Lymphocytes allantigens associated with X-chromosome-linked immune response genes

(lymphocyte membrane antigens/sex chromosome linkage/genetic regulation of immune response)

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ABSTRACT A new polymorphic allantigen system controlled by loci on the X-chromosome has been identified by using antisera from F1 hybrid mice differing in their X-chromosome. These allantigens are associated with the X-linked immune response genes controlling the immune response to the so-called "thymus independent antigens" such as type III pneumococcal polysaccharide, poly(I)-poly(C), and denatured DNA. They also show association with the histocompatibility locus present on the X-chromosome. They were mainly detected on a not yet characterized thymus-derived lymphocyte subpopulation. A certain similarity with the major histocompatibility complex of the mouse supports the possibility of additional I-like regions besides the I region of the histocompatibility-2 complex.

X-chromosome-linked regulation of immune responsiveness has been shown in mice for several so-called thymus-independent antigens (Type III pneumococcal polysaccharide (1), poly(I)-poly(C) (2), and denatured DNA (3)). The high versus low responder status to these antigens seems to be the effect of a single dominant gene and the high responsiveness is transferable by lymphocytes (2). The nature of the genetic control of X-chromosome-linked immune responsiveness is consistent with antigen specific regulation, because, e.g., the DBA/2 strain has a genetically high immune response for poly(I)-poly(C) and a similar low immune response for denatured DNA and SIII, while the SJL/J strain gives a high response to denatured DNA, and a low one to poly(I)-poly(C) and SIII (1–7). In view of the above, and in analogy to the histocompatibility-2 linked immune response (Ir) genes, we investigated whether there are lymphocyte allantigen loci associated with the X-chromosome-linked Ir (immune response) genes (8–11).

The reasoning underlying our experimental approach was as follows: if one assumes that the high and low immune response allele of the X-chromosome-linked immune response gene is expressed on lymphocytes of the respective high and low responder animal, respectively, then one can expect that the low responder animal should produce allantibodies to the X-chromosome-linked determinants on lymphocytes of the high responder animal. F1 hybrid male (low responder, female X high responder male) low responder mice were immunized with spleen cells from identical F1 high responders females. The immunization, therefore, was X(low) X Y against X(low) X X(high) with the autosomes being shared by the donors and the recipients. In this combination, the only allantigenic difference is produced by the X-chromosome controlling the high response.

In the present report, we describe three such allantiseria directed against antigens controlled by genes on the X-chromosome which carry Ir genes to the three antigens mentioned above: pneumococcal polysaccharide SIII, poly(I)-poly(C), and denatured DNA. The data to be presented show that the expression of these allantigens is restricted to a subpopulation of thymus-derived (T) lymphocytes and that the genes controlling them are associated with X-chromosome-linked Ir genes.

MATERIALS AND METHODS

Animals. Inbred mouse strains were obtained from the Experimental Animal Unit at The Weizmann Institute of Science, Rehovot, Israel. All F1 hybrid animals were bred in our laboratory.

Serum Production. For production of antisera directed against determinants controlled by genes associated to the Ir genes of the X-chromosome, different combinations of F1 hybrid male recipients (low responder) were injected with spleen cells from identical F1 hybrid females (high responder). The combinations used are listed in Table 1. Two-month-old F1 males were injected intraperitoneally with F1 female spleen cells suspended in phosphate-buffered saline at weekly intervals (1 donor spleen for 10 recipients). Mice were bled from the retroorbital sinus 7 days after the last injection.

Cytotoxic Assay. Cytotoxic activity of sera was determined by a two step semi-microcytotoxic test according to G. Hammerling et al. with some minor modifications (12). Briefly, 20 μl of a suspension containing 3 X 10⁶ cells per ml and 20 μl of serum dilution were mixed and kept at 4°C for 20 min. Then the cells were centrifuged, the supernatants discarded, and the cell pellets resuspended in 20 μl rabbit serum diluted 1:8. After 45 min at 37°C, 20 μl of 0.1% isotonic trypan blue solution was added and the samples were examined in a hemacytometer at 400-fold magnification. The medium used for all tests was M199 (Medium 199, Biolab Ltd., Jerusalem) supplemented with 5% heat-inactivated fetal calf serum (FCS). The complement source was rabbit serum selected for a high complement titer and low natural cytotoxicity against mouse thymocytes and spleen cells. In addition, complement was absorbed with agarose and subsequently with acetone-dried mouse liver, spleen, and thymus powder. Results are expressed as cytotoxic indices in percent according to the formula:

cytotoxic index (%) =

\[
\frac{\text{(percent dead cells with antiserum) - \text{(percent dead cells control)}}}{100 - \text{(percent dead cells control)}} \times 100
\]

Preparation of Cells. Lymphocyte suspensions were prepared from spleen, lymph nodes, or thymus in minimum essential medium, Biolab Ltd., Jerusalem, containing 5% FCS. T lymphocytes were prepared by passing cell suspensions through nylon wool according to Julius et al. (13). After effusion of the Thy-1.2 positive cells and washing the nylon wool with an additional 10 ml of 37°C minimum essential medium containing 5% FCS, nylon wool-adherent cells were removed by mechanical agitation. Dead cells and erythrocytes were re-
Table 1. Genetic combinations used for production of alloantisera

<table>
<thead>
<tr>
<th>Alloantisera designation</th>
<th>Recipient</th>
<th>Donor</th>
<th>Heterochromosome composition</th>
<th>Antigen to which F1 hybrid males show low response and F1 hybrid females show high response</th>
</tr>
</thead>
<tbody>
<tr>
<td>αX1</td>
<td>(DBA/2 x BALB/c)F1 × (DBA/2 x BALB/c)F1</td>
<td>XDBA/2</td>
<td>YBALB/c</td>
<td>Type III pneumococcal polysaccharide</td>
</tr>
<tr>
<td>αX2</td>
<td>(SJL/J x DBA/2)F1 × (SJL/J x DBA/2)F1</td>
<td>XDBA/2</td>
<td>XBALB/c+</td>
<td>Poly(1)-Poly(C)</td>
</tr>
<tr>
<td>αX3</td>
<td>(DBA/2 x SJL/J)F1 × (DBA/2 x SJL/J)F1</td>
<td>XLDB/2</td>
<td>XSJL/J</td>
<td>Denatured DNA</td>
</tr>
</tbody>
</table>

* The X-chromosomes in the squares are those donated by the high responder parent and are the only alloantigenic difference between donor and recipient.

moved by Ficoll-Metrizoate centrifugation (14), lymphocytes were then washed twice in minimal essential medium containing 5% FCS and resuspended for cytotoxic assay at a concentration of 3.106 lymphocytes per ml in M199 medium containing 5% heat inactivated FCS.

Anti-θ Serum. Congenic Thy-1.2 alloantisera was prepared in (A.Thy 1.1 × AKR-H-2b)F1 mice against the ASL.1 leukemia of A mice. This serum was kindly provided by Edward A. Boyse (Memorial Sloan-Kettering Cancer Center, New York).

Adsorption Experiments. Adsorption in vitro was performed by adding 100 μl of appropriately diluted antiserum to 106 packed spleen cells. After 30 min at 4°, the suspension was centrifuged and the supernatant for residual complement-dependent cytotoxicity against the appropriate target cells.

RESULTS

The three alloantisera αX1, αX2, αX3 which were produced by injections of spleen cells from F1 hybrid high responder females into identical F1 hybrid low responder males (Table 1) were tested for reactivity against lymphoid cells of homozygous females from both parental strains for each combination. Cytotoxic activity was found after the eighth weekly injection, and the maximal titer was reached after 12 injections.

Cellular expression of the X-chromosome-associated lymphocyte alloantigens

Table 2 shows the reactivity of antiserum αX2 against lymphoid cells of DBA/2 female mice [high responder to poly(I)-poly(C)] and SJL/J female mice [low responder to poly(I)-poly(C)]. No reactivity was found against lymphoid cells of the SJL/J female. Serum αX2 reacted with a minority of spleen, lymph node, and thymus cells of the high responder parental strain (DBA/2). The low cytotoxic indices seem nevertheless to be meaningful, because they were obtained consistently with several batches of sera in repeated experiments up to a serum dilution of 1:128 (Fig. 1). Considerably higher number of positive cells were found in nylon wool-purified spleen T lymphocytes (more than 85% Thy-1 positive cells) up to a 50% titer of 1:512, whereas the column adherent cells (less than 5% Thy-1 positive cells) were almost negative. No reactivity was observed against peripheral blood lymphocytes. Lymphoid cells of the (SJL/J × DBA/2)F1 male recipients were all negative which shows that no cytotoxic autoantibodies were produced. Lymphoid cells of the high responder parental male as well as lymphoid cells of the F1 female and of the F1 male containing the X-chromosome of the high responder genotype were positive. These results suggest that the alloantigens described here are controlled by genes on the X-chromosome and are not sex-limited.

Strain distribution of cytotoxic activity

To log for an eventual association of these alloantigens with the X-chromosome-linked f gene loci, we tested the reactivity of the three alloantisera αX1, αX2, and αX3 against nylon wool purified cells of a limited number of strains for which information about X-chromosome linked immune responsiveness was available (1–7). The maximum reactivity of the three alloantisera against cells of the respective high responder parental females was almost the same, namely, approximately 25% of nylon wool purified spleen cells were positive (Table 3). When the sera were tested against other high responder strains, the same number of slightly less positive cells were found, but positive cells were never found when low responder lymphocytes were tested. All the sixteen comparisons made show a definite correlation between high responsiveness and presence of these alloantisera. We suggest the following designations for the above characterized three alloantisera: LyX-1, LyX-2, and LyX-3 in correspondence to the respective alloantisera: αX1, αX2, and αX3.

Adsorption of alloantisera αX2

Cells of three mouse strains, DBA/2, BALB/c, and A/J were positive with serum αX2. To investigate whether these strains
share the same set of $\alpha_X2$-defined alloantigens, we performed adsorption studies. The results are shown in Table 4. Adsorption with cells of the parental, poly(I)-poly(C) low responder, LyX negative strain SJL/J did not change the reactivity of $\alpha_X2$ to the three positive strains. Adsorption with DBA/2 cells abolished completely the reactivity of $\alpha_X2$ against the three positive strains. After adsorption on BALB/c or A/J cells, the serum still reacted with DBA/2 cells. These results can be interpreted as evidence for the heterogeneity of $\alpha_X2$ antibodies. Alloantiserum $\alpha_X2$ defines at least two sets of alloantigens, one present on a subpopulation of T lymphocytes of DBA/2, BALB/c, and A/J and associated with the Ir locus controlling immune responsiveness to poly(I)-poly(C). We designate it LyX-2.1. The other one was only found on a subpopulation of T cells of DBA/2. Its possible association with an eventual other Ir locus has to be kept in mind. We designate it LyX-2.2.

**DISCUSSION**

The X-chromosome of mammals seems to have an important role in the immune response. The influence of the X-chromosome on IgM concentration in man has been demonstrated (15, 16). The occurrence of X-chromosome-linked recessive immune deficiency and X-linked autoimmune diseases in man also suggests an association between immunity and genes on the X-chromosomes (17–20).

The results presented in this report establish the existence of a new polymorphic system of lymphocyte alloantigens. For production of the alloantiseria, spleen cells from F1 hybrid high responder females ($X^{low}X^{high}$) were injected into the identical F1 low responder male ($X^{low}Y$). No cytotoxic autoantibodies were detected. Thus, it is reasonable to assume that all the antibodies in these sera should be directed against products of the donors X-chromosome. Sixteen comparative experiments between immune responsiveness and presence of these alloantigens were performed. These experiments show a definite correlation between high responsiveness and presence of the alloantigens (Table 3). Such a correlation for independent traits would occur randomly 1 out of 216 times. However, it must be emphasized that on the basis of the present data no conclusion can be drawn about the identity of the X-linked Ir genes and the alloantigen controlling genes, because the relative map position of these genes on the X-chromosome is not yet known.

Evidence for X-chromosome-linked histocompatibility was

<table>
<thead>
<tr>
<th>Strains</th>
<th>Immune responsiveness to S III*</th>
<th>Reactivity to serum $\alpha_X1$</th>
<th>Immune responsiveness to poly(I)·poly(C)$\dagger$</th>
<th>Reactivity to serum $\alpha_X1$</th>
<th>Immune responsiveness to denatured DNA$\S$</th>
<th>Reactivity to serum $\alpha_X1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL/J</td>
<td>Low</td>
<td>0</td>
<td>Low</td>
<td>0</td>
<td>High</td>
<td>25 (1:128)$|$</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>N.D.</td>
<td>0</td>
<td>Low</td>
<td>0</td>
<td>High</td>
<td>21</td>
</tr>
<tr>
<td>AKR/Cu</td>
<td>N.D.</td>
<td>0</td>
<td>Low</td>
<td>0</td>
<td>High</td>
<td>13</td>
</tr>
<tr>
<td>DBA/2</td>
<td>Low</td>
<td>0</td>
<td>High</td>
<td>25 (1:512)$|$</td>
<td>Low</td>
<td>0</td>
</tr>
<tr>
<td>BALB/c</td>
<td>High</td>
<td>25 (1:256)$|$</td>
<td>High</td>
<td>20</td>
<td>Low</td>
<td>0</td>
</tr>
<tr>
<td>C3H/He</td>
<td>High</td>
<td>25</td>
<td>Low</td>
<td>0</td>
<td>Low</td>
<td>0</td>
</tr>
<tr>
<td>A/J</td>
<td>N.D.</td>
<td>0</td>
<td>N.D.</td>
<td>18</td>
<td>N.D.</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are expressed as cytotoxic indices in percent. S III refers to Type III pneumococcal polysaccharide. N.D., not determined.

* See refs. 1 and 4.
† Target cells are nylon wool-filtered spleen cells of female mice (in every case more than 85% Thy 1.2-positive cells).
‡ See refs. 2, 3, 5, and 6.
§ See refs. 3, and 5–7.
¶ Fifty percent cytotoxic titer is shown in parentheses.
because second respectively C57BL/6, BALB/c, C57BL/6, and findings. Namely, may correlation histocompatibility linked loantigens defined by the sera aX2 and aX3 (Table 3). The tigens; and possible between F1 DBA/2, BALB/c, and the expressed in blood peripheral lymphocytes containing less than containing among phocytes 5% blood peripheral lymphocytes when containing the loci designated serum aX2 and aX3 (Table 3). The pattern of strain distribution of immune responsiveness to poly(I)-poly(C) and to denatured DNA was found to be opposite (3, 5, 6). Similarly, positive reactivity for αX2 and αX3 antisera, was never found together in the same strain (Table 3) suggesting a possible alleleism for these two Ir genes and for the two alloantigen controlling loci. Absorption experiments of serum αX2 with DBA/2, BALB/c, and A/J lymphocytes show heterogeneity of serum αX2 which defines at least two sets of alloantigens; one designated LyX 2.1 present on lymphocytes of DBA/2, BALB/c, and A/J and associated with the Ir locus controlling immune responsiveness to poly(I)-poly(C), and the second designated LyX 2.2 found only in DBA/2 (Table 4). The correlation between X-linked Ir genes and LyX, and between histocompatibility genes and LyX is of considerable interest, because it raises the possibility that the X-linked Ir gene systems may be similar to the (I) region of the histocompatibility-2 complex.

We have attempted to identify the subpopulation of cells bearing the LyX alloantigens. LyX-positive lymphocytes were found in the spleen, lymph nodes and thymus, but not in the peripheral blood (Table 2). The absence of LyX-positive lymphocytes among the nylon wool-adherent spleen cells (containing less than 5% Thy-1+ lymphocytes) and the relatively high percentage (25%) found in the nylon wool passed spleen cells (85–95% Thy-1+ lymphocytes) provide strong support for the expression of LyX alloantigen on a subpopulation of T lymphocytes, although it is not excluded that it could also be expressed on the 5–15% of Thy-1 negative lymphocytes present in the nylon wool passed fraction. Moreover, it is still possible that, by using other methods of immunization and techniques of detection, X-linked Ir gene-associated alloantigens could be found on B or other lymphoid cells because studies on I region-associated antigens (Ia) have stressed the importance of such procedures. For instance, localization of I region-associated antigens on resting T cells runs into considerable difficulties when tested with standard procedures used for their detection on B cells (11, 24, 25).

It is puzzling that the lymphocyte alloantigens associated with genes controlling the immune responses to so-called "thymus-independent antigens" seem to be expressed mainly on T cells. However, it should be noted that the magnitude of the antibody response to type III pneumococcal polysaccharide was shown to be regulated by two types of T cells, a suppressor cell and an amplifier cell acting in an opposing manner (1, 26, 27). For the better understanding of the X-linked regulations of immune responsiveness, it may be important to characterize the LyX bearing lymphocyte subpopulation. It is of special interest to compare this subpopulation with the functional T subpopulation characterized by the Ly alloantigens (28–30).

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