SEROLOGIC EVIDENCE FOR ANTIGENS CONTROLLED BY THE
Ir REGION IN MICE*

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The major histocompatibility complex (H-2) of the mouse consists of four regions:
H-2K, Ir, Ss, and H-2D (1). The H-2K and H-2D region genes code for cell-surface
antigens (H-2 antigens) involved in production of typical H-2 antibodies and in graft
rejection. The Ss region codes for certain protein antigens in the serum of normal
mice (2). The Ir region has been associated with at least three different functions:
It controls the level of antibody response to certain synthetic polypeptides (and probably
also to a variety of other antigens) (3, 4); it determines the degree of stimulation
in mixed lymphocyte culture (MLC) (5); and it also determines the degree of graft-vs.-
host (GVH) reaction (6). Differences in the Ir region, in at least some instances, do
not lead to skin graft rejection (6). Until recently, the products of the Ir region have
not been detected serologically. In the present communication, we describe an anti-
serum that seems to be directed against an antigen controlled by the Ir\textsuperscript{k} region of the
H-2\textsuperscript{a} chromosome. The antigen is present only on thymus-derived (T) lymphocytes,

Materials and Methods

Mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, or from the colonies
at the University of Michigan (B10.HTT, A.TE, AQR, and B10.K) and the Scripps Clinic
and Research Foundation (B10.A/SgSn, A.SW/Sn, C3H/Sr, CBA/J, and A/J). B10.A(4R)
mice were kindly provided by Dr. J. H. Stimpfling, McLaughlin Research Institute, Great
Falls, Mont. Nude mice bred onto the background of CBA (H-2\textsuperscript{a}) were a gift of Dr. M. B.
A. Oldstone, Scripps Clinic and Research Foundation. Adult thymectomized, bone marrow-
reconstituted A/J mice were prepared and kindly provided by Dr. J. Louis, Scripps Clinic.

We refer to the four regions on the H-2 chromosome (H-2K, Ir, Ss, and H-2D, in that
order) with four small letters, indicating the genetic origin of these regions. For instance, the
H-2\textsuperscript{a} chromosome carried by strain B10.A is designated kkdd; it has the H-2K and Ir regions
derived from the H-2\textsuperscript{k} chromosome, and the Ss and H-2D regions derived from the H-2\textsuperscript{d}
chromosome.

Antiserum.—Soluble lymphocyte membrane antigens (SLMA) were prepared by 3 M KCl

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extraction (7) from spleen cells of B10.A mice, and injected into three groups of B10.D2 mice. In the first group (A), the mice received subcutaneous injections of 0.1 mg per mouse of SLMA once a week for 5 wk. In the second group (B), the five injections of the antigen were accompanied by an intraperitoneal injection of polyadenylic-uridylic acid, poly A-U, (Miles Laboratories, Kankakee, Ill.), 1 µmol nucleotide phosphorus per mouse. In the third group (C), each mouse received 0.2 mg of SLMA subcutaneously and 25 mg of endotoxin (ET) (Escherichia coli lipopolysaccharide W of 0111:B4, lot 573564, Difco Laboratories, Detroit, Mich.) intravenously; 3 wk later the mice were boosted with a subcutaneous injection of 0.1 mg of SLMA and an intraperitoneal injection of 12 mg of ET. All mice were bled before the first antigen injection and then weekly up to 7 wk.

Lymphocytotoxic Tests.—The microtests under mineral oil with eosin as a dye and lymph node, spleen, thymus, or bone marrow lymphocytes as target cells were performed as described previously (7). Spleen and bone marrow lymphocytes were freed of contaminating red cells by incubation in 10 ml of 0.83% NH₄Cl per spleen or by separation on a Ficoll-Isopaque gradient (8). The latter procedure was also used to remove dead lymphocytes after incubation with anti-θ serum and complement. Purified spleen or lymph node T cells were obtained by Leuko-Pak filtration (Julius and Herzenberg, personal communication). Normal rabbit serum, selected for lack of nonspecific cytotoxicity, or agarose-absorbed guinea pig serum served as source of complement. Goat antibrain serum was kindly supplied by Dr. J. Clagett and AKR-anti-C3H serum by Dr. J. Chiller, Scripps Clinic; both sera reacted identically with thymocytes and purified T cells. A two-stage lymphocytotoxic test was performed as described elsewhere (7). All serologic results described in this paper were obtained by both one- and two-stage cytotoxic tests.

Absorptions were carried out as described earlier (7). 200,000 T cells purified from lymph nodes of B10.A mice completely abolished the cytotoxicity of 1 µl of antiserum. This cell concentration was, therefore, routinely employed.

RESULTS

Cytotoxic antibodies were detected 6–15 days after the first antigen injection in the sera of all three groups of B10.D2 mice immunized with B10.A SLMA. The strongest of nine cytotoxic antisera (86-2) was obtained 19 days after the first antigen injection with ET as adjuvant. This antiserum has been used primarily throughout this study.

Unlike H-2 alloantisera that usually kill 100% of the target cells, the antisera used in the present studies exhibited a reproducible cytotoxicity of only 20–25% above background with a titer range of 1:10 to 1:32, depending on the antiserum used. The sera were cytotoxic for B10.A lymphocytes from spleen and lymph nodes but not for B10.A lymphocytes from thymus and bone marrow or for any type of lymphocytes of B10.D2, B10, B10.M, A.SW, and DBA/1. This restricted reactivity suggested that the sera contained antibodies directed against antigens present only on the surface of a subpopulation of lymphocytes, namely the T cells. This conclusion was strengthened by the results obtained using T cell-deprived spleen or node lymphocyte populations. T cell elimination was achieved by (a) treatment of a B10.A cell suspension with anti-θ serum plus complement; (b) thymectomy of A/J mice at the age of 6 wk, lethal irradiation 5 wk later, and reconstitution with 15 × 10⁶ anti-θ serum plus complement-treated bone marrow cells; (c) using spleen cells of nude mice bred
to the background of CBA mice. None of these three cell populations reacted with the B10.D2 anti-B10.A sera. In contrast, augmentation by purification on a Leuko-Pak filter of the percentage of T cells to 85–95% in the spleen or lymph node cell suspension elevated the cytotoxic effectiveness of these sera to 40–45% above background.

To determine the strain distribution of the antigen detected by the B10.D2 anti-B10.A-SLMA antibodies, the antisera were reacted against a panel of cells from selected mouse lines. The pattern of reactivity obtained with antiserum 86-2 is depicted in Table I. The other eight antisera gave similar results. The

### Table I

Cytotoxic Reactivity of B10.D2 Anti-B10.A (86-2) Serum

| Strains | H-2 chromosome symbols | Hybrid H-2 regions | % maximal killing |
|---------|------------------------|---------------------|------------------|
| B10.D2  | d                      | d                   | <5               |
| B10.A   | a d/k                  | k                   | d 40 +           |
| B10.K   | k                      | k                   | k 40 +           |
| CBA     | k                      | k                   | k 40 +           |
| B10     | b                      | b                   | b 5               |
| B10.M   | f                      | f                   | f 5               |
| B10.A(2R) | a/b                   | k                   | b 20 +           |
| B10.A(3R) | a/b                   | b                   | b <10 –          |
| B10.BR  | k                      | k                   | k 25 +           |
| C57H    | k                      | k                   | k 45 +           |
| DBA/1   | q                      | q                   | q 5               |
| A.SW    | s                      | s                   | s <5             |
| B10.HTT | tl al/s                | s                   | k 25 +           |
| A.TE    | te tl/f                | s                   | k 30 +           |
| AQR     | ys a/q                 | q                   | k 40 +           |

* Percent maximal killing of purified lymph node T cells (90% positive) = percent killed cells with antiserum — percent killed cells without antiserum.

† Vertical bars indicate the position of crossovers (1).

cytotoxicity of the antiserum against cells of the recombinant strains AQR (qkdd), A.TE (skbf), and B10.HTT (skkd) indicates that H-2 antigens are not responsible for the observed reactions. Cells of these strains do not share the H-2K<sup>+</sup> allele with the B10.A (kkdd) mice, i.e., the donor of the immunizing antigen. Cells from mice sharing H-2K or H-2D regions with these three recombinants, specifically A.SW (ssss), DBA/1 (qqqq), and B10.M (ffff), were not killed by the serum. In addition, cells of B10.A(4R) (kk/bbb) mice were not killed by the serum although these cells carry the same H-2K region antigen as B10.A. Thus, apparently, no antibodies were present that were directed against defined H-2 antigens. The testing of the antiserum, however, revealed a reactivity directed against products of genes intercalated between H-2K and
the $S_s$, since the serum killed cells of all strains that share the $Ir$ region with B10.A mice, i.e., B10.A ($kkdd$), B10.BR, B10.K, C3H (all three have $kkkk$), B10.A(2R) ($kkdb$), B10.HTT ($skkb$), A.TE ($skbf$), and AQR ($qkdd$). A weak cytotoxic effect was also observed on cells of B10.A(5R) ($bbdd$).

To prove whether or not the antigens recognized by the antiserum in different recombinants were the same, absorptions were performed with B10.A, B10.A(2R), B10.HTT, A.TE, AQR, and B10.A(4R) cells. The results are depicted in Fig. 1. Cells of B10.A, A.TE, AQR, and B10.A(2R) mice mutually absorbed the cytotoxicity of the serum, as did B10.HTT (not shown in Fig. 1). In contrast, no change of the cytotoxicity was observed after incubation with B10.A(4R) cells.

![Graph showing absorptions of B10.D2 anti-B10.A-SLMA (86-2) serum with purified T lymph node cells of the strains B10.A (●), A.TE (▲), AQR (●), and B10.A(4R) (□). The unabsorbed serum (○) and the absorbed samples were tested for remaining cytotoxicity against cells of B10.A, B10.A(2R), A.TE, and AQR. The letters below the target cells indicate the $H-2$ region symbols for the $K$-Ir-1-$S_s$-$S_{lp}$-$D$ regions. The boxes mark the identical parts of the $H-2$ complex for the four strains tested. B10.A(4R) is characterized as $kk/bbb$.]

## DISCUSSION

The sera utilized in the present studies reacted with products of genes located in the $Ir$ region of the $H-2$ complex, as indicated by their reaction with B10.HTT, A.TE, AQR, B10.A, and B10.A(2R) but not B10.A(4R) cells. The best evidence for this conclusion is provided by the reaction pattern of the pair B10.A(2R) ($kkdb$) and B10.A(4R) ($kk/bbb$). Both strains were derived from the same parents by recombination within the $H-2$ complex. In the B10.A(4R) strain, the crossover occurred within the $Ir$ region whereas in B10.A(2R) the
crossover took place between the $Ss$ and $H-2D$ regions (1). Thus, both strains differ only in the segment of the chromosome between the $Ir$ and $Ss$ regions.

The B10.A(2R) strain is identical for the $Ir$ region with B10.A, and its cells were killed by the serum; the B10.A(4R) strain is different, and its cells were not killed. The B10.A(2R) cells are known to stimulate B10.A(4R) cells in MLC (5) and GVH reactions (6).

It is highly suggestive that the antigen detected by the B10.D2 anti-B10.A-SLMA sera relates to structures influencing immune response. This hypothesis is supported by the following observations: (a) the close linkage or identity with $Ir$ genes known to control the immune response to certain synthetic polypeptide antigens (3) and immunoglobulin allotypes (4); (b) the presence of the antigen only on T cells and in fact only on some of them; and (c) the apparent correlation of the reactivity of the antisera to MLC and GVH reactivity.

The weaker reaction of the sera against B10.BR mice as compared with the reaction against B10.K mice might be an indication for $Ir$ differences between B10.BR and B10.K. The weak reaction against B10.A(5R) cells could be explained if one assumed that the crossover took place closer to the $Ir$-I region in B10.A(5R) than in B10.A. Consequently, the B10.A(5R) strain would carry a part of the $H-2k$ chromosome between $Ir-Ik$ and $Ss^d$. In fact, lymphocytes of B10.A(5R) reduce the cytotoxicity of the antiserum 86-2 against B10.A cells but do not abolish it. This result indicates that the sera probably contain antibodies directed against at least two antigens and might suggest that there are at least two genes controlling the expression of these antigens.

The solubilization procedure employed in this study apparently keeps the antigenic structures controlled by the $Ir$ region intact with respect to their immunogenicity. The fact that it is possible to raise cytotoxic antisera against such antigens provides a valuable tool for investigation of the biochemical and immunological nature of these antigens.

An antigen of notably similar properties has been recently studied by Hauptfeld, Klein, and Klein (9). The antigen was detected by a B10.T(6R) anti-AQR antiserum that reacted only with strains carrying the $Ir^k$-region. The antigen, which seemed to be present only on T cells, was designated Ir-1.1. It is not clear whether the Ir-1.1 antigen is identical with the antigen detected with the B10.D2 anti-B10.A-SLMA and described in the present communication, but the similarity between the two antigens is remarkable.

**SUMMARY**

Antibodies produced in B10.D2 mice against soluble lymphocyte membrane antigens of B10.A ($H-2^a$) mice reacted only with lymphocytes of the strains carrying the $Ir^k$ region, i.e., B10.A(2R), B10.K, B10.BR, B10.HTT, AQR, A.TE, C3H, and CBA; they did not react with cells of strains carrying different $Ir$ regions, i.e., B10.A(4R), B10, B10.M, A.SW, DBA/1. It is therefore concluded that the antigen detected with these antibodies is apparently controlled
by the Ir region of the H-2 complex. The antigen is present on some T lymphocytes and absent on B lymphocytes. Its presence or absence seems to correlate with MLC and GVH reactivity.

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