THE ROLE OF GENE PRODUCTS OF THE *I-J* SUBREGION IN MIXED LYMPHOCYTE REACTIONS*

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Previous investigations have shown that genes controlling products which cause stimulation in the mixed lymphocyte reaction (MLR)[1] map in a number of different regions of the *H-2* gene complex (1, 2). The strongest MLR stimulation has been seen with *I* region differences. Specific loci controlling lymphocyte-activating determinants (Lad) were previously identified in the *I-A* and *I-C* subregions (3). Since then, two new *I* subregions, *I-J* and *I-E*, have been identified (4–6). Many of the haplotype combinations examined in the previous demonstrations of Lad loci in subregions *I-A* and *I-C* also included incompatibilities in the *I-J* and *I-E* subregions. Hence, Lad loci could be included in the *I-J* and/or *I-E*, as well as *I-A* and *I-C* subregions.

By using recombinant strains of mice, which differ only in the *I-J* subregion [B10.A(3R) and B10.A(5R)], or only in the *I-J, I-C, S,* and *G* regions [B10.HTT and B10.S(9R)], we have attempted to test for the existence of an Lad locus in this subregion. Although previously there have been some ambiguities concerning the expression of Ia determinants on T cells, there is now good evidence that Ia antigens are expressed on at least some subpopulations of T cells as well as on most B cells. The predominant stimulation in the MLR reported previously (1–3) was caused by B-cell determinants, but in 1975, Lonai (7) showed that T cells could also stimulate an MLR response. Since *I-J* gene products have been shown to be expressed only on T cells (5), the expression of MLR-stimulating determinants on T cells was examined in these experiments by using nylon wool purification of stimulating and responding cells. Through these approaches, we have been able to demonstrate significant MLR stimulation by T cells differing only in the *I-J* subregion. Our data suggest that the stimulating T cells may also express products of the *I-E/I-C* subregion.

Materials and Methods

Mice. Inbred strains, recombinants, and F₁ hybrids used in this study were all produced in our colony at Washington University School of Medicine, St. Louis, Mo.

Cell Preparation. Thymus, spleen, and lymph node cells were used as the T-cell sources. Purification of T cells was performed by passing the cell suspensions through nylon wool columns

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[1] *Abbreviations used in this paper:* HBSS, Hanks' Balanced Salt Solution; Lad, lymphocyte-activating determinants; MLR, mixed lymphocyte reaction; NMS, normal mouse serum.

(8). Elimination of macrophages was carried out by a modification of the method previously described by Sher and Glover (9). Briefly, 0.4 g of carbonyl iron (General Aniline & Film Corp., Linden, N. J.) was mixed with 2×10^6 nylon wool-purified T cells in Hanks' Balanced Salt Solution (HBSS). After a 20-min incubation at 37°C with shaking, carbonyl iron-phagocytized cells were removed with a strong magnet.

Antiserum Treatment of Cells. Lymph node cells (5×10^7) were reacted with anti-Thy-1.2 serum (provided by the Transplantation Immunology Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) or rabbit anti-mouse gamma globulin serum (Microbiological Associates, Bethesda, Md.) at a final concentration of 10% antiserum in 5% fetal calf serum-containing RPMI 1640 medium or in 0.01 M borate buffer (pH 8.0) in a 1-ml volume. After 30 min at 37°C, the cells were washed twice with HBSS. Agarose EDTA-absorbed undiluted rabbit complement was added and the mixture was incubated for an additional 90 min at 37°C, in a humidified atmosphere containing 5% carbon dioxide.

MLR Assay. The medium employed in this assay consisted of Hepes-buffered (30 mM) RPMI 1640, supplemented with L-glutamine (20 mM), antibiotics (penicillin, 100 IU/ml; streptomycin, 100 μg/ml), 2-mercaptoethanol (30 μM), and 5% heat-inactivated human plasma. 7×10^5 viable responder cells were cultured with 1.4×10^6 mitomycin-C-treated stimulator cells (10) in 0.2 ml of medium in microculture plates (Falcon 3040, Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Incubation was performed at 37°C in a 5% carbon dioxide, 95% air, humidified atmosphere for 72 h. 2 μCi of [³H]thymidine (sp act 1.9 Ci/mmol, New England Nuclear, Boston, Mass.) was then added and the culture was harvested after an additional 16 h.

Inhibition of MLR. MLR assays were done by the technique described above, except that human plasma was employed in the culture medium at a final concentration of 2.5, instead of 5%. 7×10^5 nylon wool-purified responder T cells were cocultured with 1.4×10^6 mitomycin-C-treated, nylon wool-purified stimulator cells in the presence of 1.25% heat-inactivated alloantiserum, or normal control serum, in a total volume of 200 μl. Percent inhibition was calculated according to the following formula:

% inhibition

$$= \left(1 - \frac{\text{cpm of allogeneic culture in antiserum-cpm of syngeneic culture in NMS}}{\text{cpm of allogeneic culture in NMS - cpm of syngeneic culture in NMS}} \right) \times 100.$$

Treatment by Ia-Antisera and Complement. Nylon wool-purified T cells were reacted with antiserum or control normal mouse serum (NMS), at a final concentration of 10%, in RPMI 1640 containing 2.5% heat-inactivated human plasma. After a 30-min incubation at 37°C, the cells were washed and suspended in agarose- and spleen cell-absorbed, EDTA-treated rabbit complement (11). After a 90-min incubation at 37°C, the dead cells were counted and separated from live cells on a Ficoll-Hypaque gradient. Dead cells in these tests were scored by a dye exclusion test. Only surviving cells, after treatment by antisera and complement (and followed by mitomycin-C treatment), were used as stimulator cells in these MLR tests. Culture media, cell numbers of stimulators and responders, the period of culture, and the method of harvesting, were all the same as those described above. No antisera were added to the media for elimination studies. Percent inhibition was estimated by the formula:

$$\% \text{ inhibition} = \left(1 - \frac{\text{cpm of allogeneic culture of treated cells - cpm syngeneic culture}}{\text{cpm of allogeneic culture - cpm of syngeneic culture}} \right) \times 100.$$

Results

Stimulating Capacity of Lymphoid Organs. Mitomycin-C-treated stimulator cells and untreated responder cells from spleens, lymph nodes, and thymuses, respectively were cocultured. These cells were derived from strains B10.A(3R) and B10.A(5R). As shown in Table I, thymus cells displayed weak or no stimulation in either direction. Spleen cells showed intermediate stimulation. Cocultures with the lymph nodes of B10.A(3R) as responders and

TABLE I

Comparison of MLR Responses in Cultures of Mouse Thymus, Spleen, and Lymph Node Cells Differing in the I-J Subregion

Lymphocytes*	Responder	Stimulator‡	[³H]TdR§	
			Experiment	Stimulation indices
			cpm	
Thymus	B10.A(3R)	B10.A(3R)	962.3 ± 63	—
	B10.A(3R)	B10.A(5R)	952.0 ± 84	0.99
	B10.A(5R)	B10.A(5R)	898.1 ± 102	—
	B10.A(5R)	B10.A(3R)	1,213.7 ± 102	1.35
Spleen	B10.A(3R)	B10.A(3R)	3,285.8 ± 367	—
	B10.A(3R)	B10.A(5R)	5,503.7 ± 450	1.67
	B10.A(5R)	B10.A(5R)	3,860.3 ± 413	—
	B10.A(5R)	B10.A(3R)	6,254.8 ± 356	1.62
Lymph nodes	B10.A(3R)	B10.A(3R)	2,046.6 ± 176	—
	B10.A(3R)	B10.A(5R)	6,317.8 ± 258	3.09
	B10.A(5R)	B10.A(5R)	1,953.1 ± 142	—
	B10.A(5R)	B10.A(3R)	4,135.0 ± 324	2.11

* Responder and stimulator lymphocytes were from the same source.
‡ Mitomycin-C-treated cells.
§ [³H]TdR stands for [³H]thymidine.

B10.A(5R) as stimulators yielded stimulation indices of about 3.0, although the stimulation indices in the reverse direction were about 2.4. This result suggested that at least one Lad locus maps in the *I-J* subregion, and also that lymph node cells are the most effective stimulators for this MLR difference. This could possibly be due to the higher concentration of T cells found in lymph nodes as compared to those found in the spleen. On the other hand, thymocytes may contain fewer mature MLR-responding cells and/or express *I-J*-determined MLR-stimulating antigens less strongly.

The Origin of the Stimulator Cell. Lymph node cells were used in subsequent experiments, since they stimulated the strongest MLR reactions. Lymph node B cells were purified by treatment with anti-Thy-1.2 serum and complement; T cells were purified by nylon wool passage (Table II). The stimulation index of the MLR response with B10.A(3R) as the responders and B10.A(5R) as stimulators was about 1.3 when B cells were used as stimulators (group 1). When nylon wool-purified T cells were employed as both responders and stimulators, the stimulation index increased to about 3.0. This suggested that the *I-J* subregion controls Lad determinants that exist on T cells. The MLR response was somewhat weaker when B10.A(5R) T cells were used as responders and B10.A(3R) T cells as stimulators.

Since there was some question as to whether a small number of B cells or macrophages might play an important role in this MLR stimulation, we attempted to further eliminate the B cells (group 4) and the macrophages (group 3) from the stimulator T-cell populations. The responder cells in groups 3 and 4 were nylon wool-purified T cells. The data with these two groups

TABLE II

Effects of Elimination of Macrophages and B Cells on MLR Stimulation by B10.A(3R) and B10.A(5R) Lymph Node Cells

Group	Responder	Stimulator	[³H]TdR incorporation*	
			Experiment (mean ± SD)	Stimulation indices
			cpm	
Group 1‡	B10.A(3R)	B10.A(3R)	4,540.1 ± 713	—
B cells	B10.A(3R)	B10.A(5R)	5,607.2 ± 409	1.23
	B10.A(5R)	B10.A(5R)	4,763.0 ± 581	—
	B10.A(5R)	B10.A(3R)	5,311.4 ± 774	1.12
Group 2§	B10.A(3R)	B10.A(3R)	852.4 ± 51	—
T cells	B10.A(3R)	B10.A(5R)	2,737.1 ± 146	3.24
	B10.A(5R)	B10.A(5R)	1,537.2 ± 131	—
	B10.A(5R)	B10.A(3R)	3,650.8 ± 209	2.38
Group 3‖	B10.A(3R)	B10.A(3R)	342.4 ± 41	—
macrophage-	B10.A(3R)	B10.A(5R)	998.3 ± 66	2.92
depleted T	B10.A(5R)	B10.A(5R)	531.3 ± 62	—
cells.	B10.A(5R)	B10.A(3R)	1,346.1 ± 107	2.52
Group 4¶	B10.A(3R)	B10.A(3R)	389.0 ± 50	—
B-cell-de-	B10.A(3R)	B10.A(5R)	1,265.1 ± 103	3.25
pleted T cells	B10.A(5R)	B10.A(5R)	560.7 ± 39	—
	B10.A(5R)	B10.A(3R)	1,305.2 ± 147	2.33

* [³H]TdR stands for [³H]thymidine.

‡ Prepared by anti-Thy-1.2 + complement treatment; 90% Ig⁺, 5% Thy-1.2⁺ by immunofluorescence.

§ Nylon wool-purified; 90% Thy-1.2⁺, 5% Ig⁺, by immunofluorescence.

‖ Prepared by treatment of group 2 cells by carbonyl iron method; 95% Thy-1.2⁺, 3% Ig⁺. Responders are same cells in group 2.

¶ Prepared by treatment of group 2 cells with anti-Ig + complement; 95% Thy-1.2⁺, 1.5% Ig⁺. Responders are same cells in group 2.

indicated that macrophages and B cells are probably not involved in the MLR stimulation in the B10.A(3R)–B10.A(5R) combination, since depletion did not significantly affect the stimulation index.

B10.S(9R) (*H-2ᵗ⁴*) and B10.HTT (*H-2ᵗ³*) differ not only in the *I-J* subregion, but also in the *I-C, S,* and *G* regions. However, previous studies have shown that differences between the *H-2ᵏ* and *H-2ᵈ* haplotypes in the *I-C, S,* and *G* regions do not cause an MLR reaction. In this combination also, the thymus cells do not give an MLR response, whereas the spleen cells and the lymph node cells show a substantial MLR response. Nylon wool-purified T cells gave the strongest MLR response in this combination also, again suggesting that the products of the Lad locus which maps in the *I-J* subregion are expressed only on T cells.

Inhibition of MLR Response by Anti-Ia Antisera.   Nylon wool-purified T cells of strain B10.A(3R) were cocultured with nylon wool-purified, mytomycin-C treated T cells of strain B10.A(5R) in the presence of various anti-Ia sera (Table III). The strongest inhibition was observed in the presence of the A.TH

TABLE III

Anti-Ia Inhibition of MLR Response by B10.A(3R) Responder Cells to B10.A(5R) Stimulator Cells

Responder*	Stimulator‡	Treatment§	Region(s) involved	[³H]TdR incorporation‖	Inhibition¶
				(mean ± SD)	%
B10.A(3R)	B10.A(3R)	NMS	–	584.8 ± 35.4	–
" "	B10.A(5R)	NMS	–	1,774.7 ± 113	–
" "	" "	A.TH anti-A.TL	I^k	504.3 ± 36.4	100
" "	" "	(C3H.Q × B10.D2) anti-AQR	(A, B, J, E)^k	873.6 ± 92.5	76
" "	" "	(A.TH × HTT) anti-A.TL	(A, B, J)^k	1,003.3 ± 72.8	65
" "	" "	(A.BY × HTT) anti-A.TL	(A, B, J)^k	988.7 ± 50.1	66
" "	" "	B10.A(3R) anti-B10.A(5R)	J^k	1,156.0 ± 62.7	52
" "	" "	B10.S(7R) anti-HTT	(E, C, S, G)^k	1,672.1 ± 143	8
" "	" "	[A.TH × B10.S(9R)] anti-A.TL	(A, B, S, G)^k	1,631.7 ± 96.5	11
" "	" "	A anti-A.AL	(C, S, G)^k	1,748.6 ± 213	0
" "	" "	A.TL anti-A.TH	I^s	1,709.1 ± 146	5
" "	" "	B10.A(2R) × C3H.Q anti-B10.A(4R)	(B, J, E, C, S, G)^b	1,538.5 ± 207	20
" "	" "	A × B10.A anti-B10	(K, I, D)^b	1,427.8 ± 153	29
" "	" "	B10.A(5R) anti-B10.A(3R)	J^b	1,793.6 ± 122	0
" "	" "	(B10 × AKR.M) anti-B10.A	(C, S, G, D)^d	1,810.7 ± 120	0
" "	" "	Anti-Thy-1.2	–	1,784.6 ± 217	0

* Nylon wool-purified T cells, 7 × 10⁵ cells per well.
‡ Mitomycin-C-treated T cells, 1.4 × 10⁶ cells per well.
§ Serum at 1.25% final dilution in the culture.
‖ [³H]TdR stands for [³H]thymidine.
¶ Inhibition = [1 – (allogeneic MLR in Ia antisera – syngeneic MLR in NMS)/(allogeneic MLR in NMS – syngeneic MLR in NMS)] × 100.

anti-A.TL serum. Substantial inhibition was also seen when (C3H.Q × B10.D2)F$_1$ anti-AQR, (A.BY × HTT)F$_1$ anti-A.TL, (A.TH × B10.HTT)F$_1$ anti-A.TL, and B10.A(3R) anti-B10.A(5R) were added to the cultures. All of these antisera contain antibodies against the *I-J* subregion gene products, with the B10.A(3R) anti-B10.A(5R) having antibodies only for the *I-J* region gene product (4). In the presence of antisera [B10.A(2R) × C3H.Q]F$_1$ anti-B10.A(4R) and (A × B10.A)F$_1$ anti-B10, a weak response was seen. These two antisera contain antibodies to antigens mapping to the left of the *I-J* subregion of B10.A(5R) and to the left of the *I-E* region of the responder strain B10.A(3R). This weak inhibition could possibly be directed against the responder cells. Antisera B10.S(7R) anti-B10.HTT, (A.TH × B10.S(9R))F$_1$ anti-A.TL, and A anti-A.AL, which do not contain antibodies against *I-J^k* products, gave no inhibition. Control antisera A.TL anti-A.TH and B10.A(5R) anti-B10.A(3R) also failed to inhibit. Anti-Thy-1.2 antiserum, as well as an anti-*H-2D^d* control

TABLE IV

Anti-Ia Inhibition of MLR Response by B10.A(5R) Responder Cells to B10.A(3R)
Stimulator Cells

Re-sponder*	Stimulator‡	Treatment§	Region	[³H]TdR incor-poration‖	Inhibi-tion¶
				(mean ± SD)	%
B10.A(5R)	B10.A(5R)	NMS	—	1,326.0 ± 208	—
B10.A(5R)	B10.A(3R)	NMS	—	2,711.2 ± 243	—
" "	" "	(A × B10.D2) anti-B10.A(4R)	(K, I, D)ᵇ	593.4 ± 176	100
" "	" "	(B10.A[2R] × C3H.Q) anti-B10.A(4R)	(B, J, E, C, S, G)ᵇ	953.7 ± 118	100
" "	" "	(B10.K × HTG) anti-B10.A(4R)	(B, J, E, C, S, G)ᵇ	1,061.5 ± 217	100
" "	" "	(A × B10.D2) anti-B10.A(5R)	(K, A, B)ᵇ	2,358.0 ± 138	25
" "	" "	B10.A(5R) anti-B10.A(3R)	Jᵇ	1,542.3 ± 172	84
" "	" "	B10.A(3R) anti-B10.A(5R)	Iᵏ	2,693.2 ± 233	5
" "	" "	A.TH anti-A.TL	Iᵏ	2,478.9 ± 215	17
" "	" "	B10.S(7R) anti-B10.HTT	(E, C, S, G)ᵏ	2,754.9 ± 215	0
" "	" "	(B10 × AKR.M) anti-B10.A	(C, S, G, D)ᵈ	2,863.3 ± 304	0
" "	" "	Anti-Thy 1.2	—	2,714.5 ± 210	4

* Nylon wool-purified T cells. 7×10^5 cells per well.
‡ Mitomycin-C-treated T cells. 1.4×10^6 cells per well.
§ Serum at 1.25% final dilution in the culture.
‖ [³H]TdR stands for [³H]thymidine.
¶ Inhibition = [1 - (allogeneic MLR in Ia antisera - syngeneic MLR in NMS)/(allogeneic MLR in NMS - syngeneic MLR in NMS)] × 100.

serum, (B10 × AKR.M)F₁ anti-B10.A, showed no inhibitory effect. These results again suggest that the stimulation in the B10.A(3R)–B10.A(5R) combination is caused by an *I-J* gene product and that anti-*I-J*ᵏ antisera contain antibodies against the MLR-stimulating antigens.

Results from the reciprocal combination, in which B10.A(5R) cells were used as responders and B10.A(3R) cells as stimulators, are shown in Table IV. In this combination, the stimulation indices were somewhat lower. Antisera (A × B10.D2)F₁ anti-B10.A(4R), [B10.A(2R) × C3H.Q]F₁ anti-B10.A(4R), (B10.K × HTG)F₁ anti-B10.A(4R), and B10.A(5R) anti-B10.A(3R), all of which could contain antibodies against the *I-J*ᵇ products, showed strong inhibition of the stimulator cell. This result suggests that the *I-J*ᵇ gene product also can stimulate in MLR, although not quite as strongly as the *I-J*ᵏ gene product in this test combination.

The specificity of the inhibition was further tested by using F₁ stimulator cells (Table V). Mitomycin-C-treated T cells from [B10.A(3R) × B10.A(5R)]F₁ animals were cocultured with the parental nylon wool-purified T cells as responders. Twice as many stimulating cells per well were used to compensate for the expected reduction in number of relevant stimulating molecules on F₁

TABLE V

Effects of Anti-Ia Sera on MLR Response between Parental Mice and Their F_1 Hybrids

Serum*	[³H]TdR incorporation‡			
	B10.A(3R)§ B10.A(5R)‖	B10.A(3R)§ B10.A(3R) × B10.A(5R) F₁‖	B10.A(5R)§ B10.A(3R) × B10.A(5R) F₁‖	B10.A(5R)§ B10.A(3R)‖
	cpm (mean ± SD)			
Syngeneic stimulation with NMS	1,741.3 ± 148	1,741.3 ± 148	2,142.0 ± 207	2,142.0 ± 207
Allogeneic stimulation in NMS	5,423.5 ± 468	5,537.8 ± 374	6,165.3 ± 576	6,203.5 ± 712
A.TH anti-A.TL (I)ᵏ	930.9 ± 119	1,265.0 ± 158	5,268.5 ± 479	5,153.5 ± 548
(A.TH × HTT) anti-A.TL (A, B, J)ᵏ	1,438.3 ± 123	1,640.7 ± 251	6,009.8 ± 1099	6,178.4 ± 733
7R anti-HTT (E, C, S, G)ᵏ	5,604.2 ± 329	5,332.0 ± 416	5,870.3 ± 396	5,992.2 ± 501
3R anti-5R (J)ᵏ	1,791.8 ± 254	1,908.3 ± 149	5,981.3 ± 1038	6,075.1 ± 562
2R × C3H.Q anti-4R (B, J, E, C, S, G)ᵇ	5,264.7 ± 591	4,960.5 ± 498	1,873.7 ± 96	2,069.9 ± 174
5R anti-3R (J)ᵇ	5,409.5 ± 296	5,237.4 ± 196	3,089.4 ± 270	3,187.3 ± 269
(B10 × AKR.M) anti-B10.A (C, S, G, D)ᵈ	5,326.0 ± 548	5,458.0 ± 607	6,231.4 ± 416	6,079.9 ± 714

* Final concentration of antisera was 1.25%.
‡ [³H]TdR stands for [³H]thymidine.
§ Responder; 7×10^5 cells per well.
‖ Stimulator; 2.8×10^6 cells per well, mitomycin-C treated.

cells. Antiserum B10.A(3R) anti-B10.A(5R) inhibited only when the responder cells were from B10.A(3R). Antiserum B10.A(5R) anti-B10.A(3R) had no effect on the stimulation of B10.A(3R) responder cells. The reciprocal result was observed when B10.A(5R) T cells were used as responding cells. These results suggest that the stimulation is specific, i.e., that inhibition by anti-Ia sera is directed against the specific MLR-stimulatory molecule and is not due to nonspecific binding or steric hindrance.

The second set of *I-J*-incompatible strains, B10.S(9R) and B10.HTT, was also used in inhibition studies with anti-Ia antisera (Table VI). The results were again consistent with the interpretation that only antibodies directed against the *I-J* gene product could inhibit the MLR response in this combination.

Elimination of Stimulating Cells with Anti-Ia Sera and Complement. Dye exclusion and ⁵¹Cr-release cytotoxic assays have so far been unsuccessful in detecting the Ia antigens controlled by the *I-J* subregion. The next series of experiments tested whether antisera directed against the *I-J* gene product, when reacted with appropriate cells in the presence of complement before establishing the mixed culture, could eliminate stimulation in *I-J*-incompatible T-cell combinations. Nylon wool-purified T cells were incubated with anti-Ia sera and complement. The dead cells were eliminated by centrifugation on Ficoll-Hypaque and the viable T cells recovered were for use as stimulators. Treatment with antisera A.TH anti-A.TL, (A.TH × B10.HTT)F₁ anti-A.TL, and B10.A(3R) anti-B10.A(5R), all of which contain anti-*I-J*ᵏ antibodies, completely eliminated the stimulating T cells in the B10.A(3R)–B10.A(5R) combination (Table VII). On the other hand, treatment with [A.TH × B10.S(9R)]

TABLE VI

Effects of Anti-Ia Antisera on MLR Responses between B10.HTT and B10.S (9R) T Cells

Responder*	Stimulator‡	Treatment§	Region	[³H]TdR incor-poration‖	Inhibi-tion¶
				(mean ± SD)	%
B10.HTT	B10.HTT	NMS	—	2,734.3 ± 356	—
B10.HTT	B10.S(9R)	NMS	—	5,968.4 ± 477	—
B10.HTT	B10.S(9R)	A.TH anti-A.TL	Iᵏ	1,964.6 ± 154	100
B10.HTT	B10.S(9R)	A.TL anti-A.TH	Iˢ	4,873.0 ± 403	34
B10.HTT	B10.S(9R)	B10.A(3R) anti-B10.A(5R)	Jᵏ	3,705.7 ± 323	70
B10.HTT	B10.S(9R)	(A.BY × B10.HTT) anti A.TL	(A, B, J)ᵏ	3,248.2 ± 216	84
B10.HTT	B10.S(9R)	(A.TH × B10.HTT) anti A.TL	(A, B, J)ᵏ	3,419.3 ± 458	79
B10.HTT	B10.S(9R)	B10.A(5R) anti-B10.A(3R)	Jᵇ	5,801.5 ± 512	5
B10.HTT	B10.S(9R)	(B10 × AKR.M) anti-B10.A	(C, S, G, D)ᵈ	5,991.1 ± 593	0
B10.S(9R)	B10.S(9R)	NMS	—	3,074.5 ± 296	—
B10.S(9R)	B10.HTT	NMS	—	7,155.3 ± 528	—
B10.S(9R)	B10.HTT	A.TL anti-A.TH	Iˢ	2,546.1 ± 142	100
B10.S(9R)	B10.HTT	(B10.K × A.TL) anti-B10.HTT	(A, B, J)ˢ	3,089.3 ± 219	100
B10.S(9R)	B10.HTT	A.TH anti-A.TL	Iᵏ	6,078.0 ± 467	26
B10.S(9R)	B10.HTT	B10.S(9R) anti-B10.HTT	Jˢ (C, S, G)ᵏ	3,893.8 ± 350	80
B10.S(9R)	B10.HTT	(3H.Q × B10.D2) anti-AQR	(A, B, J, E)ᵏ	6,476.7 ± 481	17
B10.S(9R)	B10.HTT	(B10 × AKR.M) anti-B10.A	(C, S, G, D)ᵈ	7,240.7 ± 593	0

* Nylon wool-purified T cells, 7×10^5 cells per well.
‡ Mitomycin-C-treated T cells, 1.4×10^6 cells per well.
§ Serum at 1.25% final dilution in the culture.
‖ [³H]TdR stands for [³H]thymidine.
¶ Inhibition = [1 − (allogeneic MLR in Ia antisera − syngeneic MLR in NMS)/(allogeneic MLR in NMS − syngeneic MLR in NMS)] × 100.

anti-A.TL, which could contain antibodies against products of the *I-A* and *I-B* subregions but not the *I-J* subregion, did not eliminate the stimulating cells in this combination. This suggested that the *I-J* gene products are expressed on a subpopulation of T cells that does not express the *I-A* and *I-B* subregion gene products. Surprisingly, treatment with antiserum B10.S(7R) anti-B10.HTT, directed against the *I-E* and *I-C* subregions of the H-2^k haplotype, reduced the MLR response to B10.A(5R) stimulators by 84%. This result suggests either that the I-J^k gene products and the I-E^k (I-C) gene products are expressed on the same T-cell subpopulation, or that the I-J^k antigens can stimulate only when I-E^k (I-$C)^k$-bearing cells are also present in the culture. Treatment with (B10.AKM × ASW)F₁ anti-A.TH (Anti-H-2Dᵈ) and (B10.D2 × A)F₁ anti-HTI (anti-H-2Kᵇ) completely eliminated the MLR response, indicating that as expected, the I-J^k product is expressed on cells which also carry the H-$2K$ and H-$2D$ gene products. Likewise, the cell carrying the *I-J* product expresses the

TABLE VII

Anti-Ia Elimination of B10.A(5R) MLR Stimulation of B10.A(3R) Responder Cells

Re-sponder*	Stimulator‡	Pretreatment§	Region	Dead cells	[³H]TdR incor-poration‖	Inhi-bi-tion¶
				%		%
B10.A(3R)	B10.A(3R)	NMS + C'**	—	(5)	906.7 ± 63	—
B10.A(3R)	B10.A(5R)	NMS + C'	—	(5)	2,854.5 ± 134	—
" "	" "	A.TH anti-A.TL + C'	Iᵏ	(15)	728.3 ± 51	100
" "	" "	(A.TH × HTT) anti-A.TL + C'	(A, B, J)ᵏ	(15)	943.0 ± 105	98
" "	" "	B10.A(3R) anti-B10.A(5R) + C'	Jᵏ	(15)	936.7 ± 133	99
" "	" "	(A.TH × 9R) anti-A.TL + C'	(A, B, S, G)ᵏ	(15)	2,480.3 ± 217	19
" "	" "	B10.S(7R) anti-HTT + C'	(E, C, S, G)ᵏ	(15)	1,231.8 ± 130	84
" "	" "	A anti-A.AL + C'	(C, S, G)ᵏ	(15)	2,735.3 ± 238	6
" "	" "	B10.A(5R) anti-B10.A(3R) + C'	Jᵇ	(15)	2,697.5 ± 214	8
" "	" "	(B10.AKM × ASW) anti-A.TH + C'	Dᵈ	(>90)	835	100
" "	" "	(B10.D2 × A) anti-HTI + C'	(K, A, B, J, E)ᵇ	(>90)	824	100
" "	" "	Anti-Thy 1.2 + C'	—	(>90)	851	100

* Nylon wool-purified T cells.

‡ Mitomycin-C-treated, nylon wool-purified T cells.

§ Stimulator cell treated with ¹/₁₀ serum plus agarose and spleen cell-adsorbed EDTA-treated rabbit C', then washed.

‖ [³H]TdR stands for [³H]thymidine.

¶ % Inhibition = [1 − (allogeneic MLR of antisera treatment − syngeneic MLR)/(allogeneic MLR − syngeneic MLR)] × 100.

** C' stands for complement.

Thy-1.2 antigen, since anti-Thy-1.2 treatment eliminates stimulation. The frequency of I-J^k-positive cells in the nylon wool-purified preparations is estimated to be about 10% of the lymph node T-cell population, on the basis of the observed percentage of dead cells after antiserum treatment.

When T cells from B10.A(3R) mice were treated with sera containing anti-I-J^b in the presence of complement, then used as stimulators of nylon wool-purified, responder T cells from B10.A(5R) mice, MLR stimulation was eliminated. Again, anti-H-$2D^d$ and anti-H-$2K^b$, as well as anti-Thy-1.2, completely eliminated the MLR response in this combination.

To determine whether the I-J^k and I-E^k (I-C) products are on the same cells, nylon wool-purified T cells from strain B10.A(5R) were divided into two parts;

TABLE VIII

Expression of I-J Antigens on T Cells of F_1 Hybrids

Responder*	Stimulator‡	Treatment§	Region	[³H]TdR incorporation‖
				cpm (mean ± SD)
B10.A(3R)	B10.A(3R)	NMS + C′	(−)	926.3 ± 60
B10.A(3R)	[B10.A(3R) × B10.A(5R)]F₁	NMS + C′	(−)	2,408.1 ± 254
B10.A(3R)	(B10.A(3R) × B10.A(5R))F₁	B10.A(3R) anti-B10.A(5R) + C′	(Jᵏ)	873.6 ± 116
B10.A(3R)	(B10.A(3R) × B10.A(5R))F₁	B10.A(5R) anti-B10.A(3R) + C′	(Jᵇ)	917.0 ± 130
B10.A(3R)	(B10.A(3R) × B10.A(5R))F₁	3R anti-5R + 5R anti-3R + C′	(Jᵏ + Jᵇ)	834.5 ± 77
B10.A(5R)	B10.A(5R)	NMS + C′	(−)	738.7 ± 126
B10.A(5R)	(B10.A(3R) × B10.A(5R))F₁	NMS + C′	(−)	2,310.9 ± 231
B10.A(5R)	(B10.A(3R) × B10.A(5R))F₁	B10.A(3R) anti-B10.A(5R) + C′	(Jᵏ)	945.3 ± 93
B10.A(5R)	(B10.A(3R) × B10.A(5R))F₁	B10.A(5R) anti-B10.A(3R) + C′	(Jᵇ)	734.3 ± 80
B10.A(5R)	(B10.A(3R) × B10.A(5R))F₁	3R anti-5R + 5R anti-3R + C′	(Jᵏ + Jᵇ)	820.7 ± 117

Footnotes *, ‡, §, and ‖ are the same as those in Table VII.
T cells of each F_1 and parental lymph nodes were treated with anti-Ia antisera or NMS followed by rabbit complement, and 2.8 × 10⁶ stimulator T cells were cocultured with 7 × 10⁵ parental responder T cells in a 200-µl volume.

one part was treated with antiserum B10.A(3R) anti-B10.A(5R) plus complement, to remove the *I-Jᵏ*-positive cells, the other was treated with B10.S(7R) anti-B10.HTT to remove the *I-Eᵏ* (*I-C*)ᵏ-positive cells. After removal of the dead cells, the two parts were mixed together and used as the stimulating population for B10.A(3R) nylon wool-purified responder T cells. This mixture of the two populations of cells was unable to stimulate in the MLR culture, indicating that the *I-J* gene products and the *I-E/I-C* gene products are expressed on the same T cells and arguing against a synergistic stimulatory effect by discrete *I-J/* and *I-E/I-C*-positive cells. Of course it is not excluded that the *I-E/I-C* gene product might also be expressed on other T-cell subpopulations in addition to the *I-J*-positive one. However, the inhibition studies indicate that the *I-J* gene product and the *I-E/I-C* product must be distinct molecules on the cell surface, since anti-*I-E/I-C* antibodies fail to block stimulation.

Expression of I-J Antigen on F_1 Cells. Experiments were carried out to test whether individual F_1 T cells express both parental *I-J* antigens, or whether allelic exclusion is seen. B10.A(3R) and B10.A(5R) responder cells were stimulated by [B10.A(3R) × B10.A(5R)]F₁ cells. MLR stimulation could be eliminated by adding either B10.A(3R) anti-B10.A(5R) or the reciprocal antisera in the presence of complement, indicating that both *I-Jᵏ* and *I-Jᵇ* gene products are expressed on the same cell in the F_1 hybrid, and suggesting that no allelic exclusion occurs at the *Ia-5* locus (Table VIII).

Discussion

Previous analyses of intra-*H-2* recombinants in a number of laboratories have mapped several MLR-stimulating Lad loci with the *H-2* complex (1, 2). Recent studies have defined the *I-J* subregion products as T cell-specific molecules, through detection of *I* region serological markers on soluble suppressor factors (4) and on suppressor T cells (5). Our data now demonstrate that genes located in the *I-J* subregion also specify membrane structures on T cells that are capable of stimulating in the mixed leukocyte reaction. Routine typing for the *I-J* gene products of all *H-2* haplotypes has so far not been possible, due to the rather cumbersome functional assays that have been necessary to detect them. The MLR assay described above offers a more direct and practical approach to the detection of the *I-J* subregion products and may permit a better characterization of the antigens coded by this region. Of course, at present we can only assume that the MLR is detecting the same *I-J* gene products that are involved in suppression. Three points argue rather strongly for this assumption: (*a*) both kinds of molecules are controlled by the *I-J* subregion; (*b*) they are expressed primarily or exclusively on T cells; (*c*) they react with the same panel of anti-I-J sera. However, it must be acknowledged that the evidence is still circumstantial. Further characterization of the molecules involved will be required to establish identity of the suppressor and MLR-stimulating factors.

Considerable variation was seen in the percent of inhibition of MLR obtained when different antisera were added to the cultures. For example, antiserum A.TH anti-A.TL always gave the strongest inhibition, suggesting that it might have the highest titer of anti-*I-J* antibodies. In several cases, there were suggestions that there might be some weak inhibition directed at the responder cells. Niederhuber et al. (12, 13) have presented data indicating that gene products of the *I-J* and/or *I-E* subregions on splenic macrophages are essential to the primary in vitro response to T-dependent antigens. However, the data presented here show no change in the stimulation index when macrophages are eliminated, suggesting that macrophages are not primarily responsible for the MLR reaction among *I-J*-incompatible strains. Our studies also showed that thymus cells are essentially ineffective in stimulating the MLR in this combination, suggesting a very weak representation of *I-J* antigens on these cells.

These studies resulted in the interesting finding that the *I-J* product and the *I-E/I-C* product seem to be expressed on the same T-cell subpopulation. However, there is no detectable expression of other *I* subregion products on the MLR-stimulating T cells. (The *H-2K* and *H-2D* gene products, as well as the Thy-1.2 antigen are, of course, expressed on these cells.) If these subpopulations of the T cells do in fact represent the suppressor T cells, this may have interesting implications for the roles of these two gene products. The inhibition studies implied that they represent two discrete molecular entities on the cell surface, since the anti-I-E/I-C serum failed to inhibit stimulation when added to the culture. However, it should be noted that in the combinations employed, there was no incompatibility for the *I-E/I-C* region. It remains to be determined whether an *I-E* region difference alone on either B or T cells can stimulate the

MLR. Furthermore, it has not yet been established whether or not the relevant *I-E/I-C* gene product on stimulating T cells is the same antigen detected by cytotoxicity assays on B cells.

The *I-J* gene is expressed only on T cells, whereas the *I-E/I-C* products are apparently expressed on both T and B cells. Perhaps the *I-J* genes of the T cells play a role in the generation of the T-cell suppressor factors, while the *I-E/I-C* gene products on the same cell could play a role in interaction or recognition between different cell populations. Further characterization of the various functional T-cell populations will be necessary to resolve such questions.

Summary

We have examined the MLR reaction in two sets of recombinants that differ in the *I-J* subregion. In both cases, significant stimulation was mediated by antigens controlled by genes in the *I-J* subregion. This stimulation was inhibitable by the addition of the culture of antisera directed against the *I-J* gene products on the stimulator cell. The specificity of this inhibition was shown by specific blocking of the relevant gene product on F_1 hybrid stimulator cells. MLR stimulation was also eliminated by pretreatment of the stimulator population with anti-*I-J* sera plus complement. Pretreatment of F_1 hybrid stimulator T cells with anti-*I-J* sera directed against either parental *I-J* product in the presence of complement, completely eliminated stimulation, indicating that there is no allelic exclusion of the relevant *I-J* products. Pretreatment with an anti-*I-E/I-C* serum and complement also eliminated stimulation, suggesting that the stimulating T cells express both *I-J* and *I-E/I-C* subregion products. This assay offers a potentially more direct and practical method for serological detection of the *I-J* products.

References

1. Bach, F. H., M. B. Widmer, M. Segal, M. L. Bach, and J. Klein. 1972. Genetic and immunological complexity of major histocompatibility regions. *Science (Wash. D. C.).* 176:1024.
2. Meo, T., J. Vives, V. Miggiano, and D. C. Shreffler. 1973. A major role for *Ir-1* region of the mouse *H-2* complex in the mixed leukocyte reaction. *Transplant. Proc.* 5:377.
3. Dorf, M. E., J. M. D. Plate, J. H. Stimpfling, and B. Benacerraf. 1975. Characterization of immune response and mixed lymphocyte reactions in selected intra-*H-2* recombinant strains. *J. Immunol.* 114:602.
4. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response in the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the *H-2* histocompatibility complex. *J. Exp. Med.* 144:713.
5. Murphy, D. B., L. A. Herzenberg, K. Okumura, L. A. Herzenberg, and H. O. McDevitt. 1976. A new *I* subregion (*I-J*) marked by a locus (*Ia-4*) controlling surface determinants on suppressor T lymphocytes. *J. Exp. Med.* 144:699.

6. Shreffler, D. C., C. S. David, S. E. Cullen, J. A. Frelinger, and J. E. Niederhuber. 1977. Serological and functional evidence for further subdivision of the *I* region of the *H-2* gene complex. *Cold Spring Harbor Symp. Quant. Biol.* 41:477.

7. 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. 683.

8. Julius, M. D., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* 3:645.

9. Sher, R., and A. Glover. 1976. Isolation of human eosinophils and their lymphocyte like rosetting properties. *Immunology.* 31:337.

10. Bach, F. H., and M. F. Bach. 1972. Comparison of mitomycin C and X-irradiation as blocking agents in one-way mixed leukocyte cultures. *Nat. New Biol.* 235:243.

11. Boyse, E. A., L. Hubbard, E. Stockert, and M. E. Lamm. 1970. Improved complementation in cytotoxic tests. *Transplantation (Baltimore).* 10:446.

12. Niederhuber, J. E., and D. C. Shreffler. 1977. Anti-Ia serum blocking of macrophage function in the *in vitro* humoral response. *Transplant. Proc.* 9:875.

13. Niederhuber, J. E., L. Mayo, and D. C. Shreffler. 1977. The requirement of Ia positive macrophages in the primary *in vitro* humoral response. *In* Proceedings of the Third Ir Gene Workshop. H. O. McDevitt, editor. Academic Press, Inc., New York. In press.