these genes might control a T-cell "signal" and the other a B-cell acceptor for the signal. Furthermore, it has been shown that optimal collaborations between T and B cells occur only in syngeneic combinations and genes determining this interaction were shown to map in the *I* region (11). Since the Ia antigens are thus implicated as possible candidates for the molecular recognition between T and B cells, we undertook to investigate further the expression of individual Ia specificities on the two cell populations utilizing mitogen-induced blast cells from spleen, lymph node, and thymus.

Materials and Methods

All mice used in this study were raised in our colony at the University of Michigan. Anti-Thy-1.2 and anti-Ly.4.2 were kindly provided by Dr. J. A. Frelinger. A battery of anti-Ia sera (Table I) detecting Ia specificities 1–10 was produced in our laboratory, as previously described (2). The dye exclusion microcytotoxic test and ^{51}Cr-release cytotoxic assay were performed as previously described (2). In vitro absorptions were done by incubating 50 λ of antiserum, diluted to give 80% of maximum cytotoxicity, with decreasing numbers of lymphocytes, starting from 50×10^6 cells. Antibody was eluted from the washed, sensitized cells by incubating for 20 min at room temperature in saline acidified to pH 3.0 with 0.1 N HCl. The suspension was centrifuged and the supernate decanted and neutralized (pH 7.0) with 0.004 N NaOH (12).

Lymphoid cell cultures were prepared in the following way. Spleens, lymph nodes, and thymuses were removed from CO_2-asphyxiated animals and transferred to chilled Dulbecco's medium (Ca^{++} and Mg^{++} free). Care was taken to avoid parathymic nodes in the thymus by liberating the organ from the capsular tissue before preparation of the cell suspension. Tissues were gently homogenized with a loose-fitting teflon pestle and rapidly filtered through a nylon

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TABLE I
List of Antisera Used

Antisera			Specificities detected	
Reagent no.	Recipient	Donor	H-2	Ia
1	A.TH	A.TL	—	1,2,3,7
2	A.TL	A.TH	—	4,5,[anti-T1a]
3	[B10.A(4R) × HT1]F_1	B10.A	—	6
4	[HTH × C3H.Q]F_1	C3H.B10	33	8
5	[A × B10.D2]F_1	B10.A(5R)	33	9
6	[A.CA × B10.HTT]F_1	A.TL	—	2,3
7	[A.BY × B10.HTT]F_1	A.TL	—	1,2
8	[A.TL × A.TFR 3]F_1	A.TH	—	4
9	[B10.A(4R) × HT1]F_1	B10.A(5R)	—	7
10	A.AKR	A.AL	Thy-1.2	
11	AKR	C3H	Thy-1.2, ... ,	
12	[BALB/c × SWR]F_1	B10.D2	Ly-4.2	

wool-packed syringe. The suspensions were pelleted at 600 *g* for 10 min, washed twice, and diluted to 15 × 10^6 viable cells per ml of culture medium consisting of 25 mM HEPES-buffered RPMI-1640, containing 2.5% fetal calf serum, antibiotic-antimycotic preparation (Gibco, Grand Island Biological Co., Grand Island, N. Y.), 30 μM 2-mercaptoethanol, and 20 mM L-glutamine. Suspensions were aliquoted, 7.5 ml per petri dish (60 × 15 mm, Falcon Plastics, Oxnard, Calif.), and pulsed with mitogen. Conconavalin A (Con A) (A grade, Calbiochem, Los Angeles, Calif.) was used at 5 μg/ml final concentration, lipopolysaccharide (LPS) (310-25 Bacto *Escherichia coli* OSS: BS, Difco Laboratories, Detroit, Mich.) at 100 μg/ml, and pokeweed mitogen (PWM) (Gibco N536) at 1:50 dilution of reconstituted lyophilized preparation. Cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2.

Results and Discussion

Splenic Blasts. LPS-stimulated spleen cells yielded 80–100% blasts by visual inspection at termination of the culture. Viable blast cells were separated from dead cells by layering on Ficoll-isopaque. The results obtained when blasts of haplotypes $H\text{-}2^k$ and $H\text{-}2^s$ were typed with a battery of anti-Ia sera in the ^{51}Cr cytotoxic release assay are shown in Table II. Anti-H-2, anti-Thy-1.2, and anti-Ly.4.2 sera were tested in parallel as controls. LPS-stimulated blasts reacted with anti-Ia sera at titers similar to those with normal spleen cells, but usually gave 10–20% higher ^{51}Cr release.

Con A-stimulated splenic lymphocytes gave approximately 60–70% lysis with anti-Thy-1 serum, suggesting that they were enriched in T cells. Anti-Ia sera gave variable reactions with these blasts. Against B10.K (Ia^k) targets, all anti-A.TL (Ia^k) sera gave good reactions. B10.S Con A blasts gave strong reactions with anti-Ia.4,5, but very weak reactions with antisera to Ia.9. These results suggested that Ia antigens are expressed not only on splenic LPS (B-cell) blasts, but also on splenic Con A blasts (which are T cells), although not as strongly. In general, homologous antisera gave stronger reactions than antisera detecting cross-reacting specificities.

Thymic Blasts. Thymic lymphocytes, prepared with care to exclude parathymic lymph node cells, were stimulated with PWM, Con A, and LPS. PWM and Con A-stimulated thymocytes yielded 70–100% blasts. LPS failed to stimulate detectable blast formation in thymocyte cultures, indicating lack of B-cell

TABLE II

*Cytotoxic Titers (^{51}Cr Assay) of Mitogen-Induced Spleen Blasts**

Antisera		Target Cells			
		B10.K ($H\text{-}2^k$)		B10.S ($H\text{-}2^s$)	
No.	Antibody	(Ia.1,2,3,7,15) LPS	Con A	(Ia.4,5,9,12) LPS	Con A
1	Anti-Ia. 1,2,3,7	>160(++)	>160(+)	—	—
6	" 2,3	>160(++)	>160(+)	—	—
7	" 1,2	>160(++)	>160(+)	—	—
9	" 7	>160(++)	>160(±)	—	—
2	" 4,5	—	—	>160(++)	>160(+)
5	" 9	—	—	>160(++)	20(±)
10	" Thy-1.2	0	>160(++)	0	>160(++)
12	" Ly-4.2	>160(++)	20(±)	>160(++)	20(±)

(−), not tested; (++), 20–40% release above base line; (+), 10–20% release above abase line; (±), 5–10% release above base line.

* ^{51}Cr release assay-reciprocal of titer.

contamination. Anti-Thy-I reacted with 70–90% of normal thymocytes and of PWM and Con A-stimulated blasts. Normal thymocytes gave only trace reactions with anti-Ia sera. LPS-treated thymocytes failed to react with anti-Ia sera, again suggesting that there were no contaminant B cells in the thymic cell population. The results of these tests are shown in Table III. B10.A($H\text{-}2^a$) blasts gave strong reactions with antisera to Ia.1,2,3, and 7 and a weak reaction with anti-Ia.6. B10.S($H\text{-}2^s$) blasts gave strong reactions for Ia.4,5, but a weak reaction for Ia.9. B10.D2($H\text{-}2^d$) blasts gave strong reactions with anti-Ia.6 and 7 and a weak reaction for Ia.8. These results again suggest that Ia antigens detected by cross-reacting antisera give weak reactions. However, there is no doubt that Ia antigens are expressed on T cells and are easily detectable on mitogen-activated T cells.

The reactions of [B10.A × B10.S]F_1 ($H\text{-}2^a/H\text{-}2^s$) thymic blasts with anti-Ia and control sera are shown in Fig. 1. Normal serum gave about 10% lysis, compared to anti-H-2.19, which gave 100% lysis and a titer greater than 2,560. Anti-Thy-1.2 (A.AL [Thy-1.1] anti-A.AL [Thy-1,2]) gave 90% lysis with a titer of about 320. Anti-Thy-1.2 produced in a noncongenic combination (AKR anti-C3H) gave a titer greater than 2,560 with 100% lysis. A.TH anti-A.TL (Ia^k) and A.TL anti-A.TH (Ia^s) sera gave 65–75% cell lysis. A mixture of the two antisera gave slightly higher cell lysis, but not sufficient to suggest allelic exclusion of the Ia antigens detected. Anti-Ia.9, antiserum (B10.D2 × A)F_1 anti-B10.A(5R), gave a maximum of only 50% lysis, again indicating the weaker reactivity of cross-reacting antibodies.

Lymph Node Blasts. Lymph node lymphocytes were stimulated with Con A and LPS. Lymph node Con A blasts reacted very much like thymus Con A blasts, while lymph node LPS blasts reacted like splenic LPS blasts. Both types of blasts expressed all of the Ia specificities studied.

Absorptions. Cross-absorptions were performed with Con A-stimulated thymocytes (T-Con A), Con A-stimulated lymph node cells (LN-Con A), and LPS stimulated spleen cells (S-LPS) using strain A.TL mice and antiserum A.TH anti-A.TL (Anti-Ia.1,2,3,7) (Fig. 2). LN-Con A cells (T blasts) and S-LPS (B

TABLE III

*Cytotoxic Titers (Dye Exclusion) of Mitogen-Induced Thymus Blasts**

Antisera		Target cells								
		B10.A($H\text{-}2^a$)			B10.S($H\text{-}2^s$)			*B10.D2*($H\text{-}2^d$)		
No.	Antibody	LPS	(Ia.1,2,3,6,7,15) PWM	Con A	LPS	(Ia.4,5,9,12) PWM	Con A	LPS	(Ia.6,7,8,11,16) PWM	Con A
1	Anti-Ia. 1,2,3,7	0	640(++)	640(++)	—	—	—	0	>2,560(++)	>2,560(++)
2	" 4,5,T1a	0	>2,560(++)	>2,560(++)	0	>2,560(++)	>2,560(++)	—	>2,560(++)	>2,560(++)
8	" 4	—	—	—	0	>2,560(++)	>2,560(+)	—	—	—
6	" 2,3	0	>2,560(++)	>2,560(++)	—	—	—	—	—	—
7	" 1,2	0	160(+)	160(+)	—	—	—	—	—	—
3	" 6	0	40(+)	40(+)	—	—	—	0	640(++)	160(+)
9	" 7	0	160(+)	160(+)	—	—	—	0	>2,560(++)	160(+)
4	" 8	—	—	—	—	—	—	0	40(+)	40(+)
5	" 9	—	—	—	—	20(+)	160(+)	—	—	—
10	" Thy-1.2	0	640(++)	640(++)	0	640(++)	640(++)	0	640(++)	640(++)
11	" "	0	2,560(++)	2,560(++)	0	2,560(++)	2,560(++)	0	2,560(++)	2,560(++)
12	" Ly-4.2	0	40(±)	40(±)	0	40(±)	40(±)	0	40(±)	40(±)

(−), not tested; (++), 70–90% cell lysis; (+), 50–70% cell lysis; (±), 30–50% cell lysis.

* Reciprocal of titer.

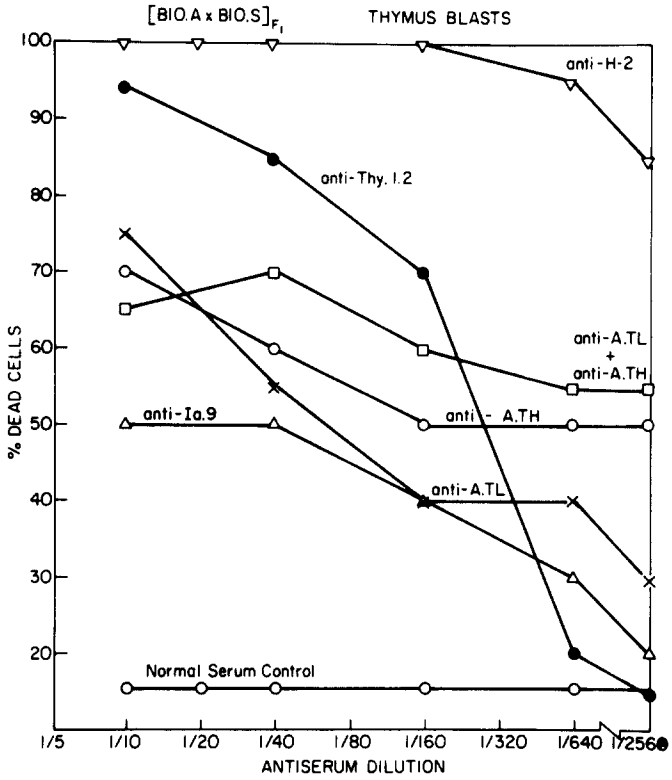

FIG. 1. Target cell [B10.A × B10.S]F_1 thymus blasts. Direct cytotoxic reaction with anti-H-2, anti-Thy-I and anti-Ia.

blasts) absorbed completely for each other, suggesting that the Ia specificities expressed on those two populations of cells are very similar or identical. T-Con A cells absorbed the antiserum less efficiently and less completely for LN-Con A and S-LPS blast target cells. This could be interpreted in two ways: (*a*) T-Con A blasts have a much lower density of one or more of the Ia specificities involved and (*b*) T-Con A blasts lack one or more of the specificities defined by the A.TH anti-A.TL sera. Conceivably, there could be two closely linked genes specifying two discrete antigens, one predominantly on T cells, the other predominantly on B cells. The two antigens could have different functional roles, e.g. one as the T-cell signal and the other as the B-cell receptor for that signal.

Absorption-Elution. Antiserum A.TH anti-A.TL was absorbed onto LPS-stimulated spleen cells from B10 (Ia.3), A.CA(Ia.1), and B10.D2(Ia.7). The lymphocytes were washed, and the antibody was eluted from the cells. The eluted antibodies reacted strongly with Con A-induced thymus blasts from the donor A.TL, suggesting expression of Ia.1,3, and 7 on both T and B blasts. The eluted anti-Ia.1 and anti-Ia.3 reacted only weakly with A.CA and B10 thymus-Con A blasts, again suggesting strong affinity of the antibody against T cells of the homologous strain compared to the cross-reacting strain. Anti-Ia.7 did not show such a difference, suggesting a common origin of Ia.7 in $H\text{-}2^k$ and $H\text{-}2^d$.

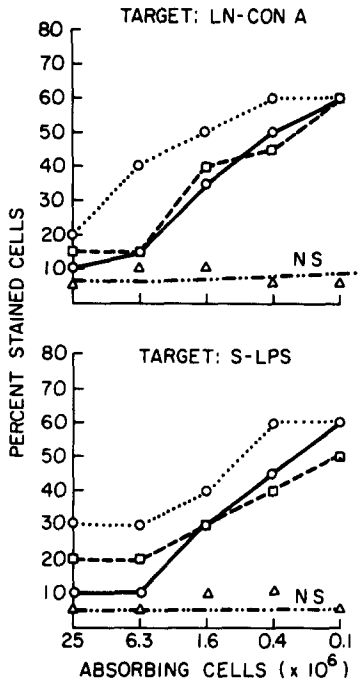

FIG. 2. A.TH anti-A.TL absorbed with A.TL Con A thymus blasts (●····●), A.TL Con A lymph node blasts (O——O), and A.TL spleen LPS blasts (□- - -□) and tested against lymph node-Con A blasts and spleen LPS blasts.

There is a possibility that mitogen-stimulated blast cells might express some viral protein. Mouse H-2 and Ia alloantisera are known to contain antiviral activity (P. Klein, personal communication). Therefore, the anti-Ia sera could conceivably be reacting with a viral antigen on the blast cells rather than with Ia antigens. There are several lines of evidence against this possibility: (*a*) Negative control antisera which are known to have antiviral activity did not give lysis of blast cells. (*b*) Antibody eluted from normal spleen cells lacks viral activity, but gave typical lysis with appropriate blast targets. (*c*) Absorption with thymus blasts removes antibody activity against normal spleen cells, which should be due to specific anti-Ia antibodies, since normal spleen cells do not express viral antigens.

Summary

Ia specificities 1–10 were detected on LPS-stimulated splenic lymphocytes and on Con A-stimulated spleen, lymph node, and thymus blasts by direct cytotoxic tests. Since Ia antigens are not readily detectable on resting thymocytes, our results suggest that T cells require some signal before they exhibit full expression of Ia specificities. Absorption-elution studies indicated that most of the Ia specificities detected on T and B cells may be identical. Ia antigens detected by homologous antisera gave much stronger reactions than those detected by cross-reacting antisera.

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