SHARING OF Ia ANTIGENS BETWEEN SPECIES

I. Detection of Ia Specificities Shared by Rats and Mice

BY DAVID H. SACHS, GLEN W. HUMPHREY, AND JOAN K. LUNNEY

(From the Transplantation Biology Section, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

The Ia antigens are defined as cell-surface alloantigens determined by genes in the I region of the major histocompatibility complex (MHC) (1). This definition can probably only be rigorously applied to the mouse, since in no other species has a distinct I region been unambiguously mapped. However, a large number of genetic, structural, and functional characteristics have now been associated with murine Ia antigens, and these may serve as correlative criteria to identify Ia-like antigens in other species. Among these characteristics are: (a) genetic association with the MHC (2, 3); (b) tissue distribution, in particular absence from platelets and relative abundance on B cells (4-6); (c) association with the Fc receptor of B lymphocytes (7); (d) chemical characterization as complex glycoproteins with subunits of mol wt approximately 28,000 and 35,000 (8); (e) presence on T-cell factors (9-11); and (f) close association or identity to MLC-stimulating determinants (12, 13). With one or more of these criteria, Ia-like antigens have now been described in a variety of species including man (14-16 a), monkey (17), guinea pig (18), and rat (19, 20). Nevertheless, production and characterization of Ia-specific antisera in species other than mice is technically difficult and prone to problems of interpretation. It was thus for both theoretical and practical reasons that we were intrigued by the chance observation of an Ia specificity apparently shared between certain strains of mice and rats. The characterization of this interspecies cross-reactive Ia antigen is the subject of this paper.

Materials and Methods

Animals. Mice were either purchased from The Jackson Laboratory, Bar Harbor, Maine or were raised in our own animal colonies. Adult female BN rats were purchased from Microbiological Associates, Bethesda, Md.

Serology. Antisera were prepared by multiple weekly intraperitoneal injections of live lymphoid cells or, in the case of ATH anti-ATL antiserum, by skin grafting followed by intraperitoneal injections of lymphoid cells, both as previously described (21).

Cytotoxicity assays were performed in disposable U plates (Cooke Laboratory Products Div., Alexandria, Va.), and percent lysis was determined by uptake of trypan blue dye, as previously described (21). Quantitative absorptions of sera were performed by first determining the number of cells necessary to clear cytotoxic activity against target cells of the absorbing strain, and then using twice this concentration of cells for the absorption of a larger sample of antiserum. Serum

1Abbreviations used in this paper: MHC, mouse histocompatibility complex; SDS, sodium dodecyl sulfate.
and cells were mixed in Eppendorf 3810 centrifuge tubes for 30 min at 4°C and then centrifuged at 15,000 rpm for 2 min in a Brinkman microcentrifuge (Brinkman Instruments, Inc., Westbury, N. Y.).

**Enriched Subpopulations of Lymphoid Cells.** Splenic lymphocyte preparations were separated into populations enriched for T cells (nonadherent) or for B cells (adherent) by fractionation on nylon wool columns according to published methods (22, 23). The percentage of immunoglobulin-bearing cells in each population was evaluated by using fluorescein-conjugated rabbit anti-mouse Ig or rabbit anti-rat Ig reagents purchased from Cappel Laboratories Inc., Downingtown, Pa. The percentage of cells staining was determined by alternate phase and fluorescence microscopy by using a Leitz Orthoplan microscope. (E. Leitz, Inc., Rochleigh, N. J.)

**Isolation of Labeled Cell Surface Antigens.** Spleen cell surface antigens were radiolabeled with $[^3H]$leucine and isolated by published methods (8). Briefly, spleen cells (5 × 10⁷/ml) from either B10.BR mice or BN rats were incubated at 37°C for 5 h in leucine-free medium (RPMI 1640 [National Institutes of Health Media Unit] supplemented with glutamine) including 200 μCi/ml of $[^3H]$leucine (New England Nuclear, Boston, Mass.). After incubation, cells were pelleted and resuspended in NP40 buffer solution (0.15 M NaCl, 0.01 M Tris pH 7.4, containing 0.5% non-Idet P-40, Particle Data Inc., Elmhurst, Ill.). After a 30-min incubation at 4°C in this buffer, the suspensions were centrifuged at 100,000 g for 1 h. The supernate was harvested and purified by affinity chromatography on a lentil lectin column by published methods (24). Antigen was eluted with 0.1 M α-methylmannoside in the same buffer, and concentrated by absorption to DE52 cellulose (Whatman Inc., Clifton, N. J.) at pH 8.8 in 0.25% NP40 followed by elution in 0.01 M Tris, pH 8.4, 0.4 M NaCl, 0.25% NP40. Aliquots of the concentrated antigens were then pretreated with *Staphylococcus aureus* Cowan I strain to remove endogenously labeled IgG. Indirect immunoprecipitation was carried out by using *S. aureus* Cowan I strain fixed bacteria as the precipitating agent, (25, 26). Precipitated immune complexes were eluted by boiling in 2% sodium dodecyl sulfate (SDS) containing 2% mercaptoethanol, and were analyzed by electrophoresis on 10% polyacrylamide SDS gels. Gels were frozen and sliced into 2-mm sections. Slices were placed in liquid scintillation fluid (Toluene containing Omnifluor and Protosol, New England Nuclear), and radioactivity was determined in a Beckman LS 255 liquid scintillation counter. (Beckman Instruments, Inc., Cedar Grove, N. J.)

**Results**

**Detection of Serologic Cross-Reactions.** The initial observation of Ia-like cross-reactivity between mice and rats was made during the serologic examination of a xenospecific antiserum, B10.D2 anti-BN, in which anti-AgB.3 activity has previously been demonstrated (27). As a specificity control, a mouse alloan-teriserum made in the same strain was required, and an available B10.D2 anti-B10.BR antiserum was chosen. Unexpectedly, the control antiserum produced a cytotoxicity pattern on BN rat splenic target cells similar to what one might expect for an anti-Ia antiserum; that is, it produced a plateau level killing of about one half of the targets (Fig. 1).

If indeed this cytotoxic reactivity were due to a shared specificity between BN rats and B10.BR mice it was reasoned that the reactivity should be detectable in the reverse direction as well. The xenoantiserum B10.D2 anti-BN was therefore tested for cytotoxic reactivity against B10.BR splenic target cells. As shown in Fig. 2, a similar pattern of reactivity was observed, the B10.D2 anti-BN xenonantiserum showing plateau killing in this case, while the alloanteriserum B10.D2 anti-B10.BR showed the expected complete lytic potential of an H-2 antiserum.

To assure that the reactions observed represented real cross-reactions rather than contamination of the antisera, absorption analyses were performed. As seen in Table I, absorption with B10.BR cells removed all allo and xenoreactivity from the alloanteriserum B10.D2 anti-B10.BR, and absorption with BN cells removed all allo and xenoreactivity from the xenoantiserum B10.D2 anti-BN.
Fig. 1. Cytotoxicity assays of three B10.D2 sera on BN splenic target cells. The B10.D2 anti-B10.BR antiserum was found to produce an expected Ia-like cytotoxicity pattern (plateau lysis of approximately 50%) on the BN splenic target cells. Complement control was 17%.

Fig. 2. Cytotoxicity titers of B10.D2 allo and xenoantisera on B10.BR splenic target cells. A cross-reaction analogous to that seen in Fig. 1 is here demonstrated for the xenoantiserum tested on B10.BR mouse splenic target cells. Complement control was less than 10%.
## Absorption of Cross-Reacting Antibodies

| Serum       | Absorbing cells (number/ml) | Target cells | Residual titer[^]* |
|-------------|-----------------------------|--------------|-------------------|
| B10.D2 α-B10.BR | 0                            | B10.BR       | 128               |
|             |                              | BN           | 64                |
| B10.BR (5 × 10⁹) |                              | B10.BR       | <2                |
|             |                              | BN           | <2                |
| B10.D2 α-BN   | 0                            | B10.BR       | 128               |
|             |                              | BN           | 512               |
| BN (10⁹)     |                              | B10.BR       | <2                |
|             |                              | BN           | <2                |

[^]* Residual titers taken as last dilution producing greater than half the maximum killing obtained on the same targets by unabsorbed serum.

These results indicated that the cross-reactions observed did not result from contamination.

**Mapping Studies.** Since the alloantiserum B10.D2 anti-B10.BR was produced between congenic resistant strains presumed to differ only at H-2, the cross-reactive specificity in this serum was thought likely to be directed against antigens determined by the MHC of the rat, which cross-reacted with MHC antigens of the mouse. Conversely, the antigen detected by B10.D2 anti-BN antiserum on B10.BR cells could be explained by cross-reactivity of a rat antigen with the allele of an H-2-linked antigen of mice present on B10.BR (H-2[^]) and not on B10.D2 (H-2[^]) lymphocytes. The availability of numerous congenic resistant and congenic recombinant strains of mice made it feasible to attempt mapping studies on this cross-reactive specificity in the mouse. Table II shows a limited strain distribution of the reactivity of the B10.D2 anti-BN antiserum with mouse spleen cells. Of the strains tested, negative results were obtained in those bearing H-2[^] and H-2[^] haplotypes and in those bearing the H-2[^] and H-2[^] recombinant H-2 haplotypes in which the left end of the H-2 complex was derived from H-2[^] and H-2[^], respectively. These results thus indicate that the cell surface antigens responsible for these cross-reactions were determined by genes to the left of the S region of H-2.

Because H-2[^] strains were positive with this antiserum, the direct reactions of the H-2[^] recombinant haplotypes were not informative for purposes of mapping the relative genes. Absorptions with A.SW (H-2[^]) were therefore performed. As seen in Table III, after complete absorption with A.SW, both H-2[^] strains as well as the B10.HTT (H-2[^]) strain were negative, while residual anti-Ia like killing was still observed on B10.BR (H-2[^]) and A.TL (H-2[^]). These absorption studies therefore indicate that at least part of the anti-Ia-like cross-reactivity must be directed toward the product of genes to the right of the K region. Since the titer of the antiserum was lowered considerably by this absorption, there must have been at least two separable cross-reactive specificities in the B10.D2 anti-BN antiserum. While the specificity removed by absorption with A.SW cells was most likely also determined by an I region gene, no definitive left-hand boundary can yet be inferred (i.e., it could also be in or to the left of the K region).
TABLE II
Strain Distribution of Reactivity

| Strain  | H-2 Type | Titer\(^{-1}\) | Maximum specific lysis |
|---------|----------|-----------------|----------------------|
| B10     | b        | <2              | 6                    |
| B10.A   | a        | 16              | 45                   |
| B10.A(2R)| h2       | 32              | 50                   |
| B10.A(4R)| h4       | 32              | 43                   |
| B10.A(5R)| i5       | <2              | 1                    |
| B10.BR  | k        | 32              | 56                   |
| C3H.OL  | o        | <2              | 2                    |
| B10.S   | s        | 16              | 50                   |
| A.SW    | s        | 32              | 46                   |
| B10.HTT | t3       | 8               | 30                   |
| A.TL    | t1       | 64              | 46                   |
| B10.D2  | d        | <2              | 0                    |

The xenoantiserum B10.D2 anti-BN was tested in a complement-mediated cytotoxicity assay on spleen cell targets of each strain indicated. Titers are expressed as reciprocal of last dilution producing greater than 50% of the maximum specific lysis. Complement controls were generally less than 15% and have been subtracted.

TABLE III
Effect of Absorption with A.SW

| Strain  | H-2 Type | Before absorption | After absorption |
|---------|----------|-------------------|------------------|
|         |          | Titer\(^{-1}\)  | Lysis  | Titer\(^{-1}\) | Lysis |
| A.SW    | s        | 32               | 46     | <2              | 0     |
| B10.S   | s        | 16               | 50     | <2              | 0     |
| A.TL    | t1       | 64               | 46     | 8               | 39    |
| B10.HTT | t3       | 8                | 30     | <2              | 0     |
| B10.BR  | k        | 32               | 56     | 8               | 42    |

The xenoantiserum B10.D2 anti-BN was quantitatively absorbed with A.SW spleen cells as described in the text. Titers and percent lysis are explained in legend to Table I.

Table IV summarizes the serologic mapping studies thus far. Arrows indicate the boundaries on the responsible genes inferred from each result. These data indicate that at least some, if not all, of the cross-reactive Ia-like specificities in the B10.D2 anti-BN antiserum tested on mouse cells is directed toward products of genes in the I-A subregion. It should be emphasized that negative results on a strain bearing part of the H-2 haplotype of a positive strain (e.g. C3H.0L and B10.A(5R)) imply definitive boundaries for mapping. However, positive results in a recombinant H-2 haplotype only imply that part of the reactivity can be mapped as indicated by the arrows (e.g. B10.A(2R) and A.TL). Thus, from the direct testing of B10.A(2R) and B10.A(5R) shown in Table IV, one can infer that genes in or to the left of the I-B subregion must be responsible for the reactivity and that at least part of the reactivity is against a product of a gene located to
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Table IV
Mapping of Ia Reactivity

| Strain         | Absorption with: | Reactivity | Position inferred |
|----------------|------------------|------------|------------------|
|                |                  | K  | I-A | I-B | I-J | I-E | I-C | S  | D  |
| B10            | 0                | b  | b   | b   | b   | b   | b   | b  | b  |
| B10.D2         | 0                | d  | d   | d   | d   | d   | d   | d  | d  |
| B10.A          | 0                | k  | k   | k   | k   | k   | k   | d  | d  |
| B10.BR         | 0                | k  | k   | k   | k   | k   | k   | k  | k  |
| B10.A(2R)      | 0                | k  | k   | k   | k   | d   | d   | d  | b  |
| B10.A(4R)      | 0                | k  | k   | b   | b   | b   | b   | b  | b  |
| B10.A(5R)      | 0                | d  | d   | d   | d   | d   | d   | d  | d  |
| B10.BR         | B10.A(4R)        | k  | k   | k   | k   | k   | k   | k  | k  |
| A.TL           | A.SW             | s  | k   | k   | k   | k   | k   | d  |   |
| B10.HTT        | A.SW             | s  | s   | s   | s   | k   | k   | k  | d  |
| C3H.OL         | 0                | d  | d   | d   | d   | d   | d   | d  | k  |

The presumed haplotype of origin of each H-2 subregion is indicated by a small letter in the table. Arrows indicate the position of the gene(s) determining reactivity relative to the position of crossover in recombinant haplotypes. Only experimentally produced recombinants (and not the H-2 presumed recombinant) have been used for mapping.

The sharing of Ia antigens between species is a complex phenomenon. To exclude I-B antigens as also being detected an absorption was necessary. Thus, the failure to find reactivity on B10.BR after absorption with B10.A(4R), also shown in Table IV, places a right-hand boundary to the left of the I-B subregion.

Testing of Fractionated Spleen Cells. One of the principle characteristics of Ia antigens is their preferential expression on B lymphocytes, in contrast to the ubiquitous expression of K and D antigens (5). It is now clear that Ia antigens are expressed on certain T-cell populations as well (28-30). However, due either to a lower quantity of Ia antigens (less than 10%) or to steric properties, the cytotoxicity of most anti-Ia antisera is almost exclusively directed toward the B-cell subpopulation.

We therefore examined the cross-reactive specificities described above on spleen cell populations fractionated on nylon wool. Table V shows the results obtained in three representative experiments. A comparison of the percentage of immunoglobulin-positive cells in each subpopulation with the maximum percent lysis obtained in that subpopulation suggests that the majority of the lysis of unseparated spleen cells by the cross-reactive antisera was due to selective lysis of B cells. While these results are similar to what has previously been described for anti-Ia reactions (5), there was low but significant killing of the nonadherent (T-cell) population (15-29% in eight experiments), for both the B10.D2 anti-BN serum tested on B10.BR and the B10.D2 anti-B10.BR serum tested on BN. This level of killing of T cells is in excess of what is generally seen.
TABLE V
Cross-Reactivities on Fractionated Cells

| Experiment | Target cells | Ig+ | Serum tested | Complement control | Maximum lysis |
|------------|--------------|-----|--------------|--------------------|---------------|
| 1          | B10.BR (pre) | 59  | B10.D2 α-B10.BR | <10                | >90           |
|            |              |     | B10.D2 α-BN    | <10                | 70            |
|            | B10.BR (Adh) | 89  | B10.D2 α-B10.BR | <10                | >90           |
|            |              |     | B10.D2 α-BN    | <10                | 87            |
|            | B10.BR (NA)  | 10  | B10.D2 α-B10.BR | 12                 | >90           |
|            |              |     | B10.D2 α-BN    | 11                 | 40            |
| 2          | B10.BR (pre) | 57  | A.TH α-A.TL    | <10                | 66            |
|            |              |     | B10.D2 α-BN    | <10                | 69            |
|            | B10.BR (Adh) | 88  | A.TH α-A.TL    | <10                | 90            |
|            |              |     | B10.D2 α-BN    | <10                | 94            |
|            | B10.BR (NA)  | 10  | A.TH α-A.TL    | 14                 | 16            |
|            |              |     | B10.D2 α-BN    | 11                 | 39            |
| 3          | BN (pre)     | 59  | B10.D2 α-BN    | 12                 | >90           |
|            |              |     | B10.D2 α-B10.BR| 15                 | 58            |
|            | BN (Adh)     | 76  | B10.D2 α-BN    | <10                | >90           |
|            |              |     | B10.D2 α-B10.BR| <10                | 84            |
|            | BN (NA)      | 4   | B10.D2 α-BN    | 18                 | >90           |
|            |              |     | B10.D2 α-B10.BR| 15                 | 33            |

Spleen cells from B10.BR (experiments 1 and 2) or BN (experiment 3) animals were tested with the antisera shown, unfractionated (pre) or after fractionation into nylon nonadherent (NA) and adherent (Adh) populations. Complement controls are shown and have not been subtracted from maximal lysis.

for murine Ia antisera (3). That this was not due to technical aspects, for example increased sensitivity of the nonadherent cell population to complement-mediated killing is evidenced by the comparison with a known anti-Ia antiserum (A.TH anti-A.TL) shown in experiment two. In this experiment the B10.D2 anti-BN antiserum showed 28%-specific lysis of B10.BR nonadherent cells while the A.TH anti-A.TL antiserum produced only about 2% specific lysis. Similar levels of lysis of nonadherent spleen cells were obtained by using the B10.D2 anti-BN antiserum on A.TL fractionated lymphocytes after absorption of the antiserum with A.SW, again confirming that the relevant genes determining this specificity were in the I region (data not shown). It thus appeared that while the cross-reactive specificities were to antigens expressed predominantly on B cells, there was also sufficient expression of the relative antigens on certain T cells to lead to specific complement-mediated lysis.

Chemical Isolation Studies. Another criterion which we have examined in attempting to characterize this Ia-like cross-reactivity between the mouse and the rat, is the chemical nature of the responsible cell surface antigens. In the mouse, anti-Ia antisera have been shown to precipitate cell surface glycoproteins of mol wt of approximately 35,000 and 28,000, as distinct from H-2 antigens which appear to be 45,000 mol wt glycoproteins (8). We have therefore analyzed both the B10.D2 anti-BN and B10.D2 anti-B10.BR antisera for their reactivities with labeled mouse and rat cell surface antigen preparations. The results are
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Fig. 3. SDS gel analyses of shared Ia specificities. The three panels show the reactivity of an alloantiserum (B10.D2 anti-B10.BR), a xenoantiserum (B10.D2 anti-BN) and a normal serum (normal B10.D2) with cell surface antigen preparations from BN rat and B10.A mouse splenic lymphocytes, as indicated in the figure. The Ia peaks form a characteristic doublet at about 4.5 and 5.5 cm of gel, indicating mol wt of approximately 35,000 and 28,000, respectively. The slight leftward displacement of all peaks in the first gel (alloantiserum tested on BN antigen) was due to a slicing artifact on that gel and was not seen in multiple replicate gels in this system.

shown in Fig. 3. When tested on B10.A antigen, the B10.D2 anti-B10.BR alloantiserum produced peaks of the three mol wt expected for H-2 (45,000) and Ia (35,000 and 28,000) antigens, which should be detected by this antiserum when tested on cells of the B10.A strain. When tested on the BN antigen preparation, however, only 2 Ia-like peaks were observed. Conversely, the xenoantiserum B10.D2 anti-BN precipitated only peaks corresponding to Ia antigens when tested on the mouse cell surface preparation, but produced peaks of a variety of sizes when tested on the BN rat cell surface preparation. Control precipitations with normal sera were negative on both preparations, as expected. It thus appeared by chemical criteria that the cross-reactive specificities were directed against Ia antigens of the mouse, and by analogy, against Ia-like antigens of the rat.

Discussion

By a variety of criteria, the cross-reactions described in this paper appear to detect Ia antigenic determinants shared between the two species examined. We have demonstrated these Ia-like reactions to be true cross-reactions by immunization in both directions (i.e., mouse anti-rat antisera tested on mouse cells and mouse anti-mouse antisera tested on rat cells) as well as by absorption analyses of the immune sera. Cross-reactions between H-2 antigens and the HLA antigens of man have previously been reported both on the basis of reactivities of mouse alloantisera with panels of human cells (31) and on the basis of the
reactivities of a rabbit anti-HLA antiserum with mouse lymphocytes (32). These
cross-reactions have generally been ascribed to sharing of H-2-like rather than
Ia-like specificities, although chemical isolation studies have not been per-
formed. Chemical analyses of the cross-reactions described in this paper, indi-
cate the detection of only Ia-like and not H-2-like antigens. A cross-reaction of a
rabbit anti-HLA-D antiserum with what appeared to be mouse Ia antigens on
SDS gels has also been reported recently (33).

Attempts to explain the basis for these interspecies cross-reactions raise some
perplexing theoretical questions, the answers to which seem worth pursuing,
since they may provide insights to the evolution of these complex antigenic
systems. The entire MHC appears by genetic, structural, and functional criteria
to be very similar among a variety of mammalian species, suggesting evolution
of this gene complex from common primordial genes. By analogy to the immuno-
globulin structural genes, one might expect that during evolution portions of the
genes determining the MHC antigens may have been conserved for functional
reasons, despite our inability at present to discern the natural function of the
products of this locus. Alloantigenic specificities, on the other hand, imply
polymorphism within a species, and usually result from divergence of sequences
at regions of a molecule which are not critical to the natural function of the gene
product.

We are thus faced with an apparent contradiction. If the shared Ia specificities
we have detected resulted from conservation of genetic information during
evolution, then the structural determinants concerned should not also be alloan-
tigens within these species. We can envision two hypotheses which might
resolve this apparent contradiction.

First, the genetic information in question may be prone to great divergence,
which could have led to extensive polymorphism in both species. By this line of
reasoning, the observed cross-reactions would reflect convergent evolution, the
random appearance of structurally similar alloantigens among the repertoire of
allotypic variants of the two species.

Second, it seems possible that the portion of the Ia molecule responsible for
this shared antigenic determinant has been conserved during the evolution, but
that the conservation has been incomplete. For example, divergence may have
been permitted in the mouse because of another, separate mutation which led to
relaxation of the mutational restrictions on the sequence in question. Then the
sequence in one of the mouse alleles (I-A\textsuperscript{k}) would be similar to the primordial
sequence which is still maintained in the rat. Alternatively, this sequence could
be slowly diverging in all species and the shared specificity detected would
reflect the common primordial sequence from which each species is diverging. In
either case, the observed cross-reaction would be the result of limited polymor-
phism and divergent evolution.

In distinguishing between these two hypotheses it is important to note that we
have so far determined an alloantigenic distribution for the cross-reacting
specificity only in the mouse. All three rat strains we have so far examined
(BN[AgB3], Lewis [AgB1] and DA [AgB4]) have reacted similarly with the
B10.D2 anti-B10.BR antiserum. Whether or not alloantigenic differences can be
distinguished between the cross-reacting determinants of different rat strains is
presently being examined, both by differential absorption studies and by the use
of different allo and xenoantisera. However, at present the apparent similarity of this specificity in the three rat strains tested favors the hypothesis of divergent rather than convergent evolution to explain the origin of these cross-reactions. Examination of the extent of polymorphism in Ia-like cross-reactivities between mouse and other species (several of which we are presently studying) should help to resolve these questions further.

It should be emphasized that the allelic distribution of the cross-reactive specificity in the mouse has permitted definitive mapping studies which indicate that the cross-reacting antigen is indeed an Ia antigen (i.e., determined by genes mapping in the I region of the MHC). However, since all three rat strains were similarly reactive with the B10.D2 anti-B10.BR antiserum, formal genetic mapping studies are not yet possible in the rat. Thus, although criteria tested indicate that the antigens detected in the rat are analogous to Ia antigens of the mouse, it remains possible (but unlikely) that the genes responsible for the cross-reacting molecules in the rat could be other than MHC linked. Coprecipitation analyses with known anti-rat Ia antisera and mapping studies in other species are currently in progress to assess MHC linkage.

In addition to these theoretical questions, there are several practical aspects to this work that we consider important for further development. First, if the sharing of Ia alloantigenic specificities is a general phenomenon, it could provide a new and readily available means of obtaining anti-Ia reagents both for typing and for functional studies in other species, including man. Second, these cross-reacting reagents may detect different I region products than those detected by alloantisera, and could thus help to further dissect the fine structure of the Ia genes. For example, the mapping studies we have performed in the mouse have indicated that the cross-reactive specificity detected is determined by genes in the I-A subregion (Table IV). It should be possible to determine by chemical precipitation studies whether or not this specificity is present on the same molecule as other I-A subregion specificities of the H-2\(^k\) haplotype. Failure of preprecipitation with the cross-reactive specificity to cause complete precipitation of all I-A\(^k\) antigens (or vice versa) would indicate that there is more than one gene product determined by the I-A subregion.

Finally, the observation that the interspecies cross-reactive Ia specificities appear to be more readily detectable on certain T cells than are alloantigenic Ia specificities, may also be of practical importance. These cross-reactive reagents could provide important tools for functional studies in which the effects of anti-Ia antisera on T-cell populations are to be examined. It will also be of interest to examine the effects of these antisera on T-cell factors which have been shown to bear Ia antigens (9-11).

**Summary**

A mouse anti-rat xenogeneic antiserum, B10.D2 anti-BN, has been found to react with a subpopulation of lymphoid cells of certain mouse strains. The corresponding alloantiserum, B10.D2 anti-B10.BR, reacted in analogous fashion with lymphoid cells of BN rats. In the case of the cross-reaction on mouse cells, mapping studies indicated that at least part of the reactivity was with the product of gene(s) determined by the I-A subregion of the H-2 complex. Chemi-
cal isolation studies with radiolabeled cell surface preparations indicated that the antigens detected in both mouse and rat had mol wt characteristic of Ia antigens (35,000 and 28,000 dalton molecules). Testing of fractionated spleen cell populations revealed that the cross-reactive antigens were expressed predominantly on B cells, but that a subpopulation of T cells were also reactive. Wider strain and species distribution studies are in progress to determine the extent of such Ia cross-reactions between species and to further assess the practical and theoretical importance of such cross-reactions.

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