A MOUSE B-CELL ALLOANTIGEN DETERMINED BY GENE(S) LINKED TO THE MAJOR HISTOCOMPATIBILITY COMPLEX

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The humoral response to transplantation across species includes the production of antibodies specific for the major histocompatibility alloantigens of the donor animal (1–3). In particular, rats immunized with mouse tissues have been shown to produce antibodies reactive against a number of individual *H-2* specificities (1). One possible advantage of this method of production of cytotoxic antisera is the possibility that rats may respond to histocompatibility alloantigens not detected by other mice, thus providing new genetic markers.

In the course of analyzing antisera from a series of cross-species immunizations, an unexpected cytotoxic reaction with C57Bl/10 lymph node cells was observed with an antiserum which, according to the current *H-2* chart, should have contained only specificity for *H-2.31*. The results of additional studies indicate: (*a*) that immunizations between appropriate congenic mouse strains produce alloimmune antisera which likewise detect this new specificity, (*b*) that these cytotoxic antisera (hereafter called anti-"β") detect a cell-surface antigen(s) (hereafter called "β") expressed preferentially on B cells, and (*c*) that tests of recombinant strains indicate that the gene(s) determining β are linked to the *K-end* of the *H-2* complex; i.e., in or to the left of the *Ir-1* region.

Materials and Methods

Animals.—Adult male mice of all strains were used. Strains A.SW/Sn, A/J, AKR/J, C57Bl/10Sn, C57BR/cdJ, B10.A/SgSn, B10.D2/nSn, B10.BR/SgSn, DBA/2J, LP/J, SJL/J, 129/J, and C3H.SW/Sn were purchased from Jackson Laboratories, Bar Harbor, Maine. Strains A/HeCr, BALB/cAnCr, CBA/JCr, C57Bl/6Cr, C3H/HeCr, C57L/Cr, NZB/Cr, B10.A(2R)/Cr, and B10.A(5R)/Cr were obtained from Mr. S. Poiley, Mammalian Genetics and Animal Production Section, National Cancer Institute. Strain B10.A(4R) was kindly provided by Dr. R. Lieberman from a breeding colony established from stock originated by Dr. J. Stimpfling. Adult male Fischer 344 rats were obtained from the Mammalian Genetics and Animal Production Section, National Cancer Institute.

Immunizations.—Rats were immunized by four weekly intraperitoneal injections of 10^8 live mouse lymphoid cells (a mixture of single cells prepared from spleens, thymuses, and lymph nodes), and were bled by cardiac puncture 1 wk after the fourth immunization (1). Mice were immunized by skin grafts according to the method of Billingham (4), followed by several biweekly intraperitoneal injections of 2×10^7 live lymphoid cells beginning the 3rd wk

after skin grafting. They were bled from the tails 1 wk after each intraperitoneal immunization and sera from successive bleedings were pooled. A potent, anti-$H\text{-}2^b$ reference antiserum was prepared by repeated intraperitoneal immunizations of BALB/c animals with 3×10^7 lymphoid cells and lymphoid tumor cells from C57Bl/6 animals. An anti-θ antiserum was prepared by repeated intraperitoneal immunizations of AKR animals with 10^7 CBA thymus cells, according to the method of Reif and Allen (5).

Serology.—Trypan blue cytotoxicity tests were performed in disposable U plates using Microdiluters (Cooke Engineering Co., Alexandria, Va.) by the two-stage method, as previously described (1). Target cells from mesenteric lymph nodes or thymuses were gently teased into Medium 199 (Media Unit, National Institutes of Health) and passed through no. 100 nylon mesh (Tobler, Ernst and Trabler, Inc.; Elmsford, N. Y.) to produce single cell suspensions which were then washed and resuspended to 5×10^6/ml in Medium 199 containing 0.1% gelatin. Bone marrow and spleen cell suspensions were prepared similarly, except that red blood cells were lysed with Tris-buffered ammonium chloride before washing and resuspending (6).

^{51}Cr cytotoxicity tests were performed according to the method of Sanderson (7). Cells were labeled with ^{51}Cr (Amersham/Searle Corp., Arlington Heights, Ill.) at 300 μCi/ml at a cell concentration of 10^8/ml, for 40 min at 37°C in Medium 199. They were then washed three times in Medium 199 containing 0.1% gelatin and resuspended in the same medium to a concentration of 5×10^6/ml. Tests were performed in plastic test tubes (Falcon No. 2052, Falcon Plastics, Division of Bio Quest, Oxnard, Calif.) using 50-μl volumes of reagents and of cell suspensions. For tests involving treatment of cells with more than one antiserum in succession, serum dilutions were chosen which had previously been determined to produce maximum killing of target cells in a standard cytotoxic assay (i.e., dilutions on the plateau of the cytotoxic curve). Cells were incubated with the first reagent for 15 min at 37°C, washed, incubated with the second reagent for an additional 15 min at 37°C, washed again, and finally treated with 50 μl of an appropriate dilution of complement for 30 min at 37°C. Supernatants and pellets from each tube were counted and the percent lysis was calculated according to the formula:

$$(\%\ \text{lysis})_n = 100 \times \frac{\dfrac{\text{supernatant}_n}{\text{total}_n} - \dfrac{\text{supernatant}_m}{\text{total}_m}}{\dfrac{\text{supernatant}_z}{\text{total}_z} - \dfrac{\text{supernatant}_m}{\text{total}_m}},$$

in which n = the experimental tube, m = medium control, and z = potent *H-2* control. Totals are calculated for each tube as the sum of supernatant and pellet.

Normal rabbit serum was used as the complement source in both kinds of cytotoxicity assays. Rabbit sera with appreciable background cytotoxicity were absorbed according to the method of Boyse et al. (8) until two serial twofold dilutions of serum with excess complement activity gave less than 10% background lysis. An appropriate complement dilution (usually about 1:4) was then selected along the plateau of complement excess.

Cell suspensions for absorptions were prepared as described above for cytotoxicity studies. Appropriate amounts of serum and cells were mixed and incubated at 4°C for 30 min and then centrifuged at 900 g for 15 min.

Enriched Subpopulations of Lymphoid Cells.—Cortisone-resistant thymocytes were prepared according to the method of Blomgren and Svedmyr (9): Mice were treated intraperitoneally with 125 mg cortisone acetate (Upjohn Co., Kalamazoo, Mich.) per kg body weight 48 h before harvesting of thymocytes. To facilitate removal of parathymic lymph nodes, carbon black ("Pelikan," Deitzgen, Eugene Co., Washington, D.C.) was injected intraperitoneally 45 min before sacrificing the animals (10).

Separation of a mesenteric lymph node cell suspension into populations enriched for T cells and for B cells was performed with the kind assistance of Doctors Schwartz and Handwerger

in our laboratory, by a modification of the method of Julius et al. (11). Briefly, the cell suspension was incubated with nylon wool according to the published method (11), the direct effluent consisting of a T-cell-enriched population as determined by fluorescence criteria, using a fluoresceinated rabbit anti-mouse IgG. The nylon wool was then mechanically agitated to obtain a B-cell-enriched population, by the same criteria. This method will be described in detail elsewhere.[1]

RESULTS

Characterization of the Reaction.—As can be seen from the abbreviated *H-2* chart in Table I, the only specificity in a Fischer anti-B10.D2 antiserum which one would expect to survive exhaustive absorption with B10.A lymphocytes

TABLE I

Abbreviated H-2 Chart

Strain	*H-2* allele	*H-2K* region specificities																	
		1	3	5	8	11	15	16	17	19	20	21	23	25	31*	33	35	36	
B10.A	*a*	+	+	+	+	+	−	−	−	−	−	−	+	+	−	−	−	−	
B10	*b*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	
B10.D2	*d*	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	

		H-2D region specificities																		
		1	2	3	4	5	6	7	9	13	18	27	28	29	30	32	35	36	41	42	43
B10.A	*a*	−	−	+	+	−	+	−	−	+	−	+	+	+	−	−	+	+	+	+	+
B10	*d*	−	+	−	−	+	+	−	−	−	−	+	+	+	−	−	−	−	−	−	−
B10.D2	*b*	−	−	+	+	−	+	−	−	+	−	+	+	+	−	−	+	+	+	+	+

Adapted from distribution charts by Shreffler and Snell (12) and Klein and Shreffler (13).

* For simplicity we have considered specificity *H-2.34* as an inclusion of *H-2.31*, since it would not be distinguishable by any of our sera.

would be anti-*H-2.31*, the so-called "private specificity" of the $H\text{-}2^d$ allele (12, 13). Fischer anti-B10.D2 serum was absorbed with 1.5×10^9 B10.A lymphocytes per ml, twice the number of cells necessary to clear the serum of cytotoxic activity against lymphocytes of the absorbing strain. Fig. 1 shows the cytotoxic reactions of this absorbed serum when titered against splenic lymphocytes from B10.D2 ($H\text{-}2^d$), B10.A ($H\text{-}2^a$), C57Bl/10 ($H\text{-}2^b$), and A/Jax ($H\text{-}2^a$). The absence of reactivity toward B10.A or A/Jax cells indicates the completeness of the absorption of species-specific antibodies (1). While the expected anti-*H-2.31* activity was indeed demonstrable in the reaction with B10.D2 cells, there was also residual activity against C57Bl/10 (B10) lymphocytes. Further absorption of this serum with four times this number of B10.A cells did not alter the pattern of these reactions.[2]

[1] Handwerger, B. S., and R. H. Schwartz. 1973. Manuscript in preparation.

[2] The initially depressed portion of the cytotoxic curve produced by this absorbed serum is probably a reflection of anticomplementary activity, despite the use of a two-stage assay (1).

The corresponding reactions of a B10.A anti-B10.D2 antiserum are shown in Fig. 2. Again there was demonstrable anti-*H-2.31* activity but also a distinct reactivity with B10 lymphocytes. For both of these sera, the reaction against B10 cells did not reach more than 50% lysis even at the most concentrated dilutions of antiserum. Furthermore, fourfold concentration of the globulin

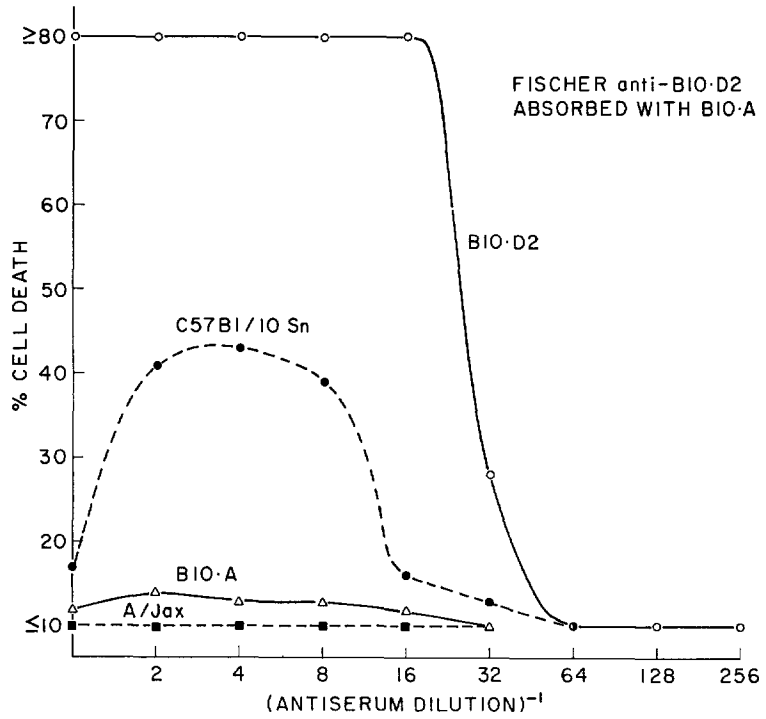

FIG. 1. Cytotoxicity assays of a Fischer anti-B10.D2 antiserum absorbed with B10.A lymphocytes. In addition to the expected anti-*H-2.31* reaction with B10.D2 lymphocytes, an unexpected reaction with C57B1/10 lymphocytes (anti-β) was observed. (Trypan blue method.)

from these sera by ultrafiltration did not increase the level of killing of B10 cells, indicating that only a subpopulation might be susceptible to lysis. Almost identical curves were obtained using lymph node lymphocytes as targets except for a slightly lower maximum percent lysis.

Since these two antisera were raised against B10.D2 tissues and yet reacted with B10 cells, the antigen being detected must be present on both B10.D2 and B10 cells. Therefore, the corresponding B10.A anti-B10 antiserum was prepared and tested against B10.D2 lymphocytes. It showed the same pattern of reactivity, with virtually complete killing of B10 cells but only 51% maximal

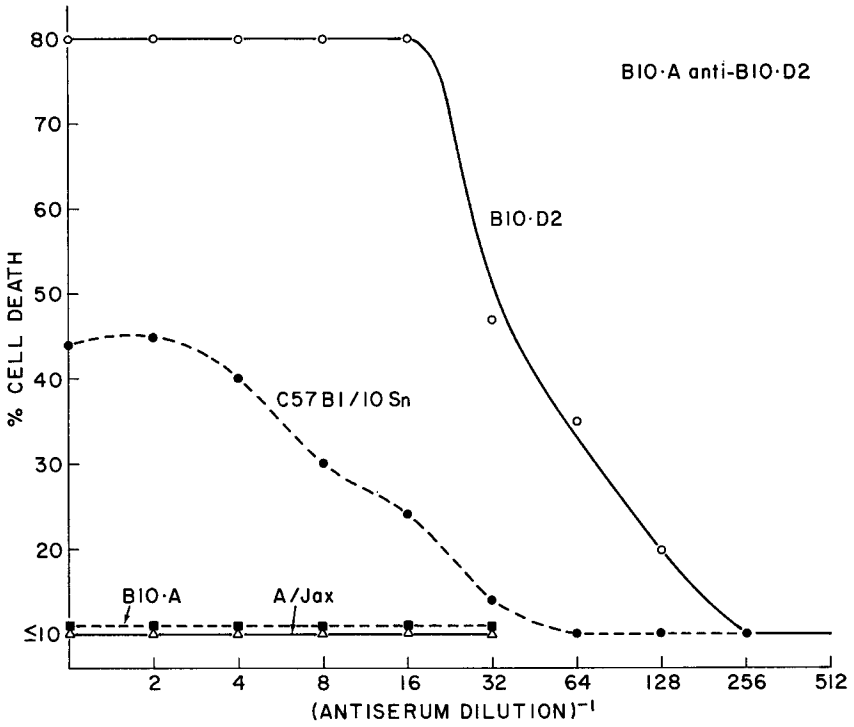

FIG. 2. Cytotoxic assays of a B10.A anti-B10.D2 alloantiserum. The reactions are analogous to those of Fig. 1 (Trypan blue method.)

killing of B10.D2 lymph node cells.[3] The titers of these sera could not be decreased by additions of normal sera from B10, B10.A, or B10.D2 animals, indicating that the antigen being detected was not the result of adsorption of some serum component to the surface of a portion of the lymphoid cells.

The congenic resistant strains of mice presumably differ genetically only at *H-2* or genes linked to *H-2*, and therefore the genes responsible for this new β specificity might also be expected to be *H-2* linked. Fortunately there are several available recombinant B10.A strains with known positions of crossover, which were derived during the production of the B10.A congenic resistant strain (14) and which make it possible to localize the genes responsible for β with respect to the *H-2* map. Fig. 3 is a schematic representation of the genetic map of the *H-2* regions of the $H\text{-}2^a$ and $H\text{-}2^b$ alleles and the correspoinding recombinant alleles. As can be seen from this diagram, if β were determined by genetic

[3] Except where specifically indicated, we have not subtracted complement background killing from reported percentages of lysis. Thus, for example, in this titer we had a 12% complement control, indicating that specific maximal lysis by the serum was actually somewhere between 29% and 41%.

material in or to the right of the *H-2*D region, it should be present in B10.A(2R) and in B10.A(4R), but not in B10.A(5R); if it were determined by material in or to the left of the *Ir-1* region, it should be present in the B10.A(5R) but not in the B10.A(2R) or B10.A(4R); other possible distributions would imply localization between *Ir-1* and the *H-2*D region. Fig. 4 shows the cytotoxic titers of our anti-β alloantiserum with lymph node cells from these recombinant strains. It is apparent that only the B10.A(5R) recombinant strain possesses the β specificity, indicating localization *in or to the left of Ir-1*.

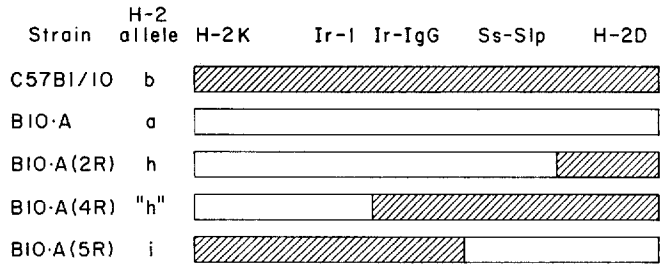

FIG. 3. Schematic representation of the genetic map of B10·A recombinants of the major histocompatibility complex of the mouse. Shaded portions of the recombinant alleles represent genetic material thought to derive from C57Bl/10 (*H-2*^{b}) and unshaded portions represent the corresponding genetic material from A/J (*H-2*^{a}). (Adapted from Lieberman [15]).

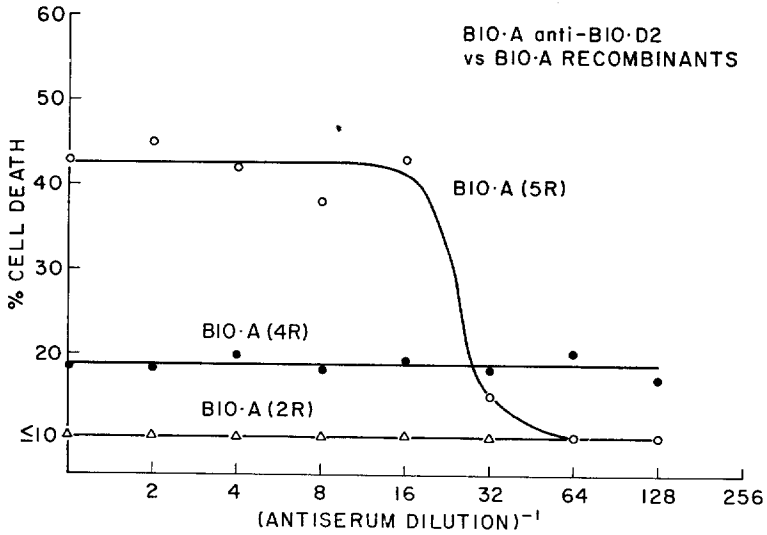

FIG. 4. Cytotoxic assays of anti-β on lymph node cells from the B10.A recombinants. The background complement cytotoxicity for B10.A(4R) cells was 22%. The cytotoxic assay on these cells is therefore regarded as negtive. (Trypan blue method.)

Strain Distribution.—The reactivity of anti-β with mesenteric lymph node cells from a variety of inbred strains is presented in Table II. Because the B10.A anti-B10.D2 serum (serum 1) contains anti-*H-2.31* activity in addition to anti-β activity, reactions with cells from animals bearing the *H-2*^{d} allele would not

TABLE II

Strain Distribution of β

Strain	*H-2* allele	Serum* tested	Complement control	Maximum % killed	Specific % lysis
C57B1/10	b	1	9	47	38
C57B1/6	b	1	12	38	26
129/J	bc	1	11	29	18
C3H.SW	b	1	14	34	20
LP/J	bc	1	12	29	17
A.BY	b	1	14	63	49
C57L	b	1	13	43	30
B10.D2	d	2	12	41	29
DBA/2	d	2	12	46	34
BALB/c	d	2	16	57	41
NZB	d	2	20	54	34
B10.BR	k	1	11	10	<10
C57BR	k	1	32	28	<10
C3H	k	1	13	14	<10
CBA	k	1	12	11	<10
AKR	k	1	14	12	<10
B10.A	a	1,2	15	13	<10
A/J	a	1,2	19	23	<10
A/He	a	1,2	8	10	<10
A.SW	s	1	12	27	15
SJL	s	1	10	26	16
B10.A(2R)	h	1	8	<10	<10
B10.A(4R)	"h"	1	22	17	<10
B10.A(5R)	i	1	15	43	28

* Serum 1 = B10.A anti-B10.D2; serum 2 = B10.A anti-B10.
Cytotoxicity tests were performed by the trypan blue method.

necessarily imply the presence of β. The presence or absence of β in these strains was therefore determined with a B10.A anti-B10 antiserum (serum 2).

It is apparent from this table that all of the *H-2*^{b} strains and *H-2*^{d} strains tested were positive for β and that all of the *H-2*^{a} strains and *H-2*^{k} strains tested were negative for β. Many of these strains bearing the same *H-2* allele were independently derived, or at least separated for many generations. The con-

cordance between the presence or absence of β and the major *H-2* allele supports the very close linkage of this antigen to the *H-2* complex.

Organ distribution.—The maximum percent killing of cell preparations from thymus, bone marrow, lymph node, and spleen of B10 animals with our anti-β alloantiserum is shown in Table III. The coresponding percent killing of these cell preparations with our reference anti-$H\text{-}2^b$ antiserum under the same conditions is also presented for comparison.

TABLE III

Organ Distribution of β in C57B1/10

Origin of cell suspension	% lysis in medium control	% lysis in complement control	Maximum % killed by anti-β	Maximum % killed by anti-$H\text{-}2^b$
Thymus	<10	25	27	67
Bone marrow	<10	11	25	48
Lymph node	<10	<10	47	>90
Spleen	<10	14	49	>90

Cytotoxicity tests were performed by the trypan blue method.

The absence of a positive cytotoxic reaction with thymus cells (i.e., less than 10% above complement control) cannot, of course, be taken as proof that the antigen is not present on those cells, since the density of antigen distribution is known to affect the extent of cytotoxic reactivity (16). Parallel absorption studies with lymph node and thymus cells from B10 and B10.D2 animals were therefore performed. The results are shown in Fig. 5. It is clear that at absorbing cell concentrations greater than 4×10^8 cells/ml, lymph node cells were able to remove all anti-β reactivity from the serum, while thymus cells at the same concentrations hardly affected this activity. One can estimate from such absorptions that thymus cells possess less than 10% of the β antigen of lymph node cells. After maximal absorption with B10 lymph node cells, the percentage of B10.D2 lymph node cells killed by this dilution of B10.A anti-B10.D2 serum was not diminished, indicating that anti-*H-2.31* activity could not be removed by B10 cells (i.e., anti-β is not a cross-reaction of anti-*H-2.31*).

The use of cortisone-resistant thymocytes provided another approach to the question of whether or not β is expressed on thymocytes. Fig. 6 compares the anti-*H-2.31* and anti-β reactivities of the B10.A anti-B10.D2 antiserum on normal and on cortisone-resistant thymocytes. Reactions with anti-θ antiserum are also shown for comparison. While cortisone-resistant B10.D2 cells showed an increase in reactivity consistent with the increase in amount of H-2 antigens which would be expected on a more mature population of thymocytes (9, 10), cortisone-resistant B10 thymocytes showed no increase in reactivity with anti-β.

Additional evidence against the presence of β on thymus cells was obtained using a fluoresceinated anti-κ light chain reagent (kindly provided by Dr. Ethan

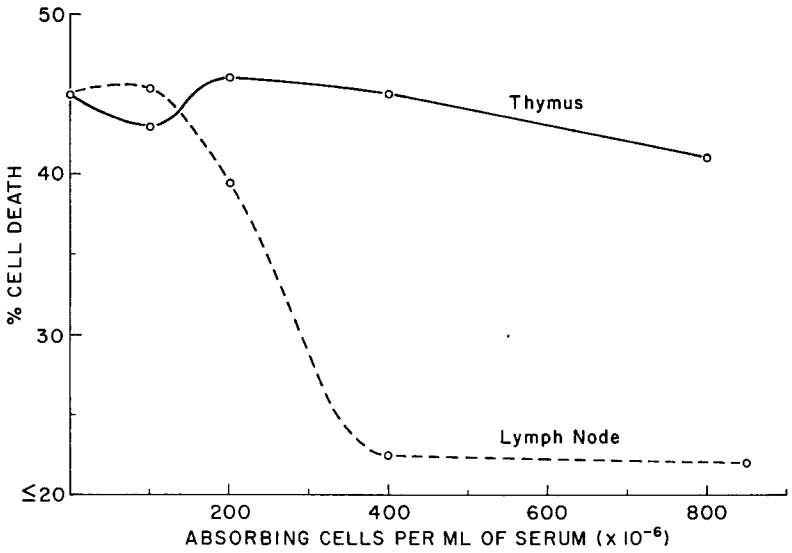

FIG. 5. Absorptions of cytotoxic activity: Shown are the residual cytotoxic activities toward B10 lymph node cells of a 1:8 dilution of B10.A anti-B10.D2 serum after absorption with increasing numbers of lymph node or thymus cells. Absorptions with B10 and B10.D2 cell suspensions gave comparable results, indicating similar amounts of β (means are shown). (Trypan blue method.)

Shevach). B10 thymus cells were first coated with excess anti-β or anti-$H\text{-}2^b$, then washed and stained with the fluorescent reagent. The percentage of cells observed to fluoresce was greater than 90% among thymocytes first coated with anti-$H\text{-}2^b$ and less than 10% among thymocytes first coated with anti-β.

Cellular Distribution.—Since β appeared to be expressed on only a portion of the peripheral lymphocyte populations tested, it was of interest to determine whether or not the population bearing this specificity could be correlated with other known categories of lymphocytes, namely B and T cells. Lymph node cells from B10 animals were fractionated on nylon columns into populations enriched for T or for B cells (11 and footnote 1). The original cell suspension and each of the enriched populations were then tested for cytotoxicity with our anti-β allo-antiserum and with our reference anti-$H\text{-}2^b$ antiserum. The results are shown in Table IV, along with the corresponding percentages of IgG positive cells (B cells) in each population by fluorescence criteria. The data indicate that the same subpopulation which was enriched for B cells showed a comparable increase in percent of cells killed by anti-β. Similarly, the subpopulation which was enriched for T cells showed a comparable decrease in the level of specific killing by anti-β. All three populations were susceptible to lysis under these conditions, as evidenced by the reactions with the anti-$H\text{-}2^b$ serum. It is also evident from this table that the nonadherent fraction of cells showed a greater level of lysis by complement alone than did the adherent fraction. This may

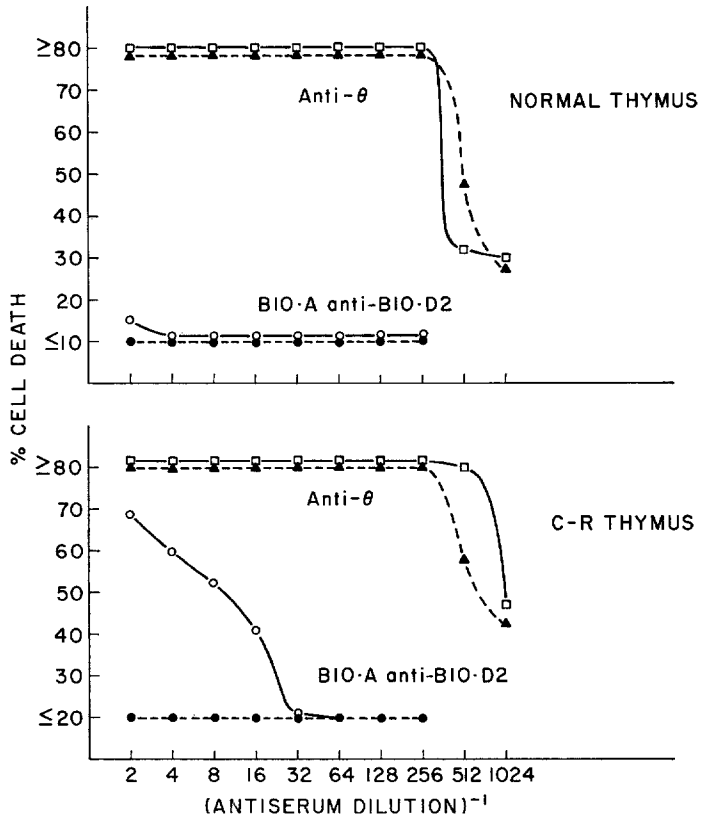

FIG. 6. Comparison of anti-*H-2.31* activity and anti-β activity of a B10.A anti-B10.D2 antiserum tested against normal and cortisone-resistant thymocytes: Anti-*H-2.31* activity is measured on B10.D2 cells (○——○) and anti-β activity on C57Bl/10 cells (●-----●). Anti-θ reactions of each cell type are shown for comparisons: B10.D2 (□——□), C57Bl/10 (▲— — —▲). 20% cell death was chosen as a lower limit for assays on cortisone-resistant thymocytes because of poorer viabilities of these cell preparations in the medium controls.

indicate that peripheral T cells, like thymocytes, are more susceptible than B cells to natural cytotoxic antibodies in rabbit serum (8).

Another approach we have taken to determine the cellular distribution of β has been to study the additivity of anti-β to the cytotoxicity of sera of known specificity for T and B cells. As a reagent for T cells we used an alloimmune anti-θ serum and as a reagent for B cells we used a rabbit antimouse κ-light chain antiserum (kindly provided by Dr. R. Mage), the specificity of which had been previously demonstrated by others (17, 18). This rabbit antiserum was absorbed with 10^9 C57BL/6 thymus cells per ml in an attempt to remove natural cytotoxic activity toward mouse cells.

The multiple incubations and washes necessary in an additivity study led to

quantitative losses of cells by the trypan blue cytotoxicity assay, presumably because of swelling of dead cells which makes them difficult to count. We therefore used a ^{51}Cr cytotoxicity assay for these studies. The results of the various combinations of antisera tested against B10 lymph node cells are shown in Table V. By this assay our anti-β anti-serum killed 38% of the lymph node cells. We attribute the high level of killing by the rabbit anti-κ serum (62%) to residual natural cytotoxic antibodies in this serum despite the absorption with thymus cells. It is apparent, nevertheless, from these data that anti-β was capable of increasing the killing by the anti-θ antiserum so that the total cell kill was comparable to that caused by addition of the anti-κ reagent, while it did not significantly increase the killing due to anti-κ. These data thus also suggest that β is a B-cell antigen.

Two other lines of evidence, although negative, should also be mentioned. When a fluoresceinated anti-κ reagent (provided by Dr. Shevach) was employed in an experiment similar to that described above for thymus cells, but using B10

TABLE IV

Activities of Fractionated Lymph Node Cells

Population from B10 lymph node	% Ig positive (by fluorescence)	% killed by*			
		Medium	Complement	Anti-β	Anti-$H\text{-}2^b$
Mixed	32	<10	<10	48	>90
Nonadherent (T)	7	<10	37	43	>90
Adherent (B)	68	12	17	77	>90

* Cytotoxicity tests were performed by the trypan blue method.

TABLE V

Additivity Studies

Combination of antisera*	% lysis ± SE	% increase of lysis	Significance of increase at $P < 0.05$
$\theta + \theta$	63.7 ± 1.1	0	—
$\theta + \kappa$	85.0 ± 3.3	21.3	Yes
$\theta + \beta$	85.6 ± 3.9	21.9	Yes
$\kappa + \kappa$	61.6 ± 3.9	0	—
$\kappa + \theta$	85.0 ± 3.9	23.4	Yes
$\kappa + \beta$	68.8 ± 3.6	7.2	No

Complement control = 22.9 ± 0.3%. Cytotoxicity tests were performed by the ^{51}Cr method.

* Each value represents the mean of duplicate pairs of assays in which the order of addition of reagents was reversed in each pair. In the case of reactions with anti-κ, somewhat higher (about 10%) killing was achieved if cells were treated with this reagent first rather than in the reverse order. This, and the high percentage of cells killed by this reagent, may reflect residual natural cytotoxic antibodies.

lymph node cells as targets, no increase in percentage of cells fluorescing was achieved by prior incubation with anti-β, while close to 100% of cells were observed to fluoresce after prior incubation with anti-$H\text{-}2^b$. Also, El-4 leukemia cells (an $H\text{-}2^b$ leukemia derived from C57Bl/6) were not killed by anti-β under conditions by which the reference anti-$H\text{-}2^b$ serum killed more than 90% of the cells. This leukemia has been shown by other criteria to be T-cell derived (18). While both of these lines of evidence consist of negative rather than positive reactions of anti-β, they point out the differences between β and other *H-2* linked specificities, and they lend presumptive support to the concept of β as a B-cell antigen.

DISCUSSION

Since the early descriptions of the functional dichotomy of lymphoid cells which cooperate in the immune response (19), a variety of cell-surface markers have been described which appear to distinguish the two major categories of cells. In the mouse, thymocytes bear one of at least two possible alleles of the cell-surface antigen-θ (5, 20) and, in TL+ strains, they also bear the TL surface antigens (20). The θ-antigen, unlike the TL antigens, is also present on peripheral T lymphocytes thought to be derived from thymocytes. The origin of the other major category of mouse lymphoid cells, B cells, is less clear, but they appear to be analogous to the bursal-derived cells of the chicken (19). Unlike T cells, these cells bear readily detectable immunoglobulin on their surface (21, 22) and they can be killed by heterologous anti-κ light chain antisera in the presence of complement (17, 18). Immunoglobulin light chains may therefore be regarded as a cell-surface marker of B cells. Another marker has been called MBLA (mouse B lymphocyte antigen) (23), an antigen detected by a rabbit antimouse lymphocyte serum absorbed exhaustively with thymocytes. By a variety of criteria these antibodies have been shown to label and/or kill mouse B cells specifically, presumably via a cell-surface marker distinct from immunoglobulin, since normal mouse serum does not inhibit their binding (23). In addition, an alloantigenic system called PC has been described for plasma cells, and the antigens of this system appear likewise to serve as B-cell markers (24). Finally, Ly-4, a recently described allelic antigen that is not linked to *H-2* may also be selectively expressed on B cells (25; I. F. C. McKenzie, personal communication[4]).

Functionally, thymocytes and their derivative T cells are thought to be involved in cellular immunity and to serve a "helper" function in humoral immune responses, while B cells are thought to be precursors of antibody-producing cells (26). However, it is important to realize that the correlations between the definitions of these cell populations by surface markers and the functional properties attributed to each cell type may not be precise (27).

The data presented here appear to reflect the presence of a cell-surface antigen expressed preferentially on B cells. The strain distribution of this antigen indicates that it is probably part of an allelic system and we are presently attempting to define other alleles This antigen has been called β, mainly for ease of presentation. A definitive nomenclature will require further investigation of

[4] McKenzie, I. F. C. Manuscript in preparation.

the other possible alleles of this antigen and of their relationship, if any, to *H-2* and Ir region products (28).

β appears to be determined by genetic material distinct from that responsible for any other known B-cell antigens. In particular, the genetic data indicate that the gene(s) responsible for β are localized *in or to the left of the Ir-1 region* of the major histocompatibility complex. Since known heavy and light chain genes are not linked genetically to *H-2*, and since normal B10 serum did not inhibit the reactivity of our anti-β sera, it seems unlikely that β is an immunoglobulin-related marker.[5] MBLA is thought to be a "species-specific" antigen rather than an allelic system, and its genetic localization is not known (23). PC and Ly-4, although allelic systems, have been shown not to be linked to *H-2*, and their strain distributions are distinct from that of β (24, 25). As for T-cell markers, only TL has been shown to be *H-2* linked; but the genetic material responsible for this antigen has been mapped to the right of the *H-2D* region, and it is therefore quite distant from the genetic material responsible for β (20).

It is interesting that the percentage of lymph node cells killed by anti-β reactions in different strains seems to vary considerably (Table II). Part of this variability may be due to variability in the proportions of relative cell types in the lymph nodes of different animals of the same strain (29), which we have noted to account for as much as $\pm 10\%$ in the reactions of anti-β with lymph node cells from normal C57Bl/10 animals. There may also be strain-related differences in the proportion of B cells in different lymphoid organs (17). Finally, part of the variability may reflect strain-related differences in susceptibility to the natural antibodies in rabbit complement, since we have found reproducibly higher complement backgrounds in certain strains. Since T cells seem to be more susceptible to the effects of complement toxicity than do B cells (vide supra) it might be reasonable to subtract complement backgrounds from our reported percentages of maximal lysis by anti-β in order to determine the actual percentage of B cells in each lymph node population. For completeness, however, we have reported both values (Table II).

It was of some concern that this specificity had not previously been found in anti-*H-2.31* antisera. However, the usual manner of producing anti-*H-2.31* antisera involves immunization of (B10 $\times$ A)F1 recipients with lymphoid tissues from B10.D2 animals (30). Unlike the B10.A, such hybrid recipients would possess β and would therefore not produce cytotoxic antibodies against it. Furthermore, it is likely that reactions similar to anti-β have been observed previously during the production of other "monospecific" antisera but, due to their low maximal percent lysis and/or titers, have been regarded as "cross-reactions" rather than as antibody specificities to antigens present on a subpopulation of lymphoid cells (31).

[5] However, cell-surface antibody may contain structural differences, as yet undetermined, from humoral antibody, and such differences could conceivably be encoded by distant genetic material.

Davies has recently described the antigenic specificity *H-2.46* on the basis of the cytotoxic reactions of an A/Jax anti-B10 antiserum with a variety of strains (32). The strain distribution reported for this specificity is not incompatible with that of our anti-β specificity. However, the serum may also have contained non-H-2 specificities, and no data were presented on the maximal percent lysis obtained. It is of course possible that a variety of *H-2* linked antigens exist which are expressed preferentially on either T or B cells. For example, Hauptfeld et al. have recently described an antiserum which purportedly reacts with an antigen specific to T cells (33). Their data indicate that this specificity is determined by genetic information in the Ir region of the major histocompatibility complex. We are presently investigating possible functional correlations for β such as cellular cytotoxicity, induction of second-set skin rejection, and effects of anti-β sera on the immune response in vitro. It would likewise seem reasonable to determine the functional relationship between the T-cell specificity described by Hauptfeld et al. and Ir gene functions before calling that specificity "*Ir-1.1*" as they have suggested (33).

H-2 antigens previously have always been found on all lymphoid cells rather than selectively distributed on subpopulations. There is probably, therefore, a qualitative difference between these new *H-2* linked T- and B-cell antigens and the classical *H-2* antigens. In this regard it is interesting to note that Shevach et al. have recently reported that alloantisera produced by reciprocal immunization of strain 2 and strain 13 guinea pigs appear to be preferentially cytotoxic to B cells (34). While they interpret their data as reflecting a higher concentration of histocompatibility antigens on B cells than on T cells, they point out the possibility that their antisera may in fact recognize B-lymphocyte antigens rather than histocompatibility antigens in the usual sense. In any case, it is interesting that guinea pig alloantisera, across what is by other criteria (e.g., MLC and *Ir*) a major histocompatibility locus, appear to react preferentially with a subpopulation of lymphoid cells. It suggests that lymphoid differentiation antigens may be found associated with the major histocompatibility complexes of a variety of species if they are intentionally sought.

SUMMARY

Antibodies cytotoxic for only a subpopulation of C57Bl/10 lymph node and spleen cells were detected when rat antiserum against B10.D2 was exhaustively absorbed with B10.A lymphocytes. Antibodies of similar specificity were also detected in B10.A anti-B10.D2 and in B10.A anti-C57Bl/10 alloantisera. Reactions with recombinant strains of mice indicate that the cell-surface antigen(s) responsible for this specificity is determined by gene(s) *in or to the left of the Ir-1 region* of the major histocompatibility complex. A variety of criteria implicate B cells as the subpopulation of lymphocytes bearing this antigen. In view of these data and the recent report by others of a T-cell alloantigen determined by gene(s) in the major histocompatibility complex, it seems possible that there may be a variety of H-2-linked alloantigens expressed preferentially on subclasses of lymphocytes.

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