

New Lymphocyte Antigen System (*Lna*) Controlled by the *Ir* Region of the Mouse *H-2* Complex

(histocompatibility/transplantation antigens/immune response genes)

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ABSTRACT A new system of lymphocyte alloantigens in mice is described. This *Lna* (lymph-node antigen) system is associated with the *Ir* region of the *H-2* (histocompatibility-2) gene complex. It has the following distinctive characteristics: (1) The gene or genes controlling these antigens has been mapped in the *Ir* (immune response) region between *H-2K* and *Ss-Slp*. (2) The antigens are most readily detectable on lymph-node cells, although they are also expressed on peripheral blood lymphocytes, splenic lymphocytes, and thymocytes. (3) Cytotoxicity against only about half of lymph-node cells is consistently observed. (4) Cytotoxic antibody titers against these antigens are strikingly high—more than 2000 by ^{51}Cr -release and up to 100,000 in the microcytotoxic test. (5) At least two, probably allelic, forms of the antigen(s) have been defined, one associated with the *H-2^k* haplotype and one with the *H-2^s* haplotype. (6) Antisera against *Lna* contain multiple antibody specificities that can be fractionated by absorption either with certain recombinants or with other *H-2* haplotypes that have crossreactive antigens. The antisera against *Lna* may be of value for definition and characterization of the products of the *Ir* and *MLR* (mixed lymphocyte reaction stimulatory) genes associated with the *H-2* complex.

The *H-2* major histocompatibility gene complex of mice is comprised of four well-defined regions, *H-2K*, *Ir*, *Ss-Slp*, and *H-2D* (1). The *H-2K* and *H-2D* loci control serologically detected cell-membrane antigens on many tissues; the two genes recombine with a frequency of about 0.5% (1). *Ss-Slp* controls quantitative and structural differences in a specific serum protein (2). Differences in immune responses to several antigens have been associated with the *H-2* complex (3), and genetic control of many of these has been mapped to the *Ir* (immune response) region (4). In one instance, an intra-*H-2* recombination appears to have divided the *Ir* region into two segments, referred to as *Ir-1* and *Ir-IgG* (5). The *Ir* region also contains genetic factors that determine strong stimulation in the mixed leukocyte culture reaction (*MLR*) (6, 7). Neither the *Ir* nor the *MLR* gene products have been defined, and it is not clear whether the *Ir* and *MLR* genetic determinants are identical, or distinct genes that happen to map together. It is also not clear whether the products of any genes in the *Ir* region function as transplantation antigens (i.e., mediate rejection of tissue transplants).

All reported *H-2*-associated, serologically detected, alloantigenic cell-surface specificities are assignable to control by either the *H-2K* or *H-2D* genes (1), based upon analyses of some 30 different intra-*H-2* recombinations. No antibodies

against alloantigenic products of genes in the *Ir* region have been reported. However, such alloantigen differences would normally be very difficult to recognize, since differences in the *Ir* region will usually be associated with differences in *H-2K* or *H-2D*. To determine whether genes in the regions between *H-2K* and *H-2D* do specify serologically detectable products and to develop reagents that might be useful for detection and characterization of *Ir* and/or *MLR* gene products, we reciprocally immunized with lymphoid tissues a pair of *H-2*-recombinant strains that are identical in *H-2K* and *H-2D*, but differ in the *Ir* and *Ss-Slp* regions. The resulting antisera react with antigenic products of genes within the *Ir* region that are distinct from *H-2K*, *H-2D*, and *Ss-Slp*. These antigens are expressed most strongly on lymph-node cells and are provisionally denoted the *Lna* (lymph-node antigen) system.

MATERIALS AND METHODS

Mouse Strains used are listed in Table 1. All were derived from the colony maintained in the Department of Human Genetics, University of Michigan, except strains B10.G, B10.T(6R), B10.HTT, and AQR, kindly provided by Dr. Jan Klein of the University of Michigan, B10.S(7R) from Dr. Hugh McDevitt of Stanford University, BRVR and BSVS supplied by Dr. Vesna Tomazic of SUNY, Buffalo, and T138 purchased from the Jackson laboratory. The two strains used for immunizations were A.TL and A.TH. A.TL carries the *H-2^{tl}* haplotype, derived from recombination between *H-2^{al}* (strain A.AL) and *H-2^s* (strain A.SW) (8). A.TH carries the *H-2th* haplotype derived from recombination between *H-2^s* (strain A) and *H-2^s* (strain A.SW) (8, 9). The compositions of the two haplotypes for the four regions of *H-2* may be represented as follows: *H-2^{tl}*—*H-2K^s* *Ir^k* *Ss-Slp^k* *H-2D^d*; *H-2th*—*H-2K^s* *Ir^s* *Ss-Slp^s* *H-2D^d*.

Immunizations. Recipients were injected intraperitoneally three times at weekly intervals with suspensions of about 6×10^6 lymph-node plus 2×10^6 thymus cells. A fourth injection was given 1 month after the third, and the animals were bled 1 week later. Antiserum against A.TL prepared in A.TH contained cytotoxic antibodies, so the recipients were bled at frequent intervals until the titer declined. They were then boosted with a single injection and bled again after 2 weeks, as before. Further boosters were given, as necessary. The same course was followed with antiserum against A.TH prepared in A.TL, except that one further injection was required before the first appearance of cytotoxic antibody.

Abbreviations: *Lna*, lymph-node antigen; *Ir*, immune response; *H-2*, histocompatibility-2; *MLR*, mixed leukocyte reaction.

The different bleedings of each antiserum were all titrated against a standard panel and had comparable activities.

Cytotoxicity Assays were done with lymph-node target cells. The ^{51}Cr -release test was performed as described (10). The two-stage dye-exclusion microcytotoxic test was done as described by Amos *et al.* (11), with some modifications. Antiserum dilutions and cell suspensions were made in Hank's Balanced Salt Solution containing 2% fetal-calf serum. Ficoll-purified cells (12) were incubated at 37° with antiserum for 30 min, followed by a wash in serum-free balanced salt solution and incubation with complement for 1 hr at 37°. Cells were stained with 0.1% trypan blue in balanced salt solution, washed once, and scored on an inverted phase microscope. Normal rabbit serum absorbed with agarose-EDTA

TABLE 1. Direct cytotoxic reactions (^{51}Cr test) of A.TH antiserum against A.TL and A.TL antiserum against A.TH lymph-node cells of inbred strains

Strain*	H-2 Haplotype	Titers† of:	
		A.TH anti-A.TL	A.TL anti-A.TH
A/SnSf; B10.A/SgSf	a	5120	0
A.AL/Sf	al	>1280	0
B10/JSf	b	640	0
C3H.B10/Sf; A.BY/SnSf	b	320	0
B10.D2/SnSf; BALB/cNSf	d	1280	0
A.CA/SnSf; B10.M/Sn	f	1280	640
B10.A(2R)/SgSf; HTH/AoSf	h	>640	0
B10.A(4R)/SgSf	h	>640	0
B10.A(5R)/SgSf; HT1/BoySf	i	>640	0
C3H/JSf; B10.K/Sf	k	>1280	0
AKR/J; BRVR	k	>1280	0
AKR.M/SnSf	m	>640	0
C3H.OH/Sf	oh	>160	0
C3H.OL/Sf	ol	>160	0
B10.P/SgSf	p	>320	>320
C3H.Q/Sf	q	>160	0
B10.G/SgKlj; T138/J	q	640	320
B10.S/SgSf; A.SW/SnSf; SJL/J	s	0	640
A.TB/Sf	tb	640	>80
A.TD/Sf	td	640	>320
A.TE/Sf	te	>1280	0
A.TL/Sf	tl	10280‡	0
B10.HTT/Ph	tl	>160	>160
A.TH/Sf	th	0	2560‡
B10.S(7R)/Sg	th	0	>320
BSVS	th	0	>320
AQR/Klj	y-Klj	>1280	0
B10.T(6R)/SgKlj	y-Sg	640	40
QSR-1/Sf; QSR-2/Sf	sq(?)	0	>320
C3H.KB/Sf	kb(?)	>640	0

* For source and haplotype information, see refs. 8, 18, and 19. BRVR and BSVS types based on unpublished data from our laboratory.

† Reciprocal of last dilution giving detectable ^{51}Cr -release above control level.

‡ Titers in dye-exclusion test = 1/100,000.

(13) was the complement source. All titers are expressed as reciprocals of last antiserum dilution giving ^{51}Cr -release above control levels or significant killing by dye exclusion. A titer of 0 means no reaction at a 1/5 or greater dilution.

Absorptions. *In vivo* absorptions were performed as described (14). Absorbing animals were injected with 0.05 ml of undiluted antiserum intraperitoneally and bled 2 $\frac{1}{2}$, 6, and 24 hr later. Residual antibody activity was assayed by both ^{51}Cr and dye-exclusion cytotoxic methods. Titers presented are uncorrected for *in vivo* dilution. For *in vitro* absorptions, 50 μl of 1/1000 dilution of antiserum was incubated for 1 hr at room temperature (25°) with packed, counted cells or tissue homogenates (13) and assayed in the dye-exclusion test for residual cytotoxic activity against appropriate target cells. Depending upon the requirements of the test, either *in vivo* or *in vitro* absorptions or both were done. Positive and negative control absorptions were conducted in parallel with all test absorptions. With very few exceptions, the absorption results reported are based upon at least two separate absorption analyses. All key *in vivo* absorptions with recombinants were also confirmed by *in vitro* absorptions.

RESULTS

Antiserum against A.TL prepared in A.TH

Direct Cytotoxic Tests. 8 Weeks after the initial immunization, antibodies were detected against lymph-node cells from the donor strain. This antiserum gave a ^{51}Cr -release of 35–40% against A.TL target cells, compared with 20–25% in normal serum controls, with a titer of 1/10,280. The same antiserum titered at 1/100,000 by the microcytotoxic dye-exclusion technique, with 50% killing of cells compared to a 10% base line. The results of tests against various *H-2*-congenic strains and *H-2*-recombinant strains are given in Table 1. The only strains that did not react were A.TH, B10.S(7R), B10.S, A.SW, QSR-1, QSR-2, and BSVS, all of which have the *Ir* region of *H-2*^a. The direct tests indicate that this antiserum has a broad reactivity among different *H-2* types.

Linkage to H-2. The pattern of reactivity against congenic strains (Table 1) indicated an *H-2*-association of the antigens detected by this antiserum. However, to exclude any non-*H-2* effects, a segregating backcross population was classified. (A x B10.S)F₁ hybrids were backcrossed to B10.S. Strain A

TABLE 2. Residual titers after *in vivo* absorptions of antibody against A.TL prepared in A.TH

Absorbed in	Tested against		Absorbed in	Tested against	
	A.TL	A.CA		A.TL	A.CA
A.TL	0	0			
B10.HTT	0	0	B10	>80	0
B10.A	0	0	B10.D2	>80	0
A	0	0	A.CA	>80	0
B10.K	0	0	C3H.Q	40	0
AQR	0	0	B10.G	>80	0
B10.A(2R)	0	0	B10.T(6R)	>80	0
A.TE	0	0	B10.A(4R)	40	0
A.TH	>80	40	B10.A(5R)	>80	0
B10.S(7R)	>80	>80	A.TB	>80	0
B10.S	>80	40	A.TD	40	0
SJL	>80	40			

TABLE 3. Results of *in vivo* absorptions of antibody against A.TL prepared in A.TH

Target cells	Titers after absorption in:							
	A.TH	A.TL	B10.A	B10.G	T138	AQR	B10.T(6R)	
(a) A.TL	>80	0	0	>80	>80	0	>80	
T138	40	0	NT*	0	0	0	0	
AQR	>80	0	NT	>80	>80	0	>80	
	A.TH	A.TL	AQR	B10.A	B10	B10.A(2R)	B10.A(4R)	B10.A(5R)
(b) A.TL	>80	0	0	0	>80	0	40†	>80
B10.A(2R)	>80	0	0	NT*	NT	0	>80†	>80
B10.A(4R)	40	0	0	NT	NT	0	0	40
B10.A(5R)	>80	0	0	NT	NT	0	0	0

* NT = not tested.

† Level of cell killing reduced to 30% in dye-exclusion test.

is positive with A.TH antiserum against A.TL and absorbs completely for A.TL, while B10.S is negative. The F₁ hybrids were positive. 24 Backcross progeny were typed in the cytotoxic test with antibody against *H-2.4* [antibody against B10.A prepared in B10 x AKR.M)F₁], as a marker for the *H-2^a* haplotype of strain A, and with A.TH antibody against A.TL. 14 Animals were positive with both antisera and 10 were negative with both, establishing *H-2*-linkage of the reactivity of A.TH antibody against A.TL and excluding any reactivity with non-*H-2* antigens.

Absorptions. Several strains that were positive in the direct test were used to absorb the antiserum *in vivo*. The results are presented in Table 2. The first eight strains completely removed activity for A.TL target cells. All of these strains derived their *Ir* region from the *H-2^k* chromosome. The next four strains, which carry the *Ir* region from the *H-2^s* chromosome, were negative on direct test and failed to absorb for A.TL. The strains in the second column, which were positive on direct test but which have *Ir* regions from non-*H-2^k*, non-*H-2^s* chromosomes, all failed to absorb reactivity for A.TL; however, all cleared for target cells from A.CA (*H-2^t*), representative of the crossreactive strains.

Absorption analyses with the *H-2^a/H-2^a* recombinant strains AQR (*H-2^{y-k1j}*) and B10.T(6R) (*H-2^{y-s6}*) and parental strains B10.A, B10.G, and T138 are presented in Table 3a. B10.A and AQR cleared completely for A.TL, while B10.G, T138, and B10.T(6R) failed to clear for either A.TL or AQR.

Absorption analyses with the *H-2^a/H-2^b* recombinant strains, B10.A(2R), B10.A(4R), and B10.A(5R), are presented in Table 3b. B10.A and B10.A(2R) cleared completely for A.TL, B10 and B10.A(5R) failed to absorb for A.TL, and B10.A(4R) reduced the titer for A.TL but failed to clear. B10.A(4R) is thought to have derived one segment (*Ir-1*) of its *Ir* region from *H-2^k* and one segment (*Ir-IgG*) from *H-2^d* (5).

These absorption results suggest that the antiserum can be fractionated in several ways. The direct reactions of cells from strains B10, B10.D2, A.CA, B10.A(5R), A.TB, and A.TD must reflect partial sharing of specificities, or cross-reactivity, with antigens of cells of strains A.TL, A, AQR, A.TE, etc., since they failed to completely absorb for these strains. A special case is strain B10.A(4R), which shares part of the *Ir* region with A.TL and differs in part. It failed to clear for A.TL, but the absorption reduced the titer and reduced the

percentage of A.TL cells lysed from 50 to 30% in the dye-exclusion test compared to 10% lysis in the controls.

Mapping of Reactivity of A.TH Antibody Against A.TL. On the basis of direct test and absorption results, reactivities of antibody against A.TL prepared in A.TH of the various strains tested can be classified as: (1) apparently identical to A.TL, i.e., positive by direct test and absorbing completely for A.TL, denoted +(+); (2) partially crossreactive with A.TL, i.e., positive by direct test but failing to absorb completely for A.TL, +(-); or (3) negative, -(-), by direct test and absorption. These data are listed in Table 4 for key parental and recombinant strains that specifically locate the genetic region responsible for this reactivity. So far as is known, A.TH and A.TL are identical in the *H-2K* and *H-2D* regions, but differ in the *Ir* and *Ss-Slp* regions. They also differ in *Tla* type (unpublished observations), but since A has the same *Tla* type as A.TH, yet absorbs completely for A.TL, this can have no bearing on the reactions observed. Thus the antigens reacting with A.TH antibody against A.TL must be controlled by a gene(s) to the left of *H-2D* and to the right of *H-2K*.

B10.A is type +(+), with this antiserum and T138 is type +(-). AQR, which is type +(+), arose from a recombination involving the *H-2^a* and *H-2^a* haplotypes of B10.A and T138, the crossover occurring to the right of *H-2K*. B10.T(6R), which typed +(-), arose from a recombination involving the *H-2* chromosomes of B10.A (*H-2^a*) and B10.G (*H-2^a*). This crossover occurred to the left of *H-2D*. These two recombinants likewise confine the controlling gene(s) between *H-2K* and *H-2D*.

Three recombinants, B10.A(2R), (4R), and (5R), arose from crossingover between haplotypes *H-2^a* (B10.A) and *H-2^b* (B10). The points of crossingover in these strains are indicated in Table 4. Reactivity of this antiserum seems to be due to genes in both the *Ir-IgG* and the *Ir-1* regions, as shown by the partial absorption by the B10.A(4R) strain. The same three sets of recombinants presented in Table 4 (i.e., A.TL-A.TH, AQR-6R, and 2R-4R) also provide the entire present evidence for the intra-*H-2* map location of the *Ir* genes (4, 5). Recombinant QSR-1 serves to further localize the controlling gene(s) to the left of *Ss-Slp*.

Antiserum against A.TH prepared in A.TL

Direct Cytotoxic Tests. About 8 weeks after the beginning of immunizations, this antiserum contained antibodies against the Tla.1,3 antigens. This reactivity was limited to thymus

TABLE 4. Mapping of genes controlling antigens detected by A.TH antiserum against A.TL and A.TL antiserum against A.TH

Strain*	H-2 Haplotype	H-2 Region†				Reaction with:	
		H-2K	Ir	Ss-Slp	H-2D	A.TH anti-A.TL	A.TL anti-A.TH
P: A	a	k	k	d	d	+(+)	-(-)
P: A.AL	al	k	k	k	d	+(+)	-(-)
P: A.SW	s	s	s	s	s	-(-)	+(+)
R: A.TL	tl	s	k	k	d	+(+)	-(-)
R: A.TH	th	s	s	s	d	-(-)	+(+)
P: B10.A	a	k	k	d	d	+(+)	-(-)
P: T138; B10.G	q	q	q	q	q	+(-)	+(-)
R: AQR	y-Klj	q	k	d	d	+(+)	-(-)
R: B10.T(6R)	y-Sg	q	q	q	d	+(-)	+(-)
P: B10	b	b	b	b	b	+(-)	-(-)
P: B10.A	a	k	k	d	d	+(+)	-(-)
R: B10.A(2R)	h-2Sg	k	k	d	d	+(+)	-(-)
R: B10.A(4R)	h-3Sg	k	k b	b	b	+(±)	-(-)
R: B10.A(5R)	i-2Sg	b	b	d	d	+(-)	-(-)
P: B10.G	q	q	q	q	q	+(-)	+(-)
P: A.SW	s	s	s	s	s	-(-)	+(+)
R: QSR-1	sq	s	s	q	q	-(-)	+(+)

* *P* indicates parental, *R* recombinant type strains. A.TL carries a recombination between haplotypes of A.AL and A.SW; A.TH carries a recombination between haplotypes of A and A.SW.

† Letter under each region indicates haplotype of origin. Vertical line indicates crossover position. Arrow indicates direction gene for reactivity maps from crossover.

cells, with a ^{51}Cr -release of 50–60% and a titer of 1/360. 12 Weeks after initiation of immunization, this antiserum also showed reactivity with donor lymph-node cells. It gave a ^{51}Cr -release of 35–45%, compared to 20–25% release in controls, and a titer of 1/2560. In the microcytotoxic dye-exclusion test, it gave 50% killing of A.TH lymph-node cells with a titer of 1/100,000. When a panel of *H-2*-congenic and -recombinant strains was tested with this antiserum, all the strains that carry the *Ir* region of the *H-2^a* chromosome [B10.S, A.SW, A.TH, B10.S(7R), BSVS, QSR-1, and QSR-2] were positive (Table 1). The other positive strains have other *Ir* regions, whose products apparently crossreact. The positive reaction of B10.HTT was unexpected, since it was thought to have the same *H-2* complex as A.TL. These reactions are not due to the antibodies against *I*ta in this serum, since tests were all against normal lymph-node cells, which do not express *I*ta antigens.

Linkage to *H-2*. Although the pattern of reactivity with *H-2*-congenic strains showed no evidence of non-*H-2* antigens, two backcross linkage tests were done to exclude this possibility. (QSR-1 x C3H) F_1 hybrids were backcrossed to C3H. QSR-1 is positive with A.TL antibody against A.TH and absorbs completely for A.TH; C3H is negative; the F_1 hybrid is positive. Backcross progeny were typed with antibody against *H-2.19* [antibody against A.SW prepared in (B6 x A) F_1] as a marker for the *H-2^{aq}* haplotype of QSR-1, and with antibody against A.TH prepared in A.TL. Eight animals were positive with both sera, six were negative with both sera. In a further test, (A x B10.S) F_1 were crossed to A.TL. B10.S is positive with A.TL antibody against A.TH and absorbs completely for A.TH, while A and A.TL are negative. The F_1 hybrid is positive. Progeny from this cross were typed with antibody against *H-2.11,23* [antibody against C3H prepared in (C3H.OH x A.SW)] as a marker for the *H-2^a* haplotype of

strain A, and with A.TL antibody against A.TH. Eight animals were positive with antibody against *H-2.11,23* and negative with antibody against A.TH, while six were negative with antibody against *H-2.11,23* and positive with antibody against A.TH. Thus, there is no evidence for antibodies to non-*H-2*-associated antigens in the A.TL antiserum against A.TH.

Absorptions. Results of extensive *in vivo* and *in vitro* absorption analyses are presented in Table 5. In Table 5a, results are presented that establish that strains B10.S, A.SW, QSR, and B10.S(7R) all absorb the reaction with A.TH, indicating apparent identity among the five strains for these antigens. However, strains B10.HTT and A.CA fractionate the antiserum. Neither strain clears for the other, or for A.TH. These results indicate two populations of antibodies, one specific for B10.HTT, the other reactive with A.CA, A.TB, A.TD, B10.G, B10.T(6R), and T138. Absorption results with the *H-2^a/H-2^a* recombinant set are presented in Table 5b. These results demonstrate that strains B10.G, T138, and B10.T(6R) have indistinguishable reactions (i.e., direct positive reactions and failure to absorb for A.TH, but complete absorption for each other), while B10.A and AQR are entirely negative with this antiserum. Only A.TH of the strains tested absorbed for B10.HTT.

Mapping of Reactivity of A.TL Antibody Against A.TH. Results bearing on the *H-2* map location of the genes controlling these reactions are summarized in Table 4 in the same fashion as for the reactions of A.TH antibodies against A.TL. Again, on the basis of all present information on the A.TL and A.TH strains, the reaction would seem to be controlled by genes located between *H-2K* and *H-2D*. This finding is supported by results with the AQR and B10.T(6R) strains; however, because strains T138, B10.G, and B10.T(6R) do not absorb completely for A.TH, these strains furnish

TABLE 5. Results of *in vivo* absorptions of A.TL antibody against A.TH

Target cells	Titers after absorption in						
	A.TE A.TL	B10.S(7R) A.TH	B10.S A.SW	QSR-1	A.TB A.TD	B10.HTT	A.CA
(a) A.TH	160	0	0	0	40	>80	>80
B10.S	>80	0	0	NT	NT	>80	NT
A.TD	>80	0	0	NT	0	>80	NT
B10.G, T138	>80	0	NT	NT	NT	>80	0
B10.HTT	>80	0	0	NT	NT	0	>80
A.CA	40	0	0	0	0	>80	0
	A.TL	A.TH	B10.G	B10.T(6R)	T138	AQR	
(b) A.TH	>80	0	>80	>80	>80	>80	
A.CA	>80	0	0	0	0	>80	
B10.HTT	>80	0	NT	>80	NT	>80	
B10.G	>80	0	0	0	0	>80	
B10.T(6R)	>80	0	0	0	0	>80	
T138	>80	0	0	0	0	>80	
AQR	0	0	0	0	0	0	
B10.A	0	NT	NT	NT	NT	NT	

mapping information only on a fraction of the specificities. The QSR-1 recombinant serves to locate the controlling genes to the left of *Ss-Slp*.

General properties of the antigens

Tissue Distribution. Absorptions of both antisera with homogenates of organs from both donor strains revealed no detectable activity in liver or kidney tissues and little, if any, in brain tissue, at up to 10 mg of wet weight of tissue homogenate per 50 μ l of antiserum diluted 1/1000. Likewise, 10^9 erythrocytes failed to absorb this level of antibody, and the antisera have no hemagglutinating activity in the polyvinyl pyridone hemagglutination test (14). In direct tests by the microcytotoxic dye-exclusion technique, only lymph-node cells are strongly reactive. Peripheral blood and thymic lymphocytes, purified on Ficoll gradients (12), give weak reactions by dye exclusion, while Ficoll-purified bone marrow and splenic lymphocytes are negative. Complete removal of reactivity from 50 μ l of either antiserum diluted 1/1000 required 6×10^6 lymph-node cells of donor type, 2×10^6 peripheral-blood lymphocytes, 10^7 splenic lymphocytes, or 10^7 thymocytes.

Age of Appearance. Lymph-node cells from donor-strain animals at 2, 3, 4, 5, and 6 weeks of age were all reactive with the appropriate antiserum, with only slightly weaker levels of reaction in the younger animals.

Tests for Reactivity in Normal Serum. Normal preimmunization sera from the A.TH and A.TL strains were unreactive in the ^{51}Cr -release test. To test whether the antisera might be reactive against some serum component of the donor strain, a constant dilution of the antiserum was mixed with doubling dilutions of normal serum from the donor strain, incubated, and centrifuged. The supernatant checked for cytotoxicity against donor lymphocytes. Controls were performed with inhibiting normal serum from the recipient strains. Both antisera reacted similarly, whether mixed with normal serum from recipient or donor strain. The antisera did not react in Ouchterlony diffusion tests against normal serum from the donor strain.

Nomenclature

Since, on the basis of strain distribution, tissue distribution, cytotoxicity of lymph-node cells, and genetic localization, the antigens detected by these sera are distinct from the *H-2K* and *H-2D* antigens, a distinctive notation for them is indicated. Since it is not clear whether they have any direct relationship to the *Ir* and/or *MLR* genes of the *Ir* region, any notation implying such relationship would be premature and possibly misleading. Therefore, because these antigens appear to be most strongly represented on lymph-node cells, we have chosen the provisional designation Lna (lymph-node antigen). The controlling gene(s) in the *Ir* region is denoted *Lna*, and the two principal "alleles" detected thus far are designated *Lna^a* in strain A.TH and *Lna^k* in strain A.TL.

DISCUSSION

The results presented establish the existence of a new system of cellular alloantigens associated with the *Ir* region of the *H-2* complex. The association of these antigens with the *Ir* region is, of course, intriguing and potentially significant. However, it must be emphasized that the present data show only genetic localization to the *Ir* region. It has not been determined whether these antigens are products of *Ir* genes, *MLR* genes, or independent genes that happen to map in the same chromosomal region. The absorption results with the B10-A(4R) recombinant present a striking parallel with the *MLR* reactivity (6, 7) and immune response characteristics (5) of this strain, but further investigations of the nature of the Lna antigens are necessary. There are, on the other hand, sufficient parallels between the *Lna* and *Ir/MLR* systems to raise the question whether the so-called lymphocyte-detected antigens of the *H-2* system may not also be serologically detected (6). However, an attempt to produce antibodies against *MLR* in humans by deliberate immunization was unsuccessful (20).

An obvious question regarding the *Lna/Ir/MLR* relationship is whether the *Lna* antigens are associated with thymus-derived cells (T-cells) or bone marrow-derived cells (B-cells). Since 50% of lymph-node cells are killed by antibody to *Lna* and only about 30% of lymph-node cells appear to be B-cells

(15), no simple relationship to B-cells seems possible. Further data (Frelinger, Niederhuber, David & Shreffler, in preparation) indicate that the Lna antigens are present on Thy-1(θ)-sensitive cells. Further, if the antisera against Lna are directed against *MLR*-stimulatory products, it may be possible to block the *MLR* reaction with these sera. If they are directed against *Ir* gene products, it may likewise be possible to block immune responses *in vitro*. These antibodies should also provide a useful tool for isolation and assay of the Lna antigens so that they may be chemically characterized.

The particular reactivity of lymph-node cells raises interesting questions concerning the expression of the Lna antigens. Are the antigens present on lymphoid cells from other sources masked or present in lower density, or are there simply very few reactive cells? Do the reactive cells in the lymph nodes represent a uniquely differentiated population?

The reactivity of strain B10.HTT with A.TL antiserum against A.TH presents an unexpected problem, since strains B10.HTT and A.TL are both derived from the same single *H-2*-recombinant animal. However, the two strains have been separated for more than 20 generations. The most probable explanation for the difference between the two strains is that one of them, possibly B10.HTT, has undergone a secondary recombination event that introduced a new, different segment of the *H-2* complex somewhere at the *H-2K* end of the complex. A mutation in one of the strains is also possible.

The relative ease with which antibodies against Lna were produced suggests that they may also occur in other antisera produced by alloimmunization with lymphoid tissues from *H-2* incompatible strains. In fact, we have found comparable antibodies in B10.A(4R) antisera against B10.A(2R) produced by immunizations similar to those reported in this paper. These two strains differ only in *Ir-IgG* (5) and *Ss-Slp* (2). Also, we have recently found antibodies against Lna in the antiserum against *H-2* [in antibody against C3H prepared (C3H.OH x A.SW)]. It seems likely that many *H-2* antisera produced against "*H-2K*-end" specificities, in combinations that are also incompatible in the *Ir* region, may contain antibodies against Lna. This hypothesis, of course, again emphasizes the questions about the specificities of antibodies in antisera against HL-A or GPL-A used to block *in vitro* proliferative responses (16, 17), already raised by the investigators concerned.

NOTE ADDED IN PROOF

Recent results indicate that anti-Lna antisera react weakly with the splenic lymphocytes in the direct micro-

cytotoxic test. Since submission of this manuscript, preliminary results suggesting an *Ir*-associated antigen have been reported [Hauptfeld *et al.* (1973), *Science* 181, 167.

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