Brief Definitive Report

I-N
A Newly Described *H-2 I* Subregion Between *K* and *I-A**

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The murine *H-2* locus was initially considered a single, transplantation antigen-encoding gene (1). However, by analysis of newly described traits and recombinant mice, it is now clear that this locus is in fact a gene complex (2, 3). Genetic, functional, and structural interrelationships exist among the *H-2* loci (3). Presently, five *H-2* regions are recognized: *K*, *I*, *S*, *G*, and *D* (2, 3). The *I* region is the most intricate of these, having been divided into *A*, *B*, *J*, *E*, and *C* subregions (3). The *Ir-1A* (immune response) locus demarcates the *I-A* subregion, which is bounded by the crossovers in recombinant haplotypes t1 (A.TL) and y1 (AQR) on the left (centromeric) side, and by h4 [B10.A(4R)] and g2 (D2.GD) on the right side (3). Several additional traits are controlled by *I-A*: immunosuppression, transplantation antigens, T lymphocyte-stimulating determinants, cell-interaction structures, Ia antigens, and determinants on soluble helper factors and their respective receptors (3). It is not clear whether these traits are manifestations of one or several structural genes. Separation of *I-A* loci by recombination is one approach whereby *I-A* complexity might be resolved.

We suggest a further subdivision of the *I* region. Specifically, *I-A* is divisible into two subregions by intercalating a new subregion, *I-N*, between *K* and *I-A*. The *I-N* region is distinguished by a locus (or loci), *Ia-7*, which controls expression of a surface structure on a lymphocyte subpopulation. A determinant, which provides a strong proliferative stimulus to responding cells in mixed leukocyte culture (MLC), maps within *I-N* as well. The left boundary of *I-N* is established by the cross-over in recombinant AQR, the right boundary by the crossover in recombinant A.TL. The designation *I-N*, rather than *N*, was chosen in view of the phenotypic traits under *I-N* control; these traits resemble more closely those that are *I* region controlled than those that are *H-2K* encoded.

Materials and Methods

Animals. Mice (male and female, 6–12 wk old) were obtained from our colony. Guinea pigs were obtained from Mogul-Ed, Oshkosh, Wis.

Media. All media and supplements were purchased from Grand Island Biological Co., Grand Island, N. Y. Fetal calf serum (FCS) was obtained from Flow Laboratories, Inc., Rockville, Md.

Antiserum. (A.TL × B10.D2)F_1 anti-B10.BR serum was produced by intraperitoneally injecting each F_1 recipient mouse with a suspension of B10.BR spleen, lymph node, and thymus

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cells (1×10^7 cells in 1 ml of phosphate-buffered saline) weekly for 5 wk. Animals were bled from the retro-orbital sinus 4 d after the last immunization. In some experiments, diluted serum (75 μl, 1:10 in medium 199 with 5% FCS) was absorbed twice with B10.T(6R) lymph node and spleen cells (1×10^8 cells/absorption, 10 min at 4°C) before assay.

Microcytotoxicity Test. A two-stage, dye-exclusion microcytotoxicity test was performed on lymph node cell targets according to Frelinger et al. (4) with modifications suggested by Dr. Donal Murphy (Yale University, New Haven, Conn. Personal communication.) (5). Fresh guinea pig serum, obtained bimonthly by heart puncture, served as complement. Complement controls were usually <5% dead cells. Between 100 and 200 cells were counted per well.

MLC. Mishell-Dutton medium supplemented with 2% FCS and 5×10^{-5} M 2-mercaptoethanol was used throughout (6). X-irradiated (2,000 R), washed splenocytes were pipeted into each of four replicate wells (0.5×10^6 cells/well) of a microtiter plate (Linbro 76-002-05, Linbro Chemical Co., Hamden, Conn.). Responding lymph node cells were added to each well (0.5×10^6 cells/well). Total culture vol was 0.2 ml. After 4 d at 37°C in a humidifed atmosphere of 5% CO_2 in air, an aliquot of [^{3}H]thymidine (New England Nuclear, Boston, Mass., 25 μl of 0.04 mCi/ml culture medium) was added to each well. Cultures were harvested 6 h later, and samples were counted in Aquasol (New England Nuclear) in a liquid scintillation counter (model 3330, Packard Instrument Co., Inc., Downers Grove, Ill.).

Results

Certain primary, MLC-proliferative responses (Fig. 1) led us to consider the possibility that an as yet undefined locus might exist between *K* and *I-A*. Table I depicts the haplotypes of strains used in these and other experiments (7). Responding (A.TL × B10.D2)F_1 cells were stimulated with cells from three strains; stimulating strains were chosen such that genetic differences were limited to discrete *H-2* regions (Fig. 1, Exp. I). Fully *H-2*-incompatible C57BL/10 cells, and *I* region-incompatible B10.S(7R) cells, provoked strong proliferative responses. B10.BR cells, which differ from the responding F_1 hybrid at *K*, *D*, *Qa*, and *Tla*, resulted in a proliferative response equal to (two experiments) or greater than (three experiments) the response either to C57BL/10 or to B10.S(7R). Other *K* and/or *D* region-different strain combinations gave relatively weak proliferative responses, compared with *H-2* or *I* region-different strains (Fig. 1, Exps. III and IV).

Studies with a second strain combination gave similar results (Fig. 1, Exp. II). *K* region-disparate A.AL cells provided a proliferative stimulus to responding A.TL cells

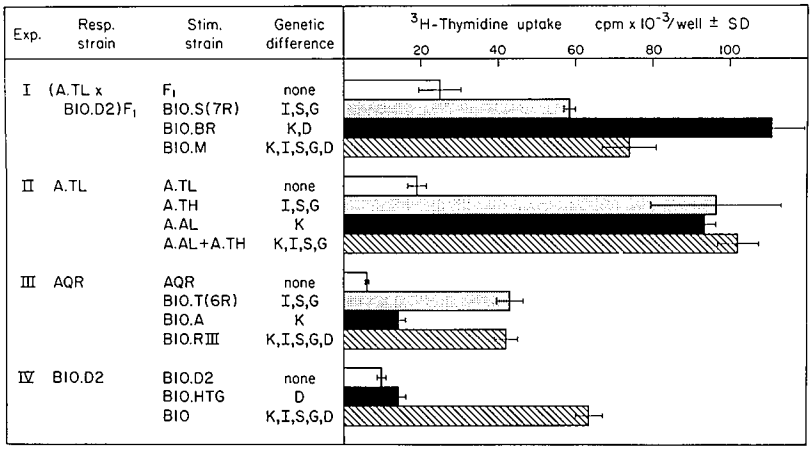

FIG. 1. Primary proliferative responses of lymphocytes stimulated by cells of strains that differ genetically by discrete *H-2* regions. Resp., responding; Stim., stimulating.

TABLE I

H-2 Haplotype of Strains Used in This Study (7)

Strain	H-2 haplotype	H-2 region										Qa region			T*la*
		I subregion										1	2	3	
		K	N	A	B	J	E	C	S	G	D				
A.AL	a1	k	k	k	k	k	k	k	k	k	d	b	a	a	c
A.TL	t1	s	?*	k	k	k	k	k	k	k	d	b	a	a	c
A.TH	t2	s	s	s	s	s	s	s	s	s	d	a	a	a	a
B10.S(7R)	t2	s	s	s	s	s	s	s	s	s	d	?	?	?	a
C57BL/10	b	b	b	b	b	b	b	b	b	b	b	b	a	a	b
B10.D2	d	d	d	d	d	d	d	d	d	d	d	a?	a	a	c
B10.BR	k	k	k	k	k	k	k	k	k	k	k	a	b	b	a
AQR	y1	q	?	k	k	k	d	d	d	d	d	a	a	a	a
B10.T(6R)	y2	q	q	q	q	q	q	q	q	?	d	a	a	a	a
B10.A	a	k	k	k	k	k	d	d	d	d	d	a	a	a	a

* Indicates that the origin of an allele or region is unknown.

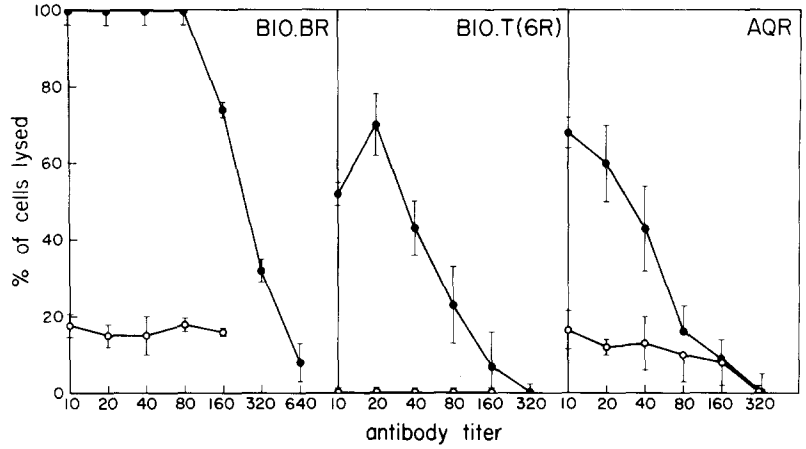

FIG. 2. Reactivity of (A.TL × B10.D2)F_1 anti-B10.BR serum with recombinant strains. (●) Unabsorbed serum; (○) B10.T(6R)-absorbed serum.

equal to that observed with *I* region-different A.TL-stimulating cells, or a combination of *I* region-different A.TH and *K* region-different A.AL cells. The results in Fig. 1 (Exps. I and II) could be reconciled with the abundance of data that suggest that the strongest proliferative responses are elicited in *I* region-different strain combinations (8–11), if an I region-like locus were postulated between *K* and *I-A*. This locus would demarcate a new *I* subregion, *I-N*. If A.TL were $I\text{-}N^s$, it would then be *I* region incompatible with B10.BR ($I\text{-}N^k$) and A.AL ($I\text{-}N^k$).

Mice were immunized with lymphocytes from appropriate strains in an attempt to produce antiserum against putative *I-N* products. One such serum, (A.TL × B10.D2)F_1 anti-B10.BR, exhibits a reactivity pattern most readily explained by postulating an *I-N* subregion. Unabsorbed serum reacts with B10.BR, B10.T(6R), and AQR; B10.T(6R)-absorbed serum retains cytotoxicity on B10.BR and AQR lymphocytes (Fig. 2 [mean lysis ± SE of the mean based on four separate experiments is shown]). Specificities encoded by K^k, $I\text{-}N^k$(?), D^k, *Qa*, and *Tla* may potentially be recognized by this antiserum (Table I). B10.T(6R) absorption removes antibody to

Qa, T*l*a, and cross-reactive H-2^q structures that might account for AQR lysis. Because reactivity to I-A^k, I-B^k, I-J^k, I-E^k, I-C^d, S^d, and G^d determinants is ruled out (because these alleles are present in the serum-producing F_1 hybrid [no autoantibody was observed]), the antigen detected on AQR must map between *K* and *I-A*. It is very unlikely that the anti-K^k and anti-D^k antibodies recognize cross-reactive, *I* region-encoded AQR antigens. These results provide serologic evidence for an antigen, Ia.W41, encoded by a locus, *Ia-7*, within a new *I* subregion, *I-N* (provisionally designated).

Discussion

The strongest proliferative stimulus in a primary MLC between cells differing for the entire *H-2* complex is encoded by genes in the *I* region (8–11). In view of this finding, the results in Fig. 1 (Exps. I and II) appear anomolous; these show strong proliferative responses between *K*- and/or *D*-incompatible strain pairs. B10.BR (Exp. I) differs from the responding F_1 at *Qa* and *Tla* in addition to *K* and *D*, but Qa and T*l*a products do not stimulate in MLC (12). Recombinant A.TL (Exp. II) occurred among the progeny of an A.AL to A.SW cross (13); it differs from A.AL at a chromosomal segment to the left of the A.TL crossover, which includes *H-2K*. If the recombination in A.TL occurred such that an *I* region-like locus were positioned to the left of the crossover, then A.TL would be *I* region disparate from both B10.BR and A.AL. The MLC proliferative results presented would then be consistent with earlier experiments (8–11).

The observation that (A.TL × B10.D2)F_1 recipients, immunized with B10.BR, recognize an antigen on AQR that is not present on B10.T(6R), demonstrates a new k haplotype-encoded structure, Ia.W41, which is shared by B10.BR but not by A.TL. AQR occurred among the progeny of a (T138 × B10.A)F_1 to B10 backcross (14); it derives genes that include *D*, *Qa*, and *Tla* from B10.A. B10.T(6R) is derived from a cross between gray-lethal test stock and B10.A (15); like AQR, it carried *D*, *Qa*, and *Tla* of B10.A. B10.T(6R) absorption, therefore, rules out the *D*, *Qa*, and *Tla* regions as sites for the Ia.W41-encoding locus. B10.T(6R) and AQR also share *K^q*. Although derived from different sources, these strains show complete cross-reactivity of major transplantation antigens (8). Thus, Ia.W41 must be encoded by a locus in the AQR genome to the right of *K* and to the left of *D*. The serum producer, (A.TL × B10.D2)F_1, carries alleles of both the k and d haplotypes from *I-A* through *G*. We conclude, therefore, that the Ia.W41-controlling gene maps between *K* and *I-A*; that A.TL carries the s allele, whereas AQR carries the k allele; and finally, that Ia.W41 and the crossovers in recombinants A.TL and AQR distinguish a new *I* subregion, *I-N*.

We suggest that the new locus is properly related to the *I* region. Its product(s) appears to stimulate a strong proliferative response in MLC, and it is present on a subpopulation of lymphoid cells in contrast to the ubiquitous H-2K products. Thus, the *I-N* subregion, which lies at the boundary between two distinct regions of the *H-2* complex in terms of traits under their genetic control, appears to be a refinement of *I*-region genetic fine structure. The intercalation of *I-N* between *H-2K* and *I-A* will necessitate a systematic reinterpretation and perhaps reevaluation of the H-2 map position of many critically important immunological traits, which have been placed within the *K* or *I-A* regions on the basis of recombinant analysis.

Summary

Strong, primary mixed leukocyte culture-proliferative responses in certain *K* region-incompatible strain combinations led us to consider whether an I region-like locus might exist between *K* and *I-A*. Results obtained with an (A.TL × B10.D2)F_1 anti-B10.BR serum provided serological evidence for a new locus; B10.T(6R)-absorbed serum retained reactivity on AQR lymphocytes. This finding demonstrates an antigen, Ia.W41, encoded by a locus, *Ia-7*, within a new I subregion, *I-N*.

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References

1. Gorer, P. A. 1938. The antigenic basis of tumour transplantation. *J. Pathol. Bacteriol.* **47:** 231.
2. Shreffler, D. C., and C. S. David. 1975. The major histocompatibility complex and the *I* immune response region: genetic variation, function and organization. *Adv. Immunol.* **20:** 125.
3. Murphy, D. B. 1979. Genetic fine structure of the *H-2* gene complex. *In* Role of the Major Histocompatibility Complex in Immunobiology. M. E. Dorf and B. Benacerraf, editors. Garland Publishing, Inc.; New York. In press.
4. Frelinger, J. A., J. E. Niederhuber, C. S. David, and D. C. Shreffler. 1974. Evidence for the expression of the Ia (*H-2*-associated) antigens on thymus-derived lymphocytes. *J. Exp. Med.* **140:**1273.
5. Hayes, C. E., and F. H. Bach. 1978. T-cell-specific murine Ia antigens. Serology of *I-J* and *I-E* subregion specificities. *J. Exp. Med.* **148:**692.
6. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126:**423.
7. Klein, J., L. Flaherty, J. L. VandeBerg, and D. C. Shreffler. 1978. *H-2* haplotypes, genes, regions, and antigens: first listing. *Immunogenetics.* **6:**489.
8. Bach, F. H., M. B. Widmer, M. L. Bach, and J. Klein. 1972. Serologically defined and lymphocyte-defined components of the major histocompatibility complex in the mouse. *J. Exp. Med.* **136:**1430.
9. 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 lymphocyte reaction. *Transplant. Proc.* **5:**377.
10. 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.
11. Lonai, P. 1975. Genetic control of the stimulator and effector function in allogeneic lymphocyte interaction. *In* Immune Recognition. Academic Press, Inc., New York. 683.
12. Widmer, M. B., D. J. Schendel, F. H. Bach, and E. A. Boyse. 1973. The H-(T1a) histocompatibility locus: a study of *in vitro* lymphocyte reactivity. *Transplant. Proc.* **5:**1663.
13. Shreffler, D. C., and C. S. David. 1972. Studies on recombination within the mouse *H-2* complex. I. Three recombinants which position the *Ss* locus within the complex. *Tissue Antigens.* **2:**232.
14. Klein, J., D. Klein, and D. C. Shreffler. 1970. *H-2* types of transplantation stocks T(2;9), 138 Ca, T(9;13) 190 Ca and an H-2 recombinant. *Transplantation (Baltimore).* **10:**309.
15. Stimpfling, J. H., and A. E. Reichert. 1970. Strain C57BL/10ScSn and its congenic resistant sublines. *Transplant. Proc.* **2:**39.