I-REGION GENES ARE EXPRESSED ON T AND B LYMPHOCYTES

Studies of the Mixed Lymphocyte Reaction (MLR)*

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A small region of the major histocompatibility complex (MHC)[1] of both the mouse and man has been found to be associated with several important immunological phenomena (1–2). This region in the mouse is situated between genes *H-2K*, the K end of *H-2*, and *Ss,Slp* (a serum protein gene) within the *H-2* complex, and is called the I region (3). The I region contains the immune response (*Ir*) genes which regulate humoral and certain aspects of cell-mediated immune responses to a number of antigens (4–7). Genes controlling strong mixed lymphocyte reactions (MLR) and graft-vs.-host reactions (GVHR), in MHC different combinations, also have been localized in the I region (8–10). Another immunological phenomenon, the interaction of T and B lymphocytes in adoptive transfer systems, also appears to be affected by genes associated with the K end of the *H-2* complex (11). In addition, the I region codes for a series of lymphocyte cell surface antigens, the Ia antigens, which can be detected by alloantisera raised in congenic mice differing only within the I region (references 12–16 and footnote 2). Whether these four phenomena—*Ir*, MLR-GVHR, "interaction barrier," and Ia—are experimental representations of only one or of several distinct gene products is presently unknown.

Because of methodological simplicity and reproducibility, anti-Ia sera and MLR are useful analytical tools for the study of the function and genetics of the I region. Additionally, the sera directed against Ia specificities are promising reagents for the detection of lymphocyte receptors used as inhibitors of various immune reactions controlled by I-region genes. However, the anti-Ia sera studied thus far have been found to be predominantly directed against B-cell specificities (15, 16). Since there is a considerable amount of evidence that *Ir* genes are expressed on T cells (1, 17), or on both T and B cells (18), we decided to perform

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[1] *Abbreviations used in this paper:* GVHR, graft-vs.-host reactions; *Ir*, immune response genes; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction.

[2] Frelinger, J. A., J. Neiderhuber, C. S. David, and D. C. Shreffler. 1974. Evidence for the expression of Ia (*H-2*-associated) antigens on thymus-derived lymphocytes. *J. Exp. Med.* **140:** 1273.

some direct experiments to study the expression of T-cell antigens controlled by the I region.

Materials and Methods

Animals. 2- to 5-mo old mice of both sexes were used. In one experiment animals of the same sex have been used. The strains and their genetic make up are summarized in Table I.

MLR Cultures. Unidirectional MLR cultures were established in Falcon Microtest II plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) in 0.2 ml/well containing 1×10^6 responder and 1×10^6 irradiated (3,000 rad from a ^{127}Cs radiation source) stimulator cells in medium RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 5% human serum, 0.002 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U penicillin and 100 μg streptomycin/ml, and 100 μCi [^{3}H]thymidine (New England Nuclear Co., Boston, Mass., 6.7 Ci/mol) for about 16 h. The cells were harvested onto glass wool fiber filters in a Mash II (Microbiological Associates, Inc., Bethesda, Md.) cell harvester, and the radioactivity was measured in a 2,5-diphenyloxazole, 1,4,-bis[2-(5-phenyloxazolyl)]benzene, and toluene scintillation mixture in a liquid scintillation spectrometer.

Preparation of Cell Suspensions. Cell suspensions were made by gentle teasing of the organs with two pairs of sharp forceps. Thymocytes were prepared from thymuses after the removal of the parathymic lymph nodes, stained previously by intraperitoneally injected india ink.

Separation of T and B lymphocytes. T and B cells were isolated from lymph node cell suspensions. T cells were separated by the nylon wool filtration technique (19) or by passing the cells through a Sepharose column coupled with polyvalent antimouse immunoglobulin. The B cells were separated by removing the T cells with anti-Thy 1.2 serum and complement followed by the removal of dead cells by centrifugation through a 35% bovine serum albumin gradient. The separated cells were tested in every experiment by anti-Thy 1.2 serum titration. No T-cell preparates under 90% and no B-cell preparations above 10% Thy 1.2 positivity were used in the experiments.

Results

The rationale of the experiments was the following: If the I region does not code for T-cell antigens, then T cells should not stimulate the MLR across differences in the I region alone. Conversely, a positive reaction would indicate that the I region does control T-cell surface antigens.

Five combinations between eight mouse strains have been used in the MLR cultures. The *H-2* map of these can be seen in Table I. The combinations have been chosen so that they should comprise a series of decreasing genetic differences within the *H-2* complex. Combination 1 (C3H and C3H.SW) differs in the whole *H-2* complex; combinations 2 and 3 [A.TL and A.TH; B10.T(6R) and B10.AQR] differ in the whole I region, but are identical in *H-2K* and *H-2D*. Finally, combinations 4 and 5 [(B10.HTT × A/J)F_1 and A.TH; B10.S(7R) and B10.HTT] differ only in one I subregion (I-C) and *Ss*,*Slp*, and the latter appears to have no effect in the MLR (8). The five combinations were tested in three different experimental sets (Tables II-IV). First, the reactions of lymph node lymphocytes against lymph node lymphocytes and thymocytes were compared (Table II). In the second set of experiments, lymph node lymphocytes were the responder cells stimulated by purified lymph node T or B cells (Table III). Finally, purified T cells as responder cells were reacted against purified T or B stimulator cells (Table IV).

The results can be seen in Tables II–IV. Five points of interest emerge from a review of these results: (*a*) In all three experimental sets, a complete MHC

TABLE 1

Genetic Combinations Used in the MLR

Combination	Strain	*H-2* haplotype	*H-2* genotype						Genetic difference
			H-2K	*Ir-1A*	*Ir-1B*	I-C	*Ss,Slp*	*H-2D*	
1	C3H/DiSn	*k*	k	k	k	k	k	k	
	C3H.SW	*b*	b	b	b	b	b	b	Whole *H-2*
2	A.TL	t^1	s	k	k	k	k	d	*Ir-1A, Ir-1B,*
	A.TH	t^2	s	s	s	s	s	d	I-C, *Ss,Slp*
3	B10.AQR	y^1	q	k	k	d	d	d	*Ir-1A, Ir-1B,*
	B10.T(6R)	y^2	q	q	q	q	q	d	I-C, *Ss,Slp*
4	(B10.HTT × A/J)F_1	t^3/a	s/k	s/k	s/k	k/d	k/d	d/d	I-C, *Ss,Slp*
	A.TH	t^2	s	s	s	s	s	d	
5	B10.HTT	t^3	s	s	s	k	k	d	I-C, *Ss,Slp*
	B10.S(7R)	t^2	s	s	s	s	s	d	

TABLE II

MLR against Lymph Node and Thymus Lymphocytes as Stimulator Cells

Combination	Strains		Cells		Syngeneic		Allogeneic		Stimulation index	*t* test *P* <
	Responder	Stimulator	Responder	Stimulator	cpm	± SD	cpm	± SD		
1	C3H/DiSn	C3H.SW	L*	L	2,517	325	62,267	8,970	24.84	0.001
	C3H/DiSn	C3H.SW	L	Thy‡	955	132	6,490	1,330	6.80	0.001
2	A.TL	A.TH	L	L	7,590	591	22,569	5,205	2.97	0.001
	A.TL	A.TH	L	Thy	8,123	3,239	23,454	3,923	2.89	0.01
	A.TH	A.TL	L	L	3,601	505	34,385	12,186	9.55	0.01
	A.TH	A.TL	L	Thy	2,630	182	9,947	1,029	3.78	0.01
3	B10.T(6R)	B10.AQR	L	L	9,184	3,430	39,208	1,746	4.27	0.001
	B10.T(6R)	B10.AQR	L	Thy	4,257	1,129	14,198	3,493	3.33	0.001
	B10.AQR	B10.T(6R)	L	L	6,957	4,546	38,877	4,504	5.59	0.001
	B10.AQR	B10.T(6R)	L	Thy	8,410	2,214	19,209	3,036	2.28	0.001
4	(B10.HTT × A/J)F_1	A.TH	L	L	924	242	1,274	156	1.38	0.05
	(B10.HTT × A/J)F_1	A.TH	L	Thy	629	117	5,099	899	8.11	0.001

* L, lymph node lymphocyte.
‡ Thy, thymocyte.

difference (C3H versus C3H.SW) gave a stronger reaction than the I-region differences, in agreement with previous findings of others (8), and our own unpublished results. (*b*) T lymphocytes (thymocytes and lymph node T cells) did stimulate in all of the combinations. (*c*) Lymph node T cells stimulated as well or better in the MLR than thymocytes. This finding rules out the possibility that the stimulation generated by thymocytes was due to a Tla-antigen difference.

TABLE III

MLR against Purified Lymph Node T and B Cells

Combination	Strains		Cells		Syngeneic		Allogeneic		Stimulation index	*t* test $P <$
	Responder	Stimulator	Responder	Stimulator	cpm	± SD	cpm	± SD		
1	C3H/DiSn	C3H.SW	L*	T‡	20,876	2,915	110,416	16,408	5.29	0.001
	C3H/DiSn	C3H.SW	L	B§	8,388	3,238	120,554	4,268	14.37	0.001
	C3H.SW	C3H	L	T	10,624	2,980	102,574	10,527	9.65	0.001
	C3H.SW	C3H	L	B	3,912	690	145,623	4,075	37.22	0.001
2	A.TL	A.TH	L	T	24,714	2,097	122,205	23,933	4.95	0.001
	A.TL	A.TH	L	B	13,200	3,251	44,308	6,544	3.36	0.001
3	B10.AQR	B10.T(6R)	L	T	2,017	464	5,134	1,580	2.55	0.01
	B10.AQR	B10.T(6R)	L	B	2,120	383	5,932	287	2.80	0.001
4	(B10.HTT × A/J)F_1	A.TH	L	T	2,924	503	13,563	978	4.64	0.001
	(B10.HTT A/J)F_1	A.TH	L	B	1,808	754	2,161	43	1.20	NS ‖
5	B10.S(7R)	B10.HTT	L	T	5,416	1,747	12,051	1,034	2.23	0.01
	B10.S(7R)	B10.HTT	L	B	18,334	4,476	17,844	2,833	0.97	NS

* L, lymph node lymphocyte.
‡ T, nylon wool purified lymph node T cells.
§ B, anti-θ + C′ purified lymph node B cells.
‖ NS, not significant.

The Tla gene is located close to *H-2D* and is expressed only in thymocytes of normal animals (20). Therefore, the stimulation generated by lymph node T cells could not be due to the Tla antigens. (*d*) Isolated lymph node T cells, as responder cells, gave essentially the same pattern of response to T- and B-cell stimulators as did lymph node cell suspensions (Table IV), suggesting that the reaction against T-stimulator cells was not the result of "back stimulation." In the case of back stimulation, the irradiated T-stimulator cells would presumably have responded towards the nonirradiated B cells in the responder lymph node cell suspension by secreting some mitogenic factor, thus triggering the responder cells to multiplication (21–22). Further, the residual B-cell content of the purified T-responder cells theoretically could have caused back stimulation. However this appears to be unlikely. Had the reaction observed between T-responder and T-stimulator cells been due to B-cell contamination only, the reciprocal combination should have produced a much stronger response (Table IV, exps. 2 and 3), which was not the case. For instance, B10.AQR T cells against B10.T(6R) T cells produced a stimulation index of 17.06, while the same B10.T(6R) T cells as responders against B10.AQR B cells gave only a stimulation index of 2.12 (Table IV, exp. 3). (*e*) In the combinations differing only in subregion I-C and in *Ss,Slp* (combinations 4 and 5), only T cells stimulated and no significant reaction was obtained using B-stimulator cells. This observation suggests that the I-C subregion in haplotypes *t2* and *t3* (Table I) may differ exclusively for T-cell surface structures. The fact that B cells do not stimulate in these combinations makes it unlikely that the stimulation obtained by T-cell fractions was due to B-cell comtamination. The same may be true for combina-

tions 3 and 4 in Table IV, since in all of these cases T cells stimulated better than B cells.

Discussion

These experiments demonstrate that the I region does code for both T- and B-cell surface structures as recognized in the MLR. Whether these antigens are expressed on all cells within the T- and B-cell populations, respectively, has not been determined. Our results are to a certain extent at variance with part of the serological evidence in the literature demonstrating preponderant B-cell expression of I-region antigens (15, 16). However in those experiments the I-region gene products were tested by only Ia sera in complement-mediated cytotoxicity reactions, and the presence of nonlytic T-cell specific antibodies cannot be ruled out. Furthermore, the sensitivity of the cytotoxic reaction is greatly influenced by the method used, e.g., Frelinger et. al.[2] using a highly sensitive microtechnique could detect anti-Ia activity directed against T cells.

The fact that, in combinations differing only in I-C and *Ss,Slp*, only T cells stimulated in the MLR suggests that some of the I-region genes may be preferentially expressed on T lymphocytes. A similar interpretation could apply to the experiments of Fathman and colleagues (23). They found that in the B10.A(4R) vs. B10.A(2R) combination, only B cells stimulated the MLR.

TABLE IV

MLR against Purified Lymph Node T and B Cells; Responder Cells Purified Lymph Node T Cells

Combination	Strains		Cells		Syngeneic		Allogeneic		Stimulation index	t test $P <$
	Responder	Stimulator	Responder	Stimulator	cpm	± SD	cpm	± SD		
1	C3H/DiSn	C3H.SW	T*	T	4,536	466	86,383	9,502	19.04	0.001
	C3H/DiSn	C3H.SW	T	B‡	13,177	4,685	160,135	15,413	12.15	0.001
	C3H.SW	C3H	T	T	2,187	295	63,810	3,655	29.18	0.001
	C3H.SW	C3H	T	B	3,260	2,163	209,222	18,727	64.18	0.001
2	A.TL	A.TH	T	T	1,892	663	5,827	699	3.08	0.001
	A.TL	A.TH	T	B	4,009	1,446	4,662	1,982	1.91	0.01
	A.TH	A.TL	T	T	28,861	5,972	351,259	50,190	12.17	0.001
	A.TH	A.TL	T	B	27,930	3,959	245,868	13,301	8.80	0.001
3	B10.T(6R)	B10.AQR	T	T	6,670	1,345	26,271	4,418	3.94	0.001
	B10.T(6R)	B10.AQR	T	B	4,138	1,184	8,783	2,839	2.12	0.05
	B10.AQR	B10.T(6R)	T	T	2,058	655	35,100	7,863	17.06	0.001
	B10.AQR	B10.T(6R)	T	B	1,080	209	3,919	1,441	3.63	0.02
4	(B10.HTT × A)F_1	A.TH	T	T	279	10	490	78	1.76	0.001
	(B10.HTT × A)F_1	A.TH	T	B	610	128	644	174	1.06	NS§
5	B10.HTT	B10.S(7R)	T	T	3,216	593	5,386	673	1.67	0.05
	B10.HTT	B10.S(7R)	T	B	4,132	1,287	4,460	1,290	1.08	NS

* T, nylon wool purified lymph node T cells.
‡ B, anti-θ + C′ purified lymph node B cells.
§ NS, not significant.

Therefore, we think it is reasonable to predict that certain I-region genes will be found to be expressed preferentially on T cells and/or others on B cells. It seems possible, furthermore, that the selective expression of I-region genes may apply for T- and B-cell subpopulations as well.

Summary

Unidirectional mixed lymphocyte reactions (MLR) were performed between mouse strains differing for various segments within the *H-2* complex. Thymocytes and purified lymph node T cells and B cells were used as stimulator cells. In three of five combinations studied, differing only within the I region, both T and B cells stimulated in the MLR. This suggests that the region codes for both T- and B-cell surface structures. However, if the difference was restricted to one I subregion (I-C), only T cells stimulated. This finding suggests that some of the I-region genes may be expressed either in T or in B cells.

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