Transplant. Rev. (1976), Vol. **30**
Published by Munksgaard, Copenhagen, Denmark

Expression of Ia Antigens on T and B Cells and their Relationship to Immune-Response Functions

JOHN E. NIEDERHUBER & JEFFREY A. FRELINGER

MLR	= mixed lymphocyte reaction
GVH	= graft versus host reactivity
H-2	= murine major histocompatibility complex
Ia	= immune response region associated antigens
Ir	= immune response
T cell	= thymus-derived lymphocyte
B cell	= bursa-equivalent-derived-lymphocyte
MBLA	= mouse-specific B-lymphocyte antigen
LPS	= lipopolysaccharide
Con-A	= concanavalin A
PHA	= phytohemagglutinin
PFC	= plaque-forming cells
SRBC	= sheep red blood cells
BRBC	= burrow red blood cells

The major histocompatibility gene complex H-2 of the mouse is composed of five regions, *K, I, S, G,* and *D* (David et al. 1975). Genes of the *H-2* complex and especially the *I* region control a variety of significant biological functions such as the level of antibody response to certain antigens (McDevitt & Sela 1965, McDevitt & Benacerraf 1969, Lieberman et al. 1972) and the susceptibility for resistance to certain viruses (Lilly 1966, 1972, Tennant & Snell 1968, Lilly & Pincus 1973). Furthermore, the *I* region has been associated with such immunologic functions as mixed lymphocyte reaction (MLR) (Bach et al. 1972, Meo et al. 1973a, b), graft versus host reactivity (GVH) (Klein & Park 1973), and cell-cell interaction (Katz et

Departments of Microbiology, Human Genetics, and Surgery, The University of Michigan Medical Center, Ann Arbor, Michigan 48109, and Department of Microbiology, University of Southern California Medical School, Los Angeles, California.

al. 1975), all of which require that the products of *I*-region genes be expressed as membrane antigens.

In 1973, several investigators (David et al. 1973, Götze et al. 1973, Hauptfeld et al. 1973, Sachs & Cone 1973, Hämmerling et al. 1974) reported the serologic detection of *I*-region gene products, later to be designated Ia for *I*-region-associated antigens (Shreffler et al. 1974). To date, 21 specificities have been identified and many of these have been mapped to specific subregions, namely I-A and I-C (Shreffler & David 1975, David et al. 1976). The Ia molecules are biochemically distinct from H-2 antigens (Cullen et al. 1974) and cap independently of *H-2K, H-2D* and Ig molecules on the cell membrane (Unanue et al. 1974). The molecules have been characterized as glycoproteins (Cullen et al. 1976) with molecular weights ranging from 25,000 to 33,000 daltons. Cullen et al. (1974, 1976) have reported that specificities mapping in either subregion I-A or subregion I-C are expressed on different molecules but specificities mapping within the same subregion appear to be associated on the same molecule.

The cellular and molecular mechanisms of the *I*-region-linked immunologic phenomena are not known, nor is it known if they are controlled by distinct genes or are phenotypic expressions of the same gene. An understanding of Ir gene control, MLR, GVH, antigen recognition and T cell–B cell or T cell-macrophage interaction is basic to immunology and cell biology. This review describes efforts to determine the tissue distribution and the relationship of the first serologically detected I-region product, the Ia antigens, to these immune functions.

METHODOLOGY

The experimental methods for the studies reported here have been described in the original manuscripts (Frelinger et al. 1974, 1975, Niederhuber et al. 1975, 1976). The congenic mice were maintained in either Dr. Niederhuber's or Dr. Shreffler's colony at The University of Michigan and are described in terms of the H-2-allelic origin of their H-2 regions and their Ia specificities in Table I.

Briefly, anti-Ia sera and specific anti-H-2 sera were prepared as described by Shreffler & David (1975) and extensively characterized by cytotoxic and absorption tests. For blocking experiments, cells were incubated with antiserum without complement for 30 minutes, washed extensively by centrifugation in media and then used in culture. In lysis experiments, cells were treated by a staged procedure, first with antibody and then with complement. Equal numbers of viable cells resistant to the antibody and com-

TABLE I

Distribution of H-2 regions and Ia specificities

Strain		Haplotype of H-2 regions						Ia specificities
			I					
		K	I-A	I-B	I-C	S	D	
A.TL	(t1)	s	k	k	k	k	d	1, 2, 3, 7, 15, 17
A.TH	(t2)	s	s	s	s	s	d	4, 5, 9, 12, 17
B10.HTT	(t3)	s	s	s	k	k	d	4, 5, 7, 9, 12, 17
B10.S(9R)	(t4)	s	s	s	d	d	d	4, 5, 6, 7, 9, 12, 17
B10.A(2R)	(h2)	k	k	k	d	d	b	1, 2, 3, 6, 7, 15, 17
B10.A(4R)	(h4)	k	k	b	b	b	b	1, 2, 3, 17
HTI	(i)	b	b	b	b	b	d	3, 8, 9, 15
B10.A(3R)	(i3)	b	b	b	d	d	d	3, 6, 7, 8, 9, ?15
B10.A(5R)	(i5)	b	b	b	d	d	d	3, 6, 7, 8, 9, 15
B10.BR	(k)	k	k	k	k	k	k	1, 2, 7, 15, 17
B10.S	(s)	s	s	s	s	s	s	4, 5, 9, 12, 17
B10.D2	(d)	d	d	d	d	d	d	6, 7, 8, 11, 16
B10	(b)	b	b	b	b	b	b	3, 7, 8, 15

List of antisera used

Recipient	Donor	Specificities detected		
		K/D	I	Ia
A.TH	A.TL	—	I-A^k, I-B^k, I-C^k	1, 2, 3, 7, 15
A.TL	A.TH	—	I-A^s, I-B^s, I-C^s	4, 5, 9, 12
[B10.A(4R) × 129]	B10.A(2R)	—	I-A^k, I-B^k, I-C^d	6, 7, 15
[ABY × B10.HTT]	A.TL	—	I-A^k, I-B^k	1, 2
[B10 × A.TH]	A.TL	—	I-A^k, I-B^k, I-C^k	1, 2
[B10 × HTI]	B10.A(5R)	—	I-C^d	6, 7
[A × A.AL]	A.TL	K^s (H-2.19)		
A.TL	A.AL	K^k (H-2.23, H-2.11)		
[B10 × AKR.M]	B10.A	D^d	?I-C^d	not detected

plement lysis were then used in culture. Most humoral response assays were made in small dishes according to the method of Mishell & Dutton (1967). Proliferative responses were assayed in microtiter plates. A pulse of tritiated thymidine was added 12–18 hours before harvesting.

RELATIONSHIP OF Ia TO DEFINABLE SUBPOPULATIONS

With the first reports describing the Ia antigens, controversy existed as to

their expression on T cells. For example, David et al. (1973), Götze et al. (1973), and Hauptfeld et al. (1973) supported the presence of Ia antigens on T cells while Sachs & Cone (1973) and Hämmerling et al. (1974) reported that Ia antigens were the exclusive property of B cells. In a previous report (Frelinger et al. 1974), a direct microcytotoxic assay with medium 199 was used to demonstrate the absorption of anti-Ia antibodies by thymocytes and the direct cytotoxicity of cortisone-resistant thymocytes. This more sensitive assay was performed in two stages, permitting the use of higher concentrations of rabbit complement.

These findings supported earlier observations (David et al. 1973) that anti-Ia sera at high concentrations had a maximum kill of 70 % of lymph-node cells and 50 % of spleen cells. These levels of cytotoxicity required lysis of at least a portion of T cells (Figure 1). Batches of the broadly specific A.TH anti-A.TL serum and A.TL anti-A.TH serum con-

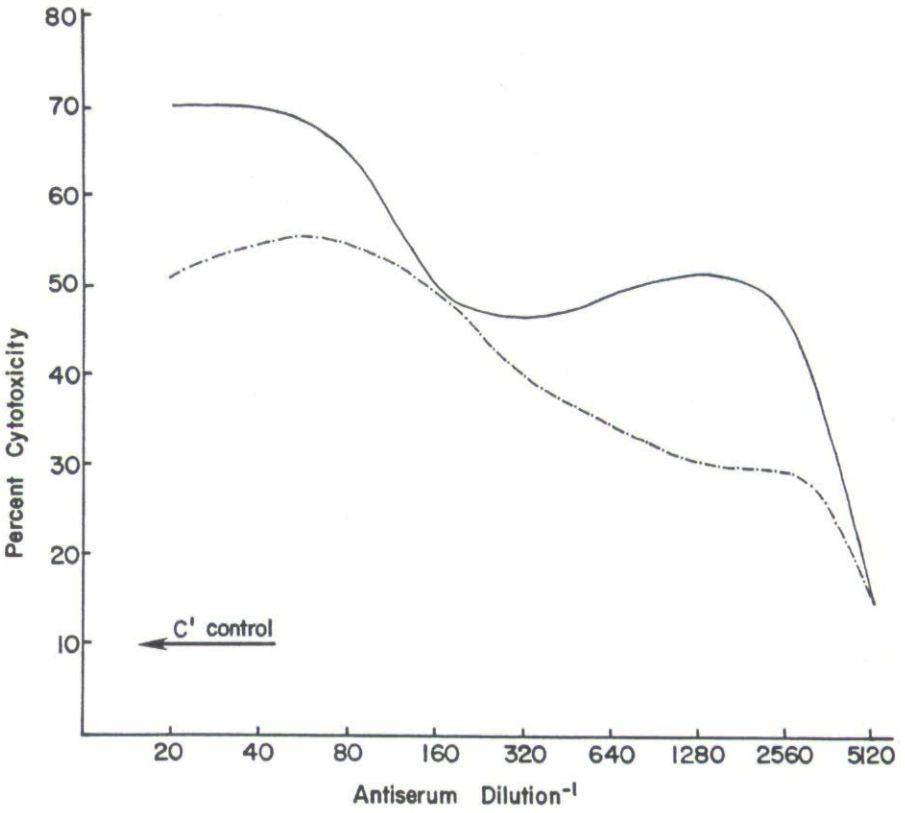

Figure 1. Direct-dye exclusion cytotoxic test of A.TH anti-A.TL serum tested with A.TL lymph-node target cells (——) and spleen lymphocyte target cells (–·–·–). The complement plus normal serum control was 10 % or less.

sistently demonstrate a biphasic cytotoxic titration curve when spleen or lymph-node cells are used as target cells.

A variety of broadly specific and monospecific anti-Ia sera were found to kill nude mouse-spleen cells and anti-Thy-1-resistant splenic B cells. Anti-Ia serum and complement also lysed antibody-forming cells. In addition, immunofluorescent studies by Unanue et al. (1974) and immunoferritin-labeling studies (Schultz et al. 1975), clearly indicated that the Ia-antigen molecule was expressed on the majority of B cells. The authors have identified a very small population of anti-Thy-1 and anti-Ia-negative splenic B cells which do not respond to the mitogen LPS. This subset of B cells has not been associated with any immune function. Recently Press (personal communication) has described a system for examining precursors of antibody-forming cells. She has evidence that Ia is expressed on IgG but not on IgM precursors of antibody-forming cells.

Efforts to demonstrate Ia antigens on T cells have been more difficult. Microcytotoxic tests using thymocytes were not consistent, with 20 % killing in some tests and no killing in others. Cortisone-resistant thymocytes, prepared by intraperitoneal injection of 10 mg cortisone 48 hours before testing (Cohen & Claman 1971), consistently had levels of 30–50 % cytotoxicity when treated with anti-Ia sera and complement. It was interesting that with these cells as targets the titer remained the same as with lymph-node or spleen cells, but the biphasic aspect was no longer present.

It was also found that normal thymus cells could absorb anti-Ia activity; however, they were much less efficient than lymph-node or spleen cells. The presence of anti-T-cell activity was further supported by first absorbing the anti-Ia sera *in vivo* in BALB/c-nu/nu mice. The resulting serum did not kill nude cells but was cytotoxic for BALB/c lymph-node cells and cortisone-resistant thymocytes.

The T- and B-cell markers, identified by anti-Thy-1 serum and anti-MBLA serum, were also used to test for Ia antigen distribution. In these cytotoxic tests the effects of mixtures of class-specific antisera and anti-Ia serum were compared with the effect of each antiserum tested separately on a given cell population. When anti-MBLA serum and anti-Ia serum (1/1000 dilution) were combined to test against lymph-node target cells, there was a marked increase in cytotoxicity from 40 % (anti-MBLA) to more than 90 % (both). When anti-Thy-1 serum was combined with anti-Ia serum, no increased killing occurred unless the anti-Ia serum was used at a concentration on the high plateau of the titration curve. With concentrated anti-Ia serum and anti-Thy-1 serum, the killing was increased from 60 % to more than 90 %.

Although it has been more difficult to detect Ia antigens on T cells, the evidence for Ia-positive T cells can be summarized as follows:

1) anti-Ia serum is cytotoxic for 30–50 % of Thy-1-positive cortisone-resistant thymocytes (Frelinger et al. 1974);

2) anti-Ia activity can be absorbed, although less efficiently by thymocytes, and when absorbed with nude (B cells) spleen cells, activity against lymph-node cells remains (Frelinger et al. 1974);

3) some Thy-1-positive tumor lines are equally sensitive to anti-Ia sera (David, personal communication);

4) Ia-positive thymocytes have been demonstrated using the fluorescence-activated cell sorter (Fathman et al. 1975);

5) Con-A-reactive spleen cells and Con-A-reactive nylon-wool-purified splenic T cells exist as an Ia-positive subpopulation (Niederhuber et al. 1976);

6) Con-A-activated thymocyte and splenic blasts are sensitive to anti-Ia antibodies and complement (Nabholz et al. 1975, David et al. 1976);

7) an anti-Ia immunoprecipitation assay of thymus cells reveals a 30,000 mol.wt. peak (Goding et al. 1975, B. Schwartz, personal communication);

8) soluble T-cell-factor activity can be absorbed by anti-Ia sera (David, personal communication).

The small number of T cells which are Ia positive (perhaps only 20 % of the peripheral T cells) and the relatively low density of the Ia antigen on the membrane are, in part, explanations for some of the difficulty in detecting Ia-positive T cells. Further work is necessary to answer questions concerning the possible existence of Ia specificities unique to T cells.

THE PRESENCE OF Ia ON MITOGEN-SENSITIVE LYMPHOCYTES

Lymphocyte subclasses can be further characterized by their sensitivity to specific mitogens. These responses have been used to determine the relationship of Ia antigens to T-cell mitogens Con-A, PHA, Leukoagglutinin (a highly purified form of PHA) and LPS, a B-cell mitogen. When spleen cells were treated with anti-Ia serum and rabbit complement to remove all Ia-positive cells, the remaining viable cells failed to respond to Con-A and LPS but responded normally to PHA and Leukoagglutinin (Figures 2 and 3).

To eliminate the possible involvement of an Ia-positive T cell in the proliferative response to LPS, spleen cells were first treated with anti-Thy-1.2 serum and rabbit complement. The anti-Thy-1.2 serum treatment was repeated a second time and the remaining viable cells were more than

95 % B cells as determined by surface markers. Furthermore, they were no longer sensitive to T-cell mitogens. These splenic B cells were then treated with anti-Ia serum and complement and the Ia-negative B cells cultured with LPS. The remaining viable cells constituted only 8 % of the original spleen-cell population and did not respond to LPS, confirming that LPS-reactive spleen cells are Ia-positive B cells (Figure 2).

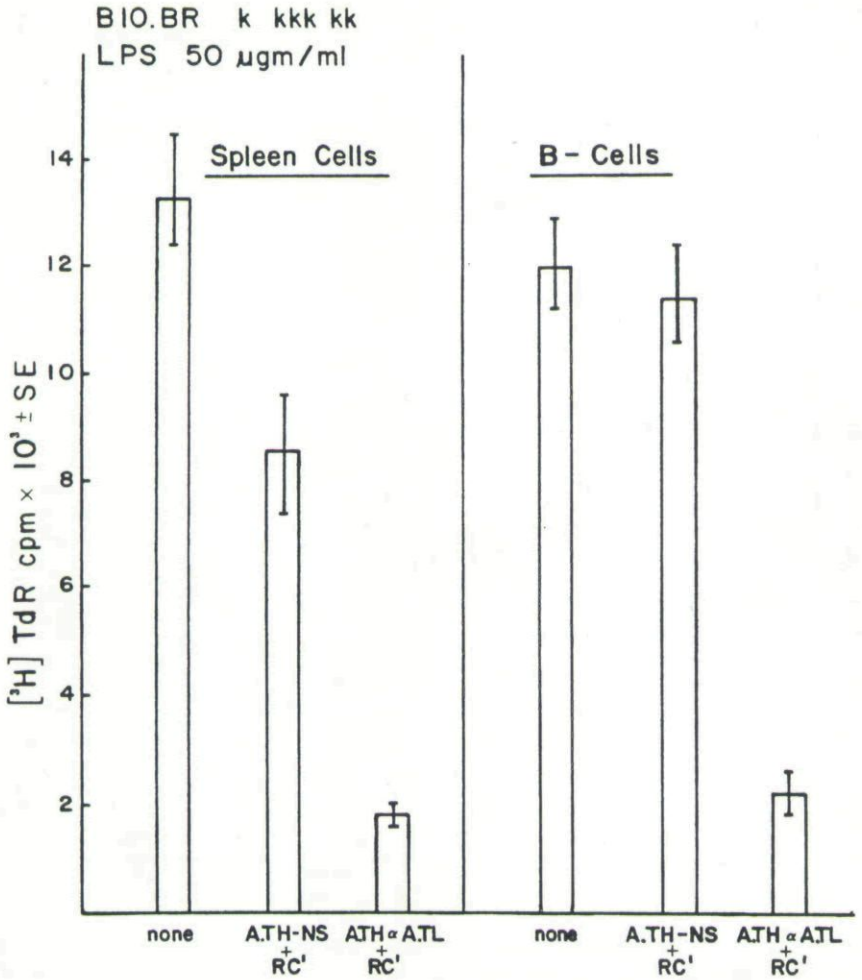

Figure 2. Proliferative response of B10.BR spleen cells to 50 μg/ml LPS. Spleen cells or anti-Thy-1.2 serum plus complement-resistant splenic B cells were treated either with A.TH normal serum plus rabbit complement or with A.TH anti-A.TL (anti-Ia^k) serum and complement. Equal numbers of viable cells (5×10^5/well) were cultured for 48 hours with LPS. Each bar represents mean of four cultures ± S.E. Reproduced from *J. Immunol.* **115**, 1672, 1975.

Experiments with Con-A and Leukoagglutinin were also performed using nylon-wool-purified splenic T cells. These cells are greater than 90 % Thy-1-positive and do not respond to LPS. When the Ia-positive cells were eliminated from this splenic T-cell population, the remaining cells did not respond to Con-A but had a normal or slightly enhanced response to PHA and Leukoagglutinin (Table II). The Con-A-sensitive T cell is, therefore, Thy-1 positive, Ia positive, and distinct from the Thy-1 positive but Ia-negative PHA-reactive T cell.

If the Ia surface molecule were in any way associated on the cell mem-

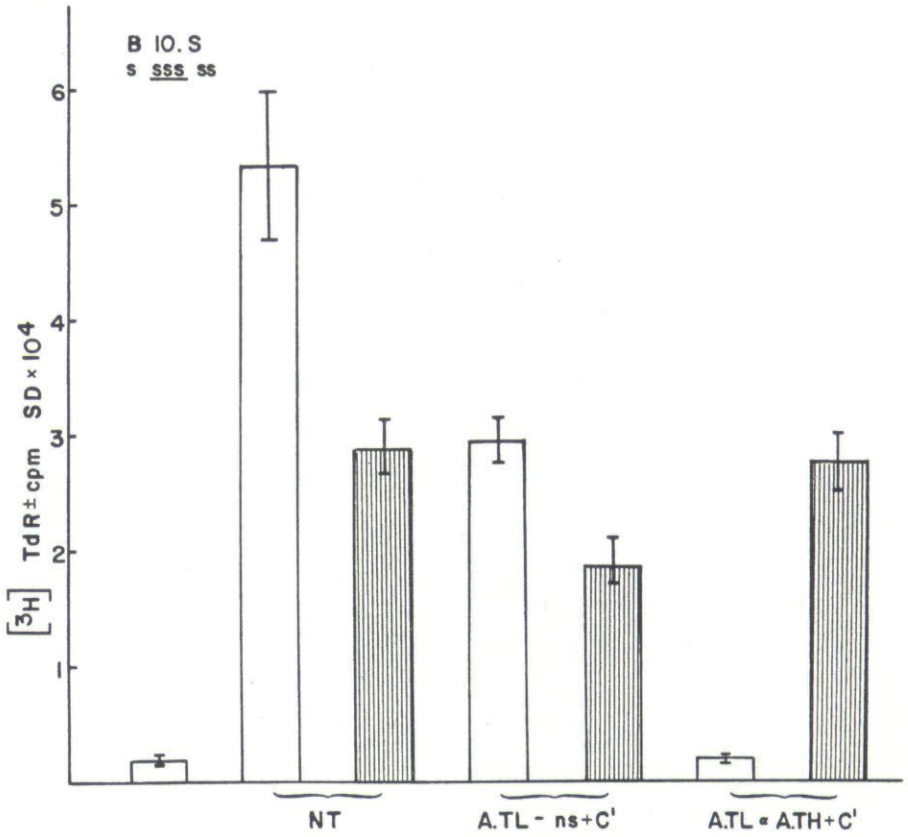

Figure 3. Con-A and Leukoagglutinin-proliferative response of A.TL anti-A.TH (anti-Ia^s) serum plus rabbit-complement-treated B10.S (*H-2*^{s}) spleen cells. NT was the response of cells exposed only to culture media; A.TL-ns + C′ was the response of cells treated with normal serum. Equal numbers of viable cells (5×10^5/well) were cultured for 72 hours with Con-A 1 μg/ml (open bar) or Leukoagglutinin 1 μg/ml (shaded bars). Each bar represents the mean of four cultures expressed as CPM ± SD. The open bar at the left of graph is the background response in unstimulated cultures. Reproduced from *J. exp. Med.* **143**, 372, 1976.

brane with the receptor sites for Con-A or LPS, then the blocking or covering of the Ia molecule with anti-Ia antibodies should interfere with the interaction of mitogen and receptor. This type of experiment obviously requires rigid controls. These controls have included the use of nonappropriate targets, normal sera, inappropriate anti-Ia sera, antisera directed at other class markers and anti-H-2K or anti-H-2D sera. When spleen cells or nylon-wool-purified splenic T cells were incubated with anti-Ia serum, anti-H-2 serum, or anti-Thy-1 serum, washed 4 times by centrifugation in media and cultured with Con-A, no difference in the dose-response curves was found.

In contrast to the lack of inhibition of the Con-A response by anti-Ia serum pretreatment, similar pretreatment of splenic B cells resulted in the partial inhibition of the LPS-proliferative response. In five experiments the mean suppression of the maximum LPS dose was 35.6 % $\pm$ 11.8 compared with a control of 0.4 % $\pm$ 16.1 ($p < 0.005$). In six additional experiments, the IgG fraction of anti-Ia serum was added directly to the cultures and was present during the entire culture period. In these experiments, the mean suppression was 35.2 % $\pm$ 12.2 compared with a control in nonappropriate cultures (B10.S) of 3.2 % $\pm$ 14.9 ($p < 0.005$) (Table IV).

This partial inhibition was a B-cell specific event, since removal of T cells by anti-Thy-1 serum plus complement gave similar results. Furthermore, inhibition was not enhanced by increasing the concentration of antibodies or extending the period of antiserum treatment. In fact, prolonged incubation with anti-Ia serum and control sera beyond 3 hours was found to produce uniform inhibition of the LPS response; treating the cells at 4°C for 24 hours was also unsuccessful. It was not until the IgG fraction of the serum had been prepared that anti-Ia antibodies could be added in significant concentration directly to the cultures.

Criticism of these experiments has focused on the use of whole antibody molecules to effect blocking, with concern that blocking could thus be nonspecific. The authors' control for this has been the use of anti-Ia sera directed against specificities not found on the target cell and specific anti-H-2K and anti-H-2D antibodies. The latter have not been observed to produce inhibition of the LPS response. In order for the partial inhibition to occur via the Fc portion of the antibody molecule, one must assume that the anti-Ia antibodies possess a unique heavy-chain-constant region, an extremely unlikely possibility because the predominant cytotoxic antibody in both anti-Ia sera and anti-H-2 sera is IgG.

In the experiments to determine whether Ia-positive T or B cells were sensitive to various mitogens, Ia-positive cells were removed from the lymphocyte population by anti-Ia serum and complement lysis. The anti-Ia

TABLE II

Mitogen response in lymphoid cells resistant to anti-Ia serum and complement treatment specific for I *subregions*

Exp.	Treatment	Target cell	Mitogen	Tested		CPM ± SD[a]
				Region	Ia specificity	
1	none	B10.S	none			1,968 ± 243
	none	„	Con A			53,470 ± 6,400
	A.TL-ns + C′	„	„			30,126 ± 2,010
	A.TL α A.TH + C′	„	„	A-C	4, 5, 9, 12	1,953 ± 65
	none	„	Leuko			29,129 ± 2,600
	A.TL-ns + C′	„	„			19,600 ± 1,956
	A.TL α A.TH + C′	„	„	A-C	4, 5, 9, 12	28,025 ± 2,520
2	none	B10.K-T[b]	none			224 ± 192
	none	„	Con A			106,259 ± 4,490
	A.TH α A.TL + C′	B10.S-T	„			156,572 ± 10,926
	A.TH α A.TL + C′	B10.K-T	„	A-C	1, 2, 7, 15	8,662 ± 3,353
	none	B10.K	Leuko			12,048 ± 1,564
	none	B10.K-T	„			13,759 ± 2,566
	A.TH α A.TL + C′	„	„	A-C	1, 2, 7, 15	22,732 ± 2,562
3	none	B10.D2	none			372 ± 80
	none	B10.D2	Con A			128,229 ± 12,094
	none	B10.D2-T	Con A			159,040 ± 14,613
	A.TH-ns + C′	„	„			60,425 ± 12,950
	A.TH α A.TL + C′	„	„	C	7	15,837 ± 3,790
4	none	B10.A(3R)	none			2,705 ± 709
	none	„	Con A			145,239 ± 11,695
	none	B10.A(3R)-T	„			75,554 ± 13,807
	B10.A(4R)-ns + C′	„	„			91,668 ± 9,759
	[129 × B10.A(4R)] α B10.A(2R) + C′	„	„	C	6, 7	61,020 ± 2,526
5	none	B10.A(4R)	none			977 ± 735
	none	„	Con A			94,625 ± 16,284
	none	B10.A(4R)-T	„			151,800 ± 7,108
	A.TH-ns + C′	„	„			170,210 ± 22,190
	(129 × A.TH) α A.TL + C′	„	„	A	1, 2	148,854 ± 6,000
6	none	B10.A(4R)	none			1,039 ± 154
	none	„	Con A			64,576 ± 5,574
	none	B10.A(4R)T	„			26,520 ± 1,802
	A.TH-ns + C′	„	„			52,666 ± 6,478
	(ABY × B10.HTT) α A.TL	„	„	A	1, 2	72,624 ± 1,768

[a] Expressed as counts per minute [^{3}H]-Thymidine incorporation for quadruplicate cultures.

[b] Nylon-wool-purified splenic T cells.

TABLE III

LPS response in lymphoid cells resistant to anti-Ia serum and complement treatment specific for I *subregions*

Exp.	Treatment	Target cell	Mitogen	Tested		CPM ± SD[a]
				Region	Ia specificity	
1	none	B10.BR	–			2,448 ± 96
	none	„	LPS[b]			13,470 ± 2,170
	A.TH-ns + C′	„	„			8,587 ± 2,209
	A.TH α A.TL	„	„	A-C	1, 2, 7, 15, 17	1,829 ± 326
2	none	B10.BR-B[c]	–	–		1,686 ± 257
	none	„	LPS	–		12,016 ± 1,728
	A.TH-ns + C′	„	„			11,472 ± 1,882
	A.TH α A.TL	„	„	A-C	1, 2, 7, 15, 17	2,228 ± 752
3	none	B10.HTT	–	–		1,753 ± 302
	none	„	LPS	–		13,047 ± 1,908
	A.TL-ns + C′	„	„			9,453 ± 976
	A.TL α A.TH + C′	„	„	A	4, 5, 9, 12, 17	2,425 ± 863
4	none	B10.A(3R)	–	–		673 ± 33
	none	„	LPS	–		6,799 ± 995
	HTI-ns + C′	„	„			5,931 ± 1,388
	(HTI × B10) α B10.A(5R) + C′	„	„	C	6, 7	5,579 ± 334
	anti-LY 4 + C′	„	„			1,306 ± 411

[a] Expressed as counts per minute [^{3}H]-Thymidine incorporation for quadruplicate cultures.
[b] Cultures were stimulated with 50 μg/ml LPS.
[c] Anti-Thy-1.2 purified splenic B cells.

serum used initially was the broadly specific A.TH anti-A.TL(Ia^k) or A.TL anti-A.TH(Ia^s). Additional experiments have been performed using antisera prepared in specific F_1 recipients to lyse selected target cells. The anti-Ia-serum test-cell combination was, therefore, specific for a defined I subregion. In these experiments, the LPS-responsive cells could be removed by antisera specific for Ia specificities mapping in the I-A subregion but in experiment 4 of Table III antibodies specific for IC^d subregion failed to remove LPS sensitive cells. Con-A-sensitive nylon-wool-purified T cells, however, gave a different pattern of sensitivity (Table II). When A.TH anti-A.TL serum and complement were used to lyse B10.D2 T-cells, the resistant viable cells responded poorly to Con-A. In this experiment, anti-Ia serum reacted with specificity Ia.7 of the I-C subregion (Table II, experi-

TABLE IV

Inhibition of the LPS-mitogenic response when the IgG fraction of A.TH anti-A.TL serum was added to cultures

Exp.	Strain	I region	cpm ± SD[a]		Per cent suppression
			Media	Anti-Ia (IgG)[b]	
1	B10.BR	k	9438 ± 2650[c]	6973 ± 1862	26 %
	B10.S	s	9552 ± 1578	8529 ± 780	11 %
2	B10.BR	k	8209 ± 506	6181 ± 750	25 %
	B10.S	s	5093 ± 1536	5414 ± 670	− 6 %
3	A.TL	k	3316 ± 664	1944 ± 346	41 %
	B10.S	s	4236 ± 1416	4443 ± 675	− 5 %
4	B10.BR	k	2246 ± 538	1738 ± 208	23 %
	B10.S	s	2817 ± 348	3272 ± 340	−16 %
5	B10.BR	k	1742 ± 611	995 ± 345	43 %
	B10.S	s	3795 ± 337	2842 ± 294	25 %
6	B10.BR	k	2626 ± 889	1393 ± 358	53 %
	B10.S	s	4163 ± 868	3732 ± 1234	10 %
				Mean suppression	35.2 ± 12.2
				Control	3.2 ± 14.9
					($p < 0.005$)

[a] Expressed as counts per minute [^{3}H]-Thymidine incorporation for quadruplicate cultures. There was no mitogen effect of anti-Ia^k (IgG) when it was added to spleen-cell cultures.

[b] Pool of inhibitory γ-fractions from agarose-block electrophoresis.

[c] Cultures were stimulated with 50 μg/ml LPS. Responses were 2.7–5.0 × control with no LPS.

ment 3). In repeated experiments using the antiserum [129 × B10.A(4R)] anti-B10.A(2R) to test specificities Ia.6 and Ia.7 on B10.A(3R) T cells, only minimal, if any, reduction in the response to Con-A was observed. Even when these target cells were retreated a second time with this antiserum and complement, the response was decreased only 30 % (Table IV, experiment 4). Of interest, however, were experiments 5 and 6 of Table II in which anti-Ia sera specific for Ia.1 and Ia.2 of the I-A subregion were used. In these experiments, there was no significant decrease in the Con-A response. These experiments suggest, but have not yet confirmed, the possibility that T cells which respond to Con-A may bear only one (or more) Ia specificity of the I-C subregion. Considerable study is required to answer the questions posed by these initial mapping studies.

Ia-POSITIVE T CELLS PROMOTE THE RESPONSE OF Ia-NEGATIVE T CELLS TO CON-A

During the course of these investigations, the authors have observed that although anti-Ia sera and complement killed only a small percentage of nylon-wool-purified splenic T cells (Niederhuber et al. 1975), it abolished their mitogenic response to Con-A. Furthermore, removal of the Ia-positive cells from a spleen-cell population prevented the generation of nonspecific suppressor T cells by Con-A stimulation (unpublished observations). Once generated, the Con-A suppressor T cells are resistant to lysis by anti-Ia antibodies and complement. These findings suggest that only a small subset of T cells respond directly to Con-A and act to recruit other T cells to respond. An alternative explanation would be the selective proliferation and cell death of the Con-A-reactive cells.

To test the ability of small numbers of Ia-positive Con-A-sensitive T cells to function as promoter cells recruiting Ia-negative cells to respond, small numbers of normal T cells were added to Ia-depleted T cells. In these reconstitution experiments, as few as 20 % normal T cells can completely restore the response of Ia-depleted T cells (Figure 4). The addition

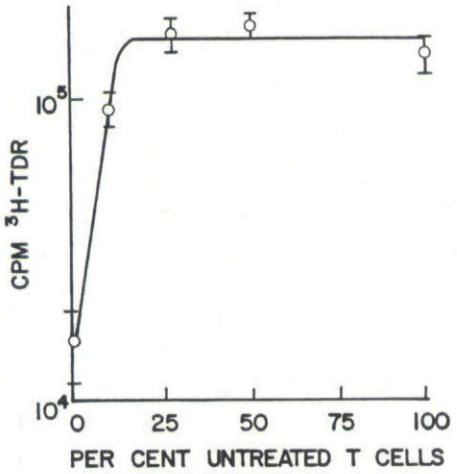

Figure 4. Reconstitution of the mitogenic response of Ia-negative T cells by Ia-positive promoter T cells. Nylon-wool T cells from CBA/J ($H\text{-}2^k$) mice were treated with A.TH anti-A.TL serum ($anti\text{-}Ia^k$) followed by complement. The remaining Ia-negative T cells were mixed with untreated (Ia-positive) T cells in different proportions. The cell mixtures were cultured at 5×10^5 cells/well with 2 μg/ml Con-A. Cultures were pulsed with 2 μCi [^{3}H]-Thymidine 2 Ci/mMole for 24 hours before harvesting on the third day of the culture period. Each point represents the mean of four cultures $\pm$ SD.

of increased numbers of normal cells did not further enhance the response. The promoter cell was nonadherent to plastic dishes, did not adhere to a nylon-wool column, and was lysed by both anti-Thy-1 serum and anti-Ia serum in the presence of complement.

In order to be certain that the Ia-negative cells were the cells responding, CBA nylon-wool-purified T cells bearing the T6 chromosomal marker were treated with anti-Ia serum and complement, added to untreated CBA T cells of normal karyotype, and stimulated with 2 μg/ml Con-A. The co-cultured cells were treated with colchicine for 16 hours before harvesting, and the proportion of T6 metaphases determined. The majority of metaphases (18/25) were T6, demonstrating that the Ia-negative cells were responding to Ia-positive Con-A-reactive cells. The Ia-negative cells did not respond in control cultures. No [^{3}H] thymidine incorporation was observed in co-cultured cells not stimulated with Con-A.

In these experiments, the Con-A-reactive T cells were Ia positive and could recruit other Ia-negative T cells to generate the observed mitogenic response to Con-A.

THE Ia GENE PRODUCT AND THE MIXED-LYMPHOCYTE REACTION

Two facts concerning the allogeneic-lymphocyte response (MLR) in the mouse have been established:

1) the major genetic control of this reaction resides in the I-A region of the H-2 complex, and

2) the Ia molecule is critical to the process of stimulation (Bach et al. 1972, Meo et al. 1973a, b). Meo has very eloquently demonstrated the specificity of anti-Ia-serum blocking of the stimulator cells using F_1 cells to stimulate parental responders. Anti-Ia serum specific for either parental haplotype comprising the F_1 stimulator cells is effective in blocking the stimulation of the appropriate responder. Responder cells are not blocked by anti-Ia-serum treatment (David et al. 1976).

Experiments carried out in which anti-Ia serum and complement were used to treat the stimulator or the responder population. Responder cells resistant to anti-Ia antibodies and complement gave an unaltered response when stimulated. However, the Ia-negative population of stimulator cells was not capable of stimulating a normal population of responders (Table V). Similar findings have been reported by Lonai (1975) who also finds the killer T cell generated by this allogeneic interaction to be resistant to lysis by anti-Ia sera and complement.

TABLE V

The effect on MLR of removing Ia-bearing cells from the stimulator population

Exp.	Stimulator	Treatment	% lysis	Responder	CPM ± SD[1]	Stimulation index
1	B10.BR[2]	—		B10.BR	4,814 ± 875	—
	A.SW	none		B10.BR	34,977 ± 6,317	7.3
	A.SW	C′	15	B10.BR	24,309 ± 3,267	5.1
	A.SW	anti-Ia^s	4	B10.BR	28,411 ± 3,192	5.9
	A.SW	anti-Ia^s + C′	46	B10.BR	6,752 ± 2,176	1.4
	A.SW	anti-Thy-1.2 + C′	45	B10.BR	20,553 ± 3,355	4.3
	A.SW	—		A.SW	8,294 ± 1,216	—
	B10.BR	none		A.SW	72,161 ± 16,266	8.7
	B10.BR	anti-Ia^s + C′	15	A.SW	92,306 ± 20,707	11.1
2	B10.BR	—		B10.BR	1,364 ± 458	—
	B10.S	none		B10.BR	17,709 ± 1,599	13.0
	B10.S	C′	45	B10.BR	6,584 ± 1,254	4.8
	B10.S	anti-Ia^s	7	B10.BR	15,491 ± 2,828	11.4
	B10.S	anti-Ia^s + C′	58	B10.BR	2,331 ± 615	1.7
	B10.S	anti-Thy-1.2 + C′	44	B10.BR	7,021 ± 1,235	5.2
	B10.S	—		B10.S	2,326 ± 191	—
	B10.BR	none		B10.S	24,682 ± 2,440	10.6
	B10.BR	anti-Ia^s + C′	25	B10.S	12,241 ± 4,309	5.3
3	A.TL	—		A.TL	23,767 ± 2,767	—
	(A.TL × A.TH)F_1	none		A.TL	109,052 ± 14,746	4.6
	(A.TL × A.TH)F_1	C′	12	A.TL	111,153 ± 32,829	4.7
	(A.TL × A.TH)F_1	anti-Ia^s + C′	40	A.TL	21,912 ± 5,105	0.9
	(A.TL × A.TH)F_1	anti-Ia^k + C′	50	A.TL	8,644 ± 2,491	0.4
	A.TH	—		A.TH	4,970 ± 6,050	—
	(A.TL × A.TH)F_1	none		A.TH	42,931 ± 2,289	8.6
	(A.TL × A.TH)F_1	anti-Ia^s + C′	40	A.TH	2,831 ± 1,908	0.6
4	A.SW	—		A.SW	2,568 ± 628	—
	B10.BR	media	10	A.SW	37,839 ± 9,878	14.7
	B10.BR	C′	19	A.SW	24,154 ± 5,033	9.4
	B10.BR	anti-Ia^k	22	A.SW	33,706 ± 2,644	13.1
	B10.BR	anti-Ia^k + C′	46	A.SW	1,774 ± 271	0.7
	B10.BR	anti-Thy-1.2 + C′	56	A.SW	9,740 ± 599	3.8

[1] Counts per minute [^{3}H]-thymidine incorporation ± standard deviation for quadruplicate cultures of 10^6 stimulator cells and 10^6 responder cells.

[2] Lymphoid cells used in these experiments were prepared from lymph nodes and stimulator cells were irradiated with 3000 R.

ANTI-Ia ANTIBODY INHIBITION OF THE *IN VITRO* HUMORAL RESPONSE

The experiments reported in this section were initiated in an attempt to discover a role for the Ia-antigen molecule in the events of the humoral response. It was predicted that if the Ia molecule was important either as an antigen-recognition structure or cell-interaction structure, covering the Ia antigen with specific anti-Ia antibodies would inhibit the generation of an immune response.

Initially Frelinger et al. (1975) reported the blocking of the IgM primary PFC response to SRBC with broadly specific anti-Ia serum but not with antibodies specific for H-2K- or H-2D-region determinants. The secondary IgG-PFC were also inhibited by anti-Ia antibodies; however, the secondary IgM response was less sensitive to anti-Ia serum.

The inhibition by anti-Ia serum added at the initiation of the cultures did not appear to be the result of differential toxicity because no differences in cell viability were observed in the different culture groups. There was, however, difficulty in obtaining consistent results when the antiserum was added directly to the culture and was present throughout the culture period. This problem was overcome by utilizing brief periods of antiserum pretreatment of the cells before initiation of the culture. The spleen cells were incubated at 37°C for 30 minutes with normal serum, anti-Ia serum, or anti-H-2 serum, washed four times by centrifugation in media, and cultured with heterologous red cells. With this method, normal mouse serum or anti-H-2 sera had essentially no effect on the primary or secondary PFC response, while anti-Ia serum produced a significant inhibition.

It was a concern had been that pretreatment with intact antiserum would result in either patching or capping of the membrane determinants, followed by shedding or endocytosis with the rapid reexpression of the Ia molecule. In these experiments, the greatest inhibition of the *in vitro* primary IgM-PFC and the secondary IgG-PFC occurred at four days, the time of maximum response in control cultures. The responses with anti-Ia antibody-blocked cells do improve by day six, suggesting a temporary blocking of the necessary reactive cells in the response to antigen. At no time did the authors observe inhibition of the response with cells pretreated with antisera specific for H-2K- or H-2D-region determinants.

In order to assess the possibility that different *I* subregions or, in fact, different Ia specificities might be critical to the observed inhibition with broadly specific anti-Ia sera, antisera-target cell combinations were used which were specific for a defined subregion. For example, to test the I-A subregion, an antiserum produced in (B10 $\times$ A.TH)F_1 mice against A.TL

TABLE VI

Effect of pretreatment with anti-Ia serum on secondary BRBC response

Exp.	Antiserum	Culture	Regions tested	PFC/culture[a]	
				IgG	IgM
1.	NS	B10.A(4R)	none	292	362
	(B10 × A.TH) α A.TL	B10.A(4R)	I-A	32	7
	A.TH α A.TL	B10.A(4R)	I-A, I-B, I-C(?)	18	8
2.	NS	B10.S(9R)	none	53	140
	(A × A.AL) α A.TL	B10.S(9R)	K	94	192
	(B10 × HTI) α B10.A(5R)	„	I-C	11	14
	A.TH α A.TL	B10.S(9R)	I-B, I-C	2	0
3.	(A × A.AL) α A.TL	B10.HTT	K	191	187
	A.TH α A.TL	B10.HTT	I-B, I-C	130	0

[a] Pool of four cultures.

cells was used. When used to pretreat B10.A(4R) spleen cells, this anti-Ia serum reacts only with products of the I-A subregion. In this experiment the IgM and IgG responses are suppressed by 90 % (Table VI). The effect of antiserum specific for the I-C subregion was tested using two combinations, B10.S(9R) spleen cells pretreated with (B10 × HTI)F_1 anti-B10.A(5R) serum and B10.HTT spleen cells pretreated with A.TH anti-A.TL serum. Both combinations showed strong inhibition of the IgG secondary response. Interestingly, only (B10 × HTI)F_1 anti-B10.A(5R) serum inhibited the secondary IgM response. The A.TH anti-A.TL serum inhibited the secondary IgM response of B10.A(4R) spleen cells but had no effect on B10.HTT spleen cells. This suggests the possibility of multiple I-C-subregion determinants.

These blocking experiments are interesting when considered in the light of experiments by Katz et al. (1975), which demonstrate that effective cooperation both *in vitro* and *in vivo* depends on compatibility only in the I-A and/or I-B subregions. The inhibitory effects of the antiserum specific for the I-C subregion suggest that this blocking may occur at a level other than that measured by Katz in his cooperation experiments.

These experiments do not determine the site of interference in the immune response or even if blocking occurs at the same level in all serum-target-cell combinations. It is possible that in blocking I-A subregion determinants, T-B cell and/or macrophage-lymphocyte interaction is impaired, while with blocking of I-C determinants only T-B cell co-

operation is blocked, explaining why both IgM and IgG secondary responses are blocked with antiserum directed at the *I-A* subregion or the entire *I* region. This would block the initial macrophage-lymphocyte interactions, while blocking of *I-C* determinants would block only the T-B cell cooperation, leaving intact the largely T-independent secondary IgM response.

Preliminary experiments have been completed which demonstrate that a brief pretreatment of the adherent cell population with anti-Ia serum before combining with the macrophage-depleted spleen-cell population inhibits the reconstituted *in vitro* primary response to BRBC (Niederhuber, unpublished observations). Although these experiments were performed with the broadly specific A.TH anti-A.TL serum, they suggest that at least some of the observed inhibition can be explained as a blocking of the macrophage-lymphocyte interaction. This interpretation is consistent with the data of Erb & Feldman (1975) which suggest that macrophages and T cells which are compatible at the *I-A* subregion can cooperate to generate helper cells, while *I-C* region differences are irrelevant.

These preliminary findings are exciting and suggest a role for the Ia molecule in the recognition of antigen by the macrophage and the presentation of antigen to the T-cell receptor, possibly as a new antigen-Ia complex. Studies are under way to determine if Ia is part of the T-cell-receptor mechanism and the relationship of this phase of immune recognition to B cell activation.

CONCLUDING REMARKS

These experiments depend a great deal on a firm serologic and genetic definition of the strains used. In a number of cases, it can not be absolutely stated that all of the reactivity in the anti-Ia serum is associated with the I region, since appropriate recombinants necessary to exhaustively test the serum do not exist. For these experiments, an attempt has been made to select antisera and target cells which have been extensively characterized. Experiments were also performed with several concentrations of antiserum, attempting to minimize differences in antibody titer or membrane-antigen(s) density. It is possible that anti-Ia sera contain undetected non-cytotoxic antibodies; these should, however, be detected in the blocking experiments.

As previously noted, the question of nonspecific blocking via Fc receptors is often raised. The use of nonappropriate targets, inappropriate anti-Ia sera or anti-H-2K sera as controls eliminates this criticism. The lack of inhibition with anti-H-2 sera indicates that blocking is not a simple alteration of membrane function, because coupling of an antibody to the cell mem-

brane was not sufficient to produce inhibition. Since both anti-H-2 antibodies and anti-Ia antibodies are largely of the IgG class, it is unlikely that the inhibition is mediated via the Fc receptor. For a difference in blocking activity to exist, one would have to assume a unique heavy-chain-constant region.

Recently, evidence for cross reactivity between I-A and I-C subregions has evolved (Murphy, Frelinger, unpublished observations). In the experiments reported here, the authors have attempted to avoid the possibility of cross reactivity in selecting the antiserum-target-cell combinations.

Recently, Cullen et al. (1976), using high-resolution gels, have characterized the anti-Ia-antibody-precipitated membrane fraction into two molecules of molecular weights 25,000 and 33,000. Investigations with the guinea pig (B. Schwartz, unpublished observations) suggest that only one of the polypeptides actually contains the Ia antigenic molecules. This raises interesting possibilities about the nature of the non-Ia molecule. It does not have to be a product of the H-2 complex, but could be the product of another locus, for example, the M locus. This would help explain the allogeneic response observed with M-locus differences. The authors are currently pursuing this possibility.

Whether the functional relationships with the Ia antigen described here reflect a quantitative variation in the expression of the Ia determinants, or whether the genetic complexity of the *I* region results in many determinants, some of which are expressed only on specific lymphocyte subsets with specific immune functions, requires further genetic analysis.

ACKNOWLEDGMENTS

This work was supported by grants from Damon Runyon DRG-1260, Michigan Kidney Foundation, NIH grant RO1-AI-12153-01,and NCI grant CA-14089. John E. Niederhuber is the recipient of U.S. Public Health Service Research Career Development Award. The excellent technical assistance of Patrica Shoffner, Laura Mayo, Carolyn Rosio and secretary Nancy Cuthbert is acknowledged. The authors would like to thank Doctors Erna Möller and Donald Shreffler for valuable advice and discussions.

REFERENCES

Bach, F. H., Widmer, M. B., Bach, M. L. & Klein, J. (1972) Serologically-defined and lymphocyte-defined components of the major histocompatibility complex in the mouse. *J. exp. Med.* **136**, 1430.

Cohen, J. J. & Claman, H. N. (1971) Thymus-marrow immunocompetence. Hydro-

cortisone-resistant cells and processes in the hemolytic antibody response of mice. *J. exp. Med.* **133**, 1026.

Cullen, S. E., David, C. S., Cone, J. L. & Sachs, D. H. (1976) Evidence for more than one Ia antigenic specificity on molecules determined by the I-A subregion of the mouse major histocompatibility complex. *J. Immunol.* In press.

Cullen, S. E., David, C. S., Shreffler, D. C. & Nathenson, S. G. (1974) Membrane molecules determined by the H-2-associated immune response region: Isolation and some properties. *Proc. nat. Acad. Sci. (Wash.)* **71**, 648.

David, C. S., Niederhuber, J. E., Frelinger, J. A., Dugan, E. P., Meo, T. & Shreffler, D. C. (1976) Murine Ia antigens. Expression on T and B cells and their role in immune response. In: *Proc. of 10th Leukocyte Culture Conference*, Academic Press Inc., New York.

David, C. S., Shreffler, D. C. & Frelinger, J. A. (1973) New lymphocyte antigen system (Lna) controlled by the *Ir* region of the mouse H-2 complex. *Proc. nat. Acad. Sci. (Wash.)* **70**, 2509.

David, C. S., Stimpfling, J. H. & Shreffler, D. C. (1975) Identification of specificity H-2.7 as an erythrocyte antigen: control by an independent locus, H-2G, between the *S* and *D* regions. *Immunogenetics* **2**, 131.

Erb, P. & Feldman, M. (1975) The role of macrophages in the generation of T helper cells. II. The genetic control of the macrophage-T-cell interaction for helper cell induction with soluble antigens. *J. exp. Med.* **142**, 460.

Fathman, C. G., Cone, J. L., Sharrow, S. O., Tyrer, H. & Sachs, D. H. (1975) Ia alloantigen(s) detected on thymocytes by use of a fluorescence-activated cell sorter. *J. Immunol.* **115**, 584.

Frelinger, J. A., Niederhuber, J. E., David, C. S. & Shreffler, D. C. (1974) Evidence for the expression of Ia (H-2-associated) antigens on thymus-derived lymphocytes. *J. exp. Med.* **140**, 1273.

Frelinger, J. A., Niederhuber, J. E. & Shreffler, D. C. (1975) Inhibition of immune responses *in vitro* by specific antiserums to Ia antigens. *Science* **188**, 268.

Goding, J. W., White, E. & Marchalonis, J. J. (1975) Partial characterization of Ia antigens on murine thymocytes. *Nature (Lond.)* **257**, 230.

Götze, D., Reisfeld, R. A. & Klein, J. (1973) Serologic evidence for antigens controlled by the Ir region in mice. *J. exp. Med.* **138**, 1003.

Hämmerling, G. J., Deak, B. D., Mauve, G., Hämmerling, U. & McDevitt, H. O. (1974) 'B' lymphocyte alloantigens controlled by the *I* region of the major histocompatibility complex in mice. *Immunogenetics* **1**, 68.

Hauptfeld, V., Klein, D. & Klein, J. (1973) Serological identification of an Ir-region product. *Science* **181**, 167.

Katz, D. H., Greaves, M., Dorf, M. E., Dimuzio, M. & Benacerraf, B. (1975) Cell interactions between histoincompatible T and B lymphocytes. VIII. Cooperative responses between lymphocytes are controlled by genes in the *I* region of the *H-2* complex. *J. exp. Med.* **141**, 263.

Klein, J. & Park, J. M. (1973) Graft-versus-host reaction across different regions of the H-2 complex of the mouse. *J. exp. Med.* **137**, 1213.

Lieberman, R., Paul, W. E., Humphrey, W. Jr. & Stimpfling, J. H. (1972) H-2-linked immune response (Ir) genes. Independent loci for Ir-IgG and Ir-IgA genes. *J. exp. Med.* **136**, 1231.

Lilly, F. (1966) The inheritance of susceptibility to the Gross leukemia virus in mice. *Genetics* **53**, 529.

Lilly, F. (1972) Mouse leukemia: a model of a multiple-gene disease. *J. nat. Cancer Inst.* **49**, 927.

Lilly, F. & Pincus, T. (1973) Genetic control of murine viral leukemogenesis. *Adv. Cancer Res.* **17**, 231.

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.* Academic Press Inc., New York.

McDevitt, H. O. & Benacerraf, B. (1969) Genetic control of specific immune responses. *Adv. Immunol.* **11**, 31.

McDevitt, H. O. & Sela, M. (1965) Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains of inbred mice. *J. exp. Med.* **122**, 517.

Meo, T., David, C. S., Nabholz, M., Miggiano, V. & Shreffler, D. C. (1973a) Demonstration by MLR test of a previously unsuspected intra-H-2 crossover in the B10.HTT strain: Implications concerning location of MLR determinants in the Ir region. *Transplant. Proc.* **5**, 1507.

Meo, T., Vives, J., Miggiano, V. & Shreffler, D. C. (1973b) A major role for Ir-1 region of the mouse H-2 complex in the mixed leukocyte reaction. *Transplant. Proc.* **5**, 377.

Mishell, R. I. & Dutton, R. W. (1967) Immunization of dissociated spleen cell cultures from normal mice. *J. exp. Med.* **126**, 423.

Nabholz, M., Young, H., Rynbeek, A., Boccardo, R., David, C. S., Meo, T., Miggiano, V. & Shreffler, D. C. (1975) I-region-associated determinants: expression on mitogen-stimulated lymphocytes and detection by cytotoxic T cells. *Europ. J. Immunol.* **5**, 594.

Niederhuber, J. E., Frelinger, J. A., Dine, M., Shoffner, P., Dugan, E. & Shreffler, D. C. (1976) Effects of anti-Ia sera on mitogenic responses. II. Differential expression of the Ia marker on PHA and Con-A reactive T cells. *J. exp. Med.* **143**, 372.

Niederhuber, J. E., Frelinger, J. A., Dugan, E., Coutinho, A. & Shreffler, D. C. (1975) Effects of anti-Ia serum on mitogenic response to B cell mitogen, LPS, by specific anti-Ia sera. *J. Immunol.* **115**, 1672.

Sachs, D. H. & Cone, J. L. (1973) A mouse B-cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *J. exp. Med.* **138**, 1289.

Shreffler, D. C. & David, C. S. (1975) The H-2 major histocompatibility complex and the *I* immune response region: genetic variation, function and organization. *Adv. Immunol.* **20**, 125.

Shreffler, D., David, C., Götze, D., Klein, J., McDevitt, H. O. & Sachs, D. H. (1974) Genetic nomenclature for nero lymphocyte antigens controlled by the *I* region of the H-2 complex. *Immunogenetics* **1**, 189.

Schultz, J. S., Frelinger, J. A., Kim, S. K. & Shreffler, D. C. (1975) The distribution of Ia antigens of the H-2 complex on lymph node cells by immunoferritin labeling. *Cell. Immunology* **16**, 125.

Tennant, J. R. & Snell, G. D. (1968) The H-2 locus and viral leukemogenesis as studied in congenic strains of mice. *J. nat. Cancer Inst.* **41**, 597.

Unanue, E. R., Dorf, M. E., David, C. S. & Benacerraf, B. (1974) Presence of I-region-associated antigens on B-cells in molecules distinct from immunoglobulin and H-2K and H-2D. *Proc. nat. Acad. Sci. (Wash.)* **71**, 5014.