THE GIX SYSTEM
A CELL SURFACE ALLO-ANTIGEN ASSOCIATED WITH MURINE LEUKEMIA VIRUS;
IMPLICATIONS REGARDING CHROMOSOMAL INTEGRATION OF THE
VIRAL GENOME*

BY ELISABETH STOCKERT, LLOYD J. OLD, M.D., AND
EDWARD A. BOYSE, M.D.
(From the Division of Immunology, Sloan-Kettering Institute for Cancer Research,
New York 10021)

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It is becoming more and more evident that infection with MuLV1 (Gross), in one
form or another, is virtually ubiquitous among mice. Mice of strains with a high in-
cidence of spontaneous leukemia (e.g. AKR and C58) are overt carriers, MuLV-
associated antigens being demonstrable in their tissues throughout life; other mice
(e.g. C3Hf/Bi) show these signs of infection only in later life; still other mice (e.g.
C57BL/6 and BALB/c) rarely or never do so even in later life, but their tumors,
induced or spontaneous, and also cultured normal tissues from these strains often
produce both MuLV virions and MuLV antigens (see 1–15 for references). Further-
more, MuLV antigen is demonstrable in probably all mouse embryos at some stage of
development (16). It seems therefore that evidence for MuLV infection could be ob-
tained at will for any mouse of any strain.

Long before these facts were appreciated, an intimate association between MuLV
and the gametes was suspected as an explanation of vertical transmission of infection,
i.e., the regular congenital transmission of virus by females and males of the AKR
and other mouse strains with a high incidence of leukemia (15, 17). The closest form
of such an association would be chromosomal integration of the viral genome as in
lysogeny, and this has been much discussed.

A number of recent findings tend to substantiate this hypothesis: (a) It is now
realized that viral ribonucleic acid (RNA) can be transcribed into deoxyribonucleic
acid (DNA) (18–21), which allows for DNA copies of RNA viral genomes like
MuLV, as proposed originally by Temin for Rous sarcoma virus (22). (b) In certain
chicken populations, the group-specific (gs) antigen of avian leukosis–sarcoma virus
can be inherited as a mendelian dominant, as if the viral gene coding for this antigen
were incorporated in one of the chicken’s chromosomes (23). (c) Bentvelzen’s demon-
stration that in mice of the GR strain the propensity to develop mammary neoplasia
is inherited as a mendelian dominant is difficult to explain on any basis other than

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1 Abbreviations used in this paper: GCSA, Gross cell surface antigen; gs, group-specific;
GSA, Gross soluble antigen; MuLV, murine leukemia virus.
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integration of mammary tumor virus genes in one of the chromosomes of this stock of mice (24).

We have been investigating a new MuLV-related cell surface antigen which is present on lymphoid cells of normal mice. It requires two unlinked chromosomal genes for its expression, and we have called it GIX (G = Gross) because one of these two genes has been located in linkage group IX.

The antigens of MuLV (Gross) include group-specific (gs:internal) and type-specific (envelope) components of the virion, recognized by immunodiffusion, complement fixation, virus neutralization, and immunoelectronmicroscopy. In addition, the cytotoxicity test identifies G (Gross) cell surface antigen (GCSA), a type-specific nonvirion antigen expressed on the surface of infected cells and rendering them susceptible to lysis by cytotoxic Gross antiserum and complement. Another type-specific nonvirion antigen, Gross soluble antigen (GSA), is found in serum and in tissue extracts and is demonstrable by immunofluorescence of viable indicator cells to which it has been adsorbed; it may represent exfoliated G cell surface antigen. (references and summary in Table I).

**TABLE I**

Previously Reported Antigens of the MuLV (Gross) System

<table>
<thead>
<tr>
<th>Description</th>
<th>Abbreviation</th>
<th>Virus or nonvirus</th>
<th>Serological technique</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-specific antigen</td>
<td>gs</td>
<td>Virion</td>
<td>Ouchterlony, C fixation</td>
<td>5, 7, 25-29</td>
</tr>
<tr>
<td>Virion envelope antigen</td>
<td>Vc</td>
<td>Virion (type-specific)</td>
<td>Virus neutralization, immunoelectronmicroscopy</td>
<td>4, 5, 30</td>
</tr>
<tr>
<td>Gross cell surface antigen</td>
<td>GCSA* †</td>
<td>Nonvirion (cellular)</td>
<td>Cytotoxic (mouse) antibody, immunoelectronmicroscopy</td>
<td>1, 3, 5, 30-32</td>
</tr>
<tr>
<td>Gross soluble antigen</td>
<td>GSA</td>
<td>Nonvirion noncellular (possibly same specificity as GCSA, may be exfoliated GCSA)</td>
<td>Immunofluorescence of indicator cells carrying adsorbed GSA</td>
<td>9</td>
</tr>
</tbody>
</table>

* Now that we recognize the new MuLV-associated antigen GIX (described in this report), we shall refer to the Gross cell surface antigen identified by mouse antiserum as GCSA rather than simply G (as previously, ref. 1) to distinguish the notation more clearly from GIX.

† Strains like AKR have a high incidence of leukemia and are overt lifelong carriers of MuLV (Gross); GCSA is demonstrable on their spleen and lymphoid tissues at all ages, and so these strains are referred to as GCSA+ in contrast to other strains (GCSA-) which only manifest GCSA late in life or not at all (1). GCSA is demonstrable on thymocytes of GCSA+ mice by serological absorption, but the amount is so small that the antiserum, anti-GCSA (rat), has only negligible cytotoxic activity on GCSA+ thymocytes. The standard test for GCSA on any cell population is therefore to test their capacity to absorb cytotoxic activity from C57BL/6 anti-K36 (AKR leukemia) serum, the absorbed serum being tested on a standard syngeneic C57BL/6 leukemia which is highly sensitive to the antiserum (1).
Past analysis has been based mainly on Gross antiserum from two sources, the mouse (1) and the rat (5, 7). The latter antiserum, prepared in inbred rats against syngeneic leukemias induced by Gross virus, identifies a more extensive range of Gross specificities than the mouse antiserum (5, and see Table I). Among these additional antibodies found in the rat antiserum is one that is strongly cytotoxic for thymocytes of certain mouse strains. We shall call it anti-G_{1X} for the reasons given above; the immunogenetic system it defines is the subject of this report.

Materials and Methods

Mice and Rats.—With the following exceptions, all mice, and rats of the W/Fu inbred strain, were from our own colonies. Strains designated /J and those based on C57BL/10 (B10) were purchased from The Jackson Laboratory, Bar Harbor, Maine. Strain 129 mice were obtained from our own colony and from The Jackson Laboratory. All strain 129 breeders for genetic tests were taken from our own colony (originating from 129 breeders supplied by Dr. D. B. Amos in 1963). Dr. L. C. Stevens kindly sent mice of his various 129 sublines for comparative tests.

Thymocytes for Serological Tests.—Where it was necessary to preserve the donors, thymocytes were obtained from anesthetized living mice by suction through a special pipette inserted into the chest via the thoracic inlet, yielding all or part of one thymic lobe (33).

Serology.—Where technical details have been described fully elsewhere, only pertinent references will be given.

Cytotoxicity test (34, 33): The maximum accepted background level of cell death in control cells exposed to complement alone or antiserum alone, or to complement and antiserum combined in the case of control cells not carrying the relevant antigen, was 15%.

Complement for the cytotoxicity test: Serum from rabbits individually selected for low toxicity for mouse thymocytes combined with a high complement level (36) was used at 1:15 (= dilution before use). Heteroantibody in these selected rabbit sera is not detectible at this dilution, therefore prior absorption with mouse cells is unnecessary.

Antiserum for G_{1X} specificity: This is the standard Gross antiserum (W/Fu × BN)F_{1} (rat) anti-W/Fu(C58NT)D (5,32). W/Fu(C58NT)D is a transplanted W/Fu leukemia induced by wild-type MuLV. Various pools were used, all heated at 56°C for 30 min to eliminate complement activity. All antibodies in this antiserum (excluding natural heteroantibody) are directed to antigens of the MuLV (Gross) system (see Table I). G_{1X} specificity is identified by the reaction of this antiserum with mouse thymocytes that carry this antigen: usual titer vs. strain 129 thymocytes = 1:400. Rat heteroantibody reactive with mouse thymocytes was removed as necessary by absorption (in the cold) with thymocytes and spleen cells of G_{1X}- strains (see Table II, prototype = C57BL/6). The natural heteroantibody has partial mouse strain specificity, therefore absorption with cells of one particular G_{1X}- strain may not remove all activity against another G_{1X}- strain; this was taken into account in absorbing antiserum for particular purposes, e.g. in linkage tests involving CBA, CBA thymocytes were included in the preparatory absorption of heteroantibody.

G_{1X}-typing: distinction of heterozygotes from positive homozygotes in segregating populations of mice from G_{1X}×G_{1X} crosses (Tables V, VII, VIII, and IX): The cytotoxic reaction on positive homozygotes is much greater than on heterozygotes in the G_{1X} system. Scoring of thymocytes is therefore easy by the direct cytotoxicity test, the former are >90% lysed and the latter <60% (maximum) lysed in a standard test with 1:20 antiserum.

G_{1X}-typing: distinction of heterozygotes from negative homozygotes in segregating populations of mice from G_{1X}×G_{1X} crosses (Tables VI, VII, and VIII): For this purpose, the relatively low sensitivity of heterozygous thymocytes (see above) sometimes makes it difficult
to distinguish heterozygotes from negative homozygotes directly by the cytotoxicity test. Therefore typing of thymocytes was in this case performed by absorption under previously standardized conditions, (0.06 ml of antiserum diluted 1:200 absorbed with $15 \times 10^6$ thymocytes of mouse to be typed and then tested on 129 thymocytes).

Quantitative estimation of $G_{IX}$ antigen (Fig. 2 and Table II): This was performed by absorption of antiserum samples (1:200) with a range of counted cell numbers followed by cytotoxicity tests of the absorbed samples on 129 thymocytes (details in 37).

Typing of cells for presence or absence of $G_{IX}$ and GCSA antigens (Tables III and IV): Except where the number of cells available was limited, as in the typing of thymocytes of individual living mice (see above), portions of the rat antiserum, diluted nearly to the endpoint, were absorbed (30 min in the cold) with equal volumes of washed packed cells of the tissue to be typed for $G_{IX}$ followed by cytotoxicity testing of the absorbed antiserum on 129 thymocytes. For GCSA-typing the standard reference antiserum C56BL/6 anti-AKR leukemia K36 (1) was used with the standard test cell C57BL/6 leukemia EφG2 induced by MuLV (Gross Passage A), the standard procedure described in reference 1.

RESULTS

I. Representation of $G_{IX}$ Antigen on Mouse Thymocytes of Various Strains (Figs. 1 and 2, Table II).—There are marked strain differences in the sensitivity

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Quantitative Representation of $G_{IX}$ Antigen on Thymocytes of Various Mouse Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>$G_{IX}^+$</td>
<td>129 (and 129/Sv, 129/Sv-SiRCP+/+, 129/Sv-SiRCP/+, 129/Rt, 129/Re-J-dy</td>
</tr>
<tr>
<td>$G_{IX}^+$</td>
<td>CE/J</td>
</tr>
<tr>
<td>$G_{IX}^-$</td>
<td>C58; AKR (and AKR.K, AKR/H-2k); A (and A/TL-, A/0-AKR); I; C3H/He</td>
</tr>
<tr>
<td>$G_{IX}^+$</td>
<td>SJL/J; DBA/2; C3H/An; 101</td>
</tr>
<tr>
<td>$G_{IX}^-$</td>
<td>C57BL/6 (and C57BL/H-2k, C57BL/TL+, C57BL/Ly-A.1, C57BL/Ly-B.1); C57BL/10/J (and B10.129(5M), B10.129(9M), B10.129(10M), B10.129(12M), B10.129(13M)); BALB/c (and T'T+/+); CBA/J; CBA-T6(Lyon); RF/J; DBA/1/J; MA/J; SWR; H-2H; H-2I; H-2G; C57BR/J; C3H/Bi (when young; see text)</td>
</tr>
</tbody>
</table>

Convention for expressing quantitatively the representation of $G_{IX}$ antigen on thymocytes of various strains:

- $G_{IX}^+$: Highest quantity (129 and CE strains only)
- $G_{IX}^+$: Intermediate quantity (approximately $\frac{3}{5}$ of 129 quantity)
- $G_{IX}^+$: Low quantity (approximately $\frac{2}{5}$ of 129 quantity)
- $G_{IX}^-$: No demonstrable antigen

* Congenic strains are grouped in parenthesis after the respective strains whose main genotype they share.

of mouse thymocytes to anti-$G_{IX}$ serum in the cytotoxicity test. Most strains fall into one or another of four categories indicated in Fig. 1: $G_{IX}^+$ with (a) high, (b) intermediate or (c) low sensitivity, or (d) $G_{IX}^-$ (completely insensitive).

This suggested either that there are quantitative strain differences in representation of $G_{IX}$ or that more than one specificity is involved. The latter was ruled out by cross-absorption, which showed that thymocytes of each $G_{IX}^+$ strain absorbed all cytotoxic activity for all other $G_{IX}^+$ strains. Quantitative
absorptions (Fig. 2) confirm that these strain differences are in fact due to characteristic differences in the quantity of GIX on thymocytes of different strains. Fig. 2 also illustrates that the amounts of GIX in the three GIX+ categories stand approximately in the ratios 3:2:1, so we shall adopt the notation GIX³, GIX², and GIX¹ for distinguishing the three GIX+ phenotypes. For convenience, we shall use the same symbols for the genotypes.

Fig. 2 includes two examples of 50% representation of GIX on thymocytes of GIX+ × GIX− hybrids as compared with the homozygous GIX+ parent.

Fig. 1. Strain differences in sensitivity of thymocytes to anti-GIX serum. Cytotoxic activity of anti-GIX serum on thymocytes of four mouse strains representing the three GIX+ categories GIX³ (129), GIX² (I), GIX¹ (C3H/An), and the fourth category GIX− (C57BL/6).

Table II shows the strain distribution of the four GIX phenotypes. These do not correspond with GCSA phenotypes, many strains being GIX+:GCSA−. Neither is there correspondence with any known system of allo-antigens, and in any event the quantitative strain differences in GIX representation have no close counterpart in known systems of allo-antigens.

II. Tissue Representation of GIX Antigen (Determined by Absorption).—In normal mice, GIX is exclusively an antigen of lymphoid cells. The extent to which it is represented on lymphocytes from different lymphoid organs depends upon whether or not the mouse belongs to a GCSA− strain or to a GCSA+ strain (strain carrying MuLV as a lifelong overt infection, prototype AKR, see definition in Table I).
(a) In 129, which is the prototype $G_{1x}^+$:GCSA$^-$ strain, $G_{1x}$ is demonstrable only on thymocytes, except in aged mice when it may be detectible also on cells from spleen and lymph nodes. (b) In AKR, which is the prototype $G_{1x}^+$: GCSA$^+$ strain, $G_{1x}$ is easily demonstrable in all lymphoid tissues throughout

![Graphs illustrating the estimation of G_{1x} antigen on thymocytes of various mouse strains and their hybrids by quantitative absorption](image)

**Fig. 2.** Estimation of the amount of $G_{1x}$ antigen on thymocytes of various mouse strains and their hybrids by quantitative absorption (four selected representative tests). Four categories are recognizable, according to the amount of $G_{1x}$ antigen expressed: $G_{1x}^3$ (high; 129 and CE), $G_{1x}^2$ (intermediate; C58, A and I), $G_{1x}^1$ (low; SJL and C3H/An), and $G_{1x}^-$ (C57BL/6 and young C3H/Bl mice). Figs. A, B, C, and D refer to four separate tests, therefore comparison of cell numbers required for absorption (arrows) are valid only internally within each of the four figures. The bold numbers (arrows) indicate how many cells were required to reduce the cytotoxicity of the anti-$G_{1x}$ serum sample to 50% lysis of 129 thymocytes in the standard cytotoxicity test. Note that these numbers signify ratios of approximately 3:2:1 for quantitative representation of $G_{1x}$ on the three $G_{1x}$ categories, $G_{1x}^3$: $G_{1x}^2$: $G_{1x}^1$.

Figs. C and D illustrate that on thymocytes of reciprocal hybrids from $G_{1x}^+$ X $G_{1x}^-$ matings there is 50% representation of $G_{1x}$ in comparison with the $G_{1x}^+$ parent.
life. (c) At least one of the GCSA- strains, C3Hf/Bi (Gix-), can be called a GCSA conversion strain (10) because these mice usually become GCSA+ in later life, at which time they also show the other signs of overt MuLV infection, i.e., appearance of virions and of MuLV-gs antigens. The lymphoid tissues of such mice also become Gix+. The complete change in phenotype can be written MuLV-:GCSA-:Gix- → MuLV+:GCSA+:Gix+.

### TABLE III

<table>
<thead>
<tr>
<th>Phenotypes of malignant cells</th>
<th>Numbers observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leukemias</td>
</tr>
<tr>
<td>GCSA/Gix</td>
<td></td>
</tr>
<tr>
<td>GCSA- Gix+ strains</td>
<td>6 (incl. 1 Passage A Gross virus)</td>
</tr>
<tr>
<td></td>
<td>5 (incl. 1 Passage A Gross virus)</td>
</tr>
<tr>
<td>GCSA- Gix- strains</td>
<td>8 (incl. 1 Rauscher virus)</td>
</tr>
<tr>
<td></td>
<td>2 (Friend virus)</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

* All spontaneous leukemias arising in GCSA+ strains (prototype AKR) were GCSA+: Gix+. The purpose of this table is to illustrate that GCSA and Gix antigens can occur independently of one another on malignant cells of both Gix+ and Gix- mouse strains.

### III. Gix Phenotypes of Leukemias and Other Tumors (Table III): Appearance of Gix Antigen on Malignant Cells of Gix- Mice.—All spontaneous leukemias of the high-incidence AKR and C58 strains were Gix+:GCSA+, as are the normal lymphoid tissues of these GCSA+ strains.

Scoring for presence vs. absence of GCSA and Gix antigens gives four possible phenotypes: ++, +−, −+, and −−. All four were observed, both in Gix- strains and in Gix+ strains, as Table III shows.

Thus Gix is often expressed on the malignant tissues of Gix- mice, as has already been shown for GCSA in GCSA- mice (1). Leukemias induced by Friend, Moloney, and Rauscher, variants of MuLV, were Gix- showing that the Gix- → Gix+ conversion is peculiar to infection with MuLV (Gross),...
which is the common wild-type leukemia virus of the mouse. Of the four sarcomas and one melanoma tested, all were G$_{IX^-}$ although four of them were GCSA$^+$. This GCSA$^-$$\rightarrow$$GCSA^+$ conversion in sarcomas is familiar from earlier studies showing the appearance of MuLV virions and MuLV antigens in sarcomas of GCSA$^-$ mice (see 11 and 12). Three spontaneous mammary tumors, of C3H/An (2) and A(1), were GCSA$^+:G_{IX^+}$.

IV. Induction of $G_{IX}$ Antigen in Rats Inoculated with MuLV at Birth (Table IV).—Newborn W/Fu rats are exquisitely sensitive to leukemogenesis by MuLV (Gross); inoculation of thymocytes from GCSA$^+$ mice induce leukemia in virtually 100% of recipients (5, 38). No trace of $G_{IX}$ antigen is demonstrable in any tissue of normal W/Fu rats.

Table IV summarizes numerous experiments in which thymocytes of W/Fu rats were tested for $G_{IX}$ antigen after neonatal injection of mouse thymocytes of various strains. $G_{IX^-}$$\rightarrow$$G_{IX^+}$ conversion occurred in the thymocytes and spleen cells of all rats receiving thymocytes from the GCSA$^+$ strains AKR and C58. $G_{IX^-}$$\rightarrow$$G_{IX^+}$ conversion of recipient rat thymocytes was also brought about by inoculation of filtrates of GCSA$^+$ thymocytes (Millipore No. 22), and of Passage A Gross virus. Inoculation of thymocytes from GCSA$^-$ strains did not produce conversion, regardless of whether the donor of the thymocytes came from a G$_{IX^+}$ strain, like 129, or from one of the G$_{IX^-}$ strains. Thus the $G_{IX^-}$$\rightarrow$$G_{IX^+}$ conversion is seen to be restricted to those preparations that contain infective MuLV (Gross) and induce leukemia in the rat.

<table>
<thead>
<tr>
<th>Donor (mouse)</th>
<th>Recipient (rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Phenotype</td>
</tr>
<tr>
<td>C57BL/6, BALB/c</td>
<td>GCSA$^-$ G$_{IX^-}$</td>
</tr>
<tr>
<td>129, A, I</td>
<td>GCSA$^-$ G$_{IX^-}$</td>
</tr>
<tr>
<td>C58, AKR</td>
<td>GCSA$^+$ G$_{IX^+}$</td>
</tr>
<tr>
<td>Gross Passage A virus</td>
<td>—</td>
</tr>
</tbody>
</table>

* Based on data from 70 recipient W/Fu rats tested 10-90 days after birth. In every instance thymocytes and spleen cells were tested separately and gave the same result. Recipient thymocytes were tested with H-2 and TL antiserum and shown to be of recipient (rat) not donor (mouse) type.

† $(8-15) \times 10^7$ viable thymocytes injected i.p. into each newborn (<24 hr old) W/Fu rat recipient. In the case of AKR and BALB/c, both viable thymocytes and thymocyte filtrates were tested, and gave similar results. $G_{IX^-}$$\rightarrow$$G_{IX^+}$ conversion is seen to be produced by mouse thymocytes which produce MuLV, but not by mouse thymocytes that do not produce MuLV.
V. Genetics of \( G_{ix} \) Antigen Expression on Thymocytes

(a) Table V: 747 mice of backcross matings of the type \( G_{ix}^3/G_{ix}^- \times G_{ix}^3/G_{ix}^- \) have been scored for 100% vs. 50% expression of \( G_{ix}^3 \). The ratios were 370:377. These data indicate segregation of a single gene. This gene is semidominant, i.e., \( +/+ \) is distinguishable from \( +/- \). Quantitative absorption with thymocytes of selected individual mice showed no variation in the amount of \( G_{ix} \) on thymocytes of different mice within each class, i.e., subclasses of the \( +/+ \) and \( +/- \) categories were not distinguishable.

<table>
<thead>
<tr>
<th>( G_{ix} ) phenotype</th>
<th>Serial backcross* generation number</th>
<th>Total</th>
<th>Genotype inferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>++ (100% ( G_{ix}^3 ))</td>
<td>20 4 5 4 19 21 20 18 13 33</td>
<td>157</td>
<td>( G_{ix}^3/G_{ix}^3 )</td>
</tr>
<tr>
<td>+ (50% ( G_{ix}^3 ))</td>
<td>18 9 4 9 20‡ 15 14 18 18 25‡</td>
<td>150</td>
<td>( G_{ix}^3/G_{ix}^- )</td>
</tr>
</tbody>
</table>

* The successive matings were set up from typed positive (50\% \( G_{ix}^3 \)) mice of the preceding generations; use of \( \delta \) vs. \( \sigma \) backcross parents had no effect on the segregation ratio.
‡ \( G_{ix}^- \) mice, presumed genotype \( G_{ix}^-/G_{ix}^- \) were selected from the progeny of matings among these groups and inbred to produce a 129/\( G_{ix}^- \) congenic line (from the 5th and later from the 10th backcross generation). Further data are available from first generation backcrosses of CBA/J, C57BR/J, and BALB/c (all \( G_{ix}^- \)) to 129; these bring the total 129 strain backcross segregation data (including the figures in this table) to 370++ and 377+ (747 mice in all).

The serial backcross data shown in detail in Table V confirm determination by a single mendelian gene, because the 1:1 ratio was maintained through 10 generations of backcrossing. Intercrossing of positive (50\% \( G_{ix}^3 \)) segregants at the fifth backcross generation (= N5), and later at the 10th, yielded a proportion of \( G_{ix}^- \) (\(-/-\)) progeny as anticipated. Some of these negative segregants were inbred to provide the 129/\( G_{ix}^- \) (N5) congenic stock, which is now being replaced with 129/\( G_{ix}^- \) (N10).

(b) Table VI: In mice of the reciprocal backcross, \( G_{ix}^3/G_{ix}^- \times G_{ix}^-/G_{ix}^- \), the segregation data fit very closely to the expectation for two unlinked genes; 40 positive: 116 negative (observed) vs. 39 positive: 117 negative (expected).

(c) Table VII: To define the two-gene requirement further, \( G_{ix}^+ \) segregants from the fifth serial backcross to C57BL/6 \( (G_{ix}^-) \) identified in Table VI were intercrossed and typed for \( G_{ix} \). These matings yielded the anticipated three classes in the ratios expected on the basis of a two-gene requirement for expres-
sion of G\textsubscript{IX}, one gene semidominant and the other fully dominant. Segregants of ++ (100\% G\textsubscript{IX}\textsuperscript{3}) phenotype are being inbred to produce a C57BL/G\textsubscript{IX}\textsuperscript{3} congenic stock.

\textit{(d) Table VIII:} Further confirmation of the two-gene requirement for G\textsubscript{IX} expression was obtained by typing 192 F\textsubscript{2} mice from G\textsubscript{IX}\textsuperscript{3}/G\textsubscript{IX}\textsuperscript{-} × G\textsubscript{IX}\textsuperscript{3}/G\textsubscript{IX}\textsuperscript{-} matings. Again the ratios of the three classes conform to control of G\textsubscript{IX} expression by one fully dominant gene and one semidominant gene.

\textit{(e) Table IX:} Linkage tests have located the semidominant gene in group IX, suggesting the symbol G\textsubscript{IX} (Gross\textsubscript{IX}). The distance of G\textsubscript{IX} from H-2 is 36.4 ± 2.7 units. (Linkage tests with the following markers were negative: Ly-A, Ly-B, Gpd-1, Id-1, Lv, and Mdh-1. Typing for biochemical markers was
performed by Dr. J. J. Hutton, Roche Institute of Molecular Biology, Nutley, N.J.) The results of three-point tests to determine whether Gix is distal or proximal to H-2 are not yet available.

Note Added in Proof.—A three-point cross involving T (brachyury), H-2, and Gix gives the order T:H-2:Gix.

**TABLE VIII**

Two-Gene Determination of Gix Phenotype in F₁ Generation* of Crosses between Strain 129 (Gix³) and C57BL/6 (Gix⁻)

<table>
<thead>
<tr>
<th>GIX phenotype</th>
<th>Observed</th>
<th>Expected†</th>
<th>Genotype inferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>++ (100% Gix³)</td>
<td>37</td>
<td>36</td>
<td>Gix³/Gix³</td>
</tr>
<tr>
<td>+  (50% Gix³)</td>
<td>70</td>
<td>72</td>
<td>Gix³/Gix⁻</td>
</tr>
<tr>
<td>−  (Gix⁻)</td>
<td>85</td>
<td>84</td>
<td>Gix⁻/Gix⁻</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>192</td>
<td></td>
</tr>
</tbody>
</table>

* From matings of (129 × C57BL/6) or reciprocal F₁ mice; separate calculations for the reciprocal matings showed no significant difference in segregation ratios.

† On the assumption that expression of GIX depends on two unlinked genes, the first semidominant (+/+ distinguished from +/−) and the second fully dominant (+/+ indistinguishable from +/−), as the data in Tables V, VI, and VII require (see Appendix).

**TABLE IX**

Linkage of Gix with H-2

<table>
<thead>
<tr>
<th>Parental</th>
<th>Recombinant</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Combined data for the crosses (CBA/J × 129) ♀♀ × 129 and (C57BR/J × 129) ♀♀ × 129*</td>
<td></td>
</tr>
<tr>
<td>H⁻²⁺:Gix⁺</td>
<td>64</td>
</tr>
<tr>
<td>H⁻²⁻:Gix⁺⁺</td>
<td>78</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
</tr>
<tr>
<td>(b) Combined data for the crosses 129 ♀♀ × (CBA/J × 129) and 129 ♀♀ × (C57BR/J × 129)*</td>
<td></td>
</tr>
<tr>
<td>H⁻²⁺:Gix⁺</td>
<td>37</td>
</tr>
<tr>
<td>H⁻²⁻:Gix⁺⁺</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
</tr>
</tbody>
</table>

Mice typed for H⁻² by the cytotoxicity test on lymph node lymphocytes. Estimated map distance 36.4 ± 2.7 units, χ² = 25.4, P < 0.001 (combined data, 208 parental: 119 recombinant).

* Phenotypes, CBA/J and C57BR/J are H⁻²⁺:Gix⁻. 129 is H⁻²⁻:Gix⁺⁺.

Gix⁺⁺ = 100% Gix³ phenotype
Gix⁺ = 50% Gix³ phenotype
DISCUSSION

G<sub>x</sub> as an Allo-Antigen.—G<sub>x</sub> meets the criteria of an allo-antigen; it is present in some strains and absent in others, it is expressed in half-quantity in heterozygotes, and the segregation data are mendelian.

A two-gene requirement for expression of a cell surface antigen is a new finding in the mouse, but has precedents elsewhere; e.g. expression of the A and B blood group phenotypes in man requires that the allele H is present at the unlinked H<sub>H</sub> locus (39). Restriction of G<sub>x</sub> antigen to lymphoid tissue is not unusual, in fact the majority of serologically demonstrable lymphoid cell surface antigens of the mouse are not represented on other tissues (40). These two properties therefore are not exceptional.

But G<sub>x</sub> has other properties which are not those of conventional allo-antigens, and these should be noted particularly for their possible relevance to the question whether G<sub>x</sub> is coded by a viral or a cellular gene. (a) Foremost among these is the induction of G<sub>x</sub> antigen in the lymphoid cells of newborn rats inoculated with MuLV. (b) Although the amount of G<sub>x</sub> antigen on thymocytes of normal mice is a constant property characteristic of each strain of mouse, mouse strains differ in the amount of G<sub>x</sub> antigen which they express. Most G<sub>x</sub><sup>+</sup> mouse strains fall into one of three categories, possessing a high, intermediate, or low amount of G<sub>x</sub>, approximately in the ratios 3:2:1. This may be compared with the reduced amount of H-2 (D) antigen on thymocytes of TL<sup>+</sup> strain mice as compared with TL<sup>-</sup> strain mice (41). But with this exception there is no parallel with these fixed quantitative differences in representation of an antigen from strain to strain. Possibly the ratios 3:2:1 reflect the number of MuLV gene copies present in the respective strains. (c) A third exceptional property is the appearance of G<sub>x</sub> on leukemias and other malignant cells of G<sub>x</sub><sup>-</sup> strains. Similarly, in mice of certain strains that show signs of overt MuLV infection in later life (conversion strains, see footnote to Table I) this event is accompanied by phenotypic conversion of their lymphoid cells from G<sub>x</sub><sup>-</sup> → G<sub>x</sub><sup>+</sup>. A comparable conversion from negative to positive is seen in the TL system of allo-antigens, where TL<sup>+</sup> leukemias occur in strains of mice that are TL<sup>-</sup>, i.e., in mouse strains that normally express no TL antigens (40). The same explanation is offered in both cases, namely that the relevant structural genes are present throughout the species and are normally controlled by other genes with alleles that govern expression vs. nonexpression of the structural genes coding for the G<sub>x</sub> and TL antigens. (d) In conventional systems of allo-antigens it is usual to find that every member of a species expresses one or another of the number of alternative specificities coded by alternative alleles. In the G<sub>x</sub> and TL systems however, alternative antigens representing alleles of the structural genes have not been observed, and indeed their occurrence would be incompatible with the ubiquity of the same structural genes throughout the species, as discussed above (and 40). Strains therefore differ in
respect of expression vs. nonexpression of \( G_{IX} \) and TL antigens, rather than in expressing alternative specificities. This does not itself signify that the structural genes are likely to be viral rather than cellular, because for example an amorph of a cellular structural gene (defined as an allele producing no recognizable product) gives the same negative phenotype as that produced when the pertinent structural gene is present but not expressed because there is a non-expression allele at a controlling locus. The Bombay blood type in man is a case in point, genes for A and B antigens being present but not expressed because of the absence of the allele H at the distant \( Hh \) locus (39). And furthermore there is a priori no reason why a viral gene coding for a cell surface antigen should not exhibit variation and so specify alternative antigens.

The \( G_{IX} \) and \( Tla \) Loci Compared.—Ever since the TL antigens were first described, suggestions have been made about their possible origin from a viral genome integrated in chromosome IX (42, 43). The fact that all known leukemia viruses are of RNA type seemed to tell against this at the time, for no mechanism of making DNA copies seemed to be available. Not only has such a mechanism since been recognized, but we can now point to an important similarity to the \( G_{IX} \) system which we know is intimately associated with a leukemia virus. The resemblance is that both \( G_{IX} \) and TL antigens appear anomalously in the leukemias of mouse strains that normally do not express them. Thus there is now a somewhat better case for regarding \( Tla \) as an integrated viral genome. (Note that \( G_{IX}^{-} \rightarrow G_{IX}^{+} \) conversion implies productive MuLV infection which may or may not be immediately associated with malignant transformation, whereas \( TL^{-} \rightarrow TL^{+} \) conversion is absolutely diagnostic of malignancy.)

The \( G_{IX} \) and \( Tla \) loci, both of which specify antigens confined to lymphoid cells and both of which are intimately connected with leukemogenesis, are also both situated in group IX [as is also a third locus, \( R_{GV} \) (44), a strong determinant of susceptibility to leukemia induction by Gross virus (45) situated at the \( K \) end of \( H-2 \) (46)]. But no obviously useful conclusion seems to follow from this linkage at the moment. The \( G_{IX} \) and \( Tla \) loci are in any event widely separated, and anomalous expression of genes at these loci occurs independently during leukemogenesis i.e., leukemias of \( G_{IX}^{-}; TL^{-} \) strains may be \( G_{IX}^{-}; TL^{-}, G_{IX}^{+}; TL^{-}, G_{IX}^{-}; TL^{+}, \) or \( G_{IX}^{+}; TL^{+} \).

With regard to oncornaviruses as a group (47), (oncogenic RNA viruses including the leukemia–sarcoma viruses of chicken, the mouse mammary tumor virus, and leukemia and sarcoma viruses of several mammalian species including the mouse), it is known that sometimes only a part of the viral genome is expressed in infected cells. Hamster tumors induced by Rous sarcoma virus, for example, have gs viral antigen but produce no virions or envelope antigens (48). Thus the occurrence of \( G_{IX} \) antigen in the absence of other MuLV antigens, as in the thymocytes of 129 mice or in myeloma cells of BALB/c mice, is not an argument against viral coding for \( G_{IX} \).
system, no connection between virions and the presence or induction of antigen has ever been established.) Thus there is a tantalizing correspondence between the two systems that awaits further elucidation.

The Nature of the Two Chromosomal Genes.—To recapitulate, the segregation data, gathered from reciprocal backcrosses, F₂ populations, and intercrosses of serially backcrossed heterozygotes, indicate unmistakably (a) that two unlinked dominant genes are required for the expression of G₁x antigen, (b) that one gene is fully dominant (+/− indistinguishable from +/+ ) and the other semidominant (+/− quantitatively distinguishable from +/+ , and (c) that the latter is loosely linked with H-2 in strain 129 mice.

But the G₁x⁺ phenotype may also be acquired by the cells of G₁x⁻ mice, i.e. mice that do not normally express this antigen; this we refer to as phenotypic conversion, G₁x⁻ → G₁x⁺. With the exceptions indicated by Table III this happens only when productive MuLV infection occurs in the cell population concerned (which in turn is believed to represent release of MuLV from a previous latent state rather than primary exogenous infection, (see refs. 2, 10, 49). This event, MuLV⁻ → MuLV⁺ conversion, occurs (a) in the lymphoid tissues of older mice belonging to certain G₁x⁻ strains (10, 13), and (b) frequently in leukemias and other tumors of G₁x⁻ mouse strains (11, 13).

In summary, the G₁x⁺ phenotype is either inherited as a mendelian trait with full penetrance, like any other allo-antigen, or it is acquired as a consequence of productive MuLV infection. [Because it is not immediately germane to the G₁x system, we shall not discuss why MuLV⁻ → MuLV⁺ conversion so commonly accompanies malignancy (2, 11, 13). Nor shall we discuss why some malignant cells have the unusual phenotype G₁x⁺:GCSA⁻ or G₁x⁻:GCSA⁺ (Table III) for the significance of this is far from clear.]

These findings invite the following interrelated questions: (a) Is the gene coding for G₁x antigen viral or cellular? (b) Can it be identified with either of the two chromosomal genes which are necessary for its expression in 129 mice? (c) Do the data indicate that MuLV is integrated at either of the chromosomal sites identified in the 129 genome?

There are two reasons for not undertaking a lengthy discussion of these questions now. First, no highly probable answer to any one of them can be given at the moment. Secondly, continued study along the lines discussed in this report may provide the data necessary for reaching these important decisions relatively soon, and so obviate a necessarily inconclusive discussion. A few comments will suffice to indicate the outstanding problems to be solved.

If G₁x antigen were a component of the virion, many of the following considerations would be redundant. But this is unlikely, although it is not yet quite excluded. The evidence against it so far is: (a) Monospecific rat antisera prepared against five structural proteins of MuLV (Gross) by R. Nowinski were not cytotoxic for 129 thymocytes (G₁x⁺:GCSA⁻:MuLV-g⁻); these same anti-
sera are cytotoxic for cells productively infected with MuLV. (b) Myelomas producing MuLV (Gross) may be GCSA\(^++\); \(G_{\text{IX}}^-\); [but it could well be argued that \(G_{\text{IX}}^-\) happens to be lacking from a so-far-unclassified subtype of MuLV (Gross) responsible for this exceptional phenotype]. (c) Thymocytes of 129 mice \((G_{\text{IX}}^3)\) do not produce virions (Aoki, T., unpublished work) and lack all MuLV antigens other than \(G_{\text{IX}}^3\); [but this could be attributed to differential expression of MuLV genes]. More critical tests are now in hand to confirm that \(G_{\text{IX}}^3\) is not a component of the virion. The likelihood that \(G_{\text{IX}}^-\) antigen is not a component of the virion leaves us with no hint as to whether the gene that specifies it belongs to the cell or to MuLV.

According to one hypothesis, \(G_{\text{IX}}^-\) antigen may be specified by a cellular gene which is normally expressed in some \((G_{\text{IX}}^+\) strains of mice and not in others (nor in the rat), this gene being activated or derepressed in the cells of \(G_{\text{IX}}^-\) mouse strains (and of the rat) as a result of productive MuLV infection. It is particularly necessary to entertain this possibility in view of precedents elsewhere, notably: (a) The \(T^{-}\rightarrow T^{+}\) conversion accompanying leukemogenesis (40) which signifies aberrant expression of chromosomal genes at the \(T\la\) locus and (b) the appearance on malignant cells of antigens that are normally apparent only on cells that have been trypsinized (50) or on embryo cells (51). There is in fact no cogent evidence against the hypothesis that \(G_{\text{IX}}^-\) antigen may be coded by a cellular gene that can be switched on by MuLV, although this entails some complexities of specification, as we shall see.

But before going further, let's consider whether either of the two identified chromosomal genes can be recognized as the gene coding directly for \(G_{\text{IX}}^-\) antigen. [It will be convenient from now on to refer to this gene, i.e., the gene directly specifying \(G_{\text{IX}}^-\) antigen, as \(\text{str}\), in contrast to epistatic genes governing its expression. In the case of carbohydrate antigens such as the AB blood group substances there can be no structural gene coding for the antigen directly, but it is implicit in this discussion that if \(G_{\text{IX}}^-\) antigen were a carbohydrate rather than a protein, then the gene referred to as structural would in fact code for the enzyme responsible for imparting \(G_{\text{IX}}^-\) specificity to the backbone molecule.] This question is complicated by uncertainty as to whether \(\text{str}\) belongs to the genome of the mouse or to the genome of MuLV. In either event it must be present in all mouse strains because all strains of mice may express it, either congenitally as a mendelian trait or sporadically in MuLV-infected normal or malignant cells, as outlined above.

Accordingly suppose (a) that \(\text{str}\) is a cellular gene. Since it is present in all mice and so cannot segregate in crosses, it automatically is distinguished from both of the chromosomal genes, for the latter were identified by the fact of their segregation. This would mean that the two chromosomal genes control the \(G_{\text{IX}}^+\) phenotype \textit{indirectly} and that \(\text{str}\) is a third gene (which may or may not be linked to one of the other two genes).
Alternatively, suppose (b) that \(str\) is an MuLV gene. The interpretation in this case is less obvious, for the reason that it is not safe to assume (as one can in the case of a cellular gene) that the only possible location for \(str\) is the place where it resides in 129 mice. In other words there is no certainty that \(str\), if it belongs to MuLV, must necessarily be integrated at the same place in all strains of mice or even that it must always be integrated at all. This being so, it follows that expression vs. nonexpression of \(str\) might be bound up with the state or site of the MuLV genome to which it belongs; in this case its expression in \(G_{1x}^+\) mice might be conditional on integration at a particular chromosomal site, as in 129 mice. So \(str\), although common to all mice, could in this way yield conventional segregatin ratios for the phenotypes \(G_{1x}^+\) versus \(G_{1x}^-\).

This argument is entirely speculative, but it serves the purpose of showing that the genetic data involving expression of \(G_{1x}\) antigen in 129 mice can be accommodated within a framework of two rather than three genes, one controlling expression and the other \(str\), if the latter is assumed to constitute part of the MuLV genome.

As to how the expression of a cell surface antigen may be controlled by a gene epistatic to its structural gene, the first choice lies between (a) mechanisms typified by the regulation of protein synthesis in bacteria and (b) those that involve sequential steps in biosynthesis. The latter are illustrated by the human AB blood group specificities, which can be produced only after \(H\) specificity has been conferred on the backbone molecule by the action of the unlinked \(Hh\) locus (39). In the case of \(G_{1x}\), either of these mechanisms is possible and neither is specially favored by the data available.

In studying the nature of the two chromosomal loci further, given our now extensive knowledge of the ecology and immunology of MuLV, we may hope to obtain further insight into the intimate relations of indigenous leukemia viruses with the cells of the host species.

**SUMMARY**

This report concerns a cell surface antigen (\(G_{1x}\); \(G =\) Gross) which exhibits mendelian inheritance but which also appears *de novo* in cells that become productively infected with MuLV (Gross), the wild-type leukemia virus of the mouse.

In normal mice, \(G_{1x}\) is a cell surface allo-antigen confined to lymphoid cells and found in highest amount on thymocytes. Four categories of inbred mouse strains can be distinguished according to how much \(G_{1x}\) antigen is expressed on their thymocytes. \(G_{1x}^-\) strains have none; in the three \(G_{1x}^+\) categories, \(G_{1x}^3\), \(G_{1x}^2\), and \(G_{1x}^1\), the amounts of \(G_{1x}\) antigen present (per thymocyte) are approximately in the ratios 3:2:1.

A study of segregating populations derived mainly from strain 129 (the prototype \(G_{1x}^3\) strain) and C57BL/6 (the prototype \(G_{1x}^-\) strain) revealed that
two unlinked chromosomal genes are required for expression of GIx on normal lymphoid cells. The phenotype GIx + is expressed only when both genes are present, as in 129 mice. C57BL/6 carries neither of them.

At one locus, expression of GIx is fully dominant over nonexpression (GIx fully expressed in heterozygotes). At the second locus, which is linked with H-2 (at a distance of 36.4 ± 2.7 units) in group IX (locus symbol GIx), expression is semidominant (50% expression of GIx in heterozygotes); gene order T:H-2: Tla:GIx.

As a rule, when cells of GIx− mice or rats become overtly infected with MuLV (Gross), an event which occurs spontaneously in older mice of certain strains and which also commonly accompanies malignant transformation, their phenotype is converted to GIx+. This invites comparison with the emergence of TL+ leukemia cells in TL− mouse strains which has been observed in previous studies and which implies that TL− → TL+ conversion has accompanied leukemic transformation of such cells. So far the only example of GIx− → GIx+ conversion taking place without overt MuLV infection is represented by the occurrence of GCSA−:GIx+ myelomas in BALB/c (GCSA−:GIx−) mice.

Unlike the other Gross cell surface antigen described earlier, GCSA, which is invariably associated with MuLV (Gross) infection and never occurs in its absence, GIx− antigen sometimes occurs independently of productive MuLV infection; for example, thymocytes and some leukemias of 129 mice are GCSA−:GIx+, and MuLV-producing sarcomas may be GCSA+:GIx−.

The frequent emergence of cells of GIx+ phenotype in all mouse strains implies that the structural gene coding for GIx antigen is common to all mice. There is precedent for this in the TL system, in which two of the Tla genes in linkage group IX appear to be ubiquitous among mice, but are normally expressed only in strains of mice carrying a second (expression) gene.

It is not yet certain whether either of the two segregating genes belongs to the MuLV genome rather than to the cellular genome. This leaves the question whether MuLV may have a chromosomal integration site still debatable. But there is a good prospect that further genetic analysis will provide the answer and so elucidate the special relationship of leukemia viruses to the cells of their natural hosts.

Discussions with Drs. K. Itakura and R. C. Nowinski have been most helpful. The assistance of Miss C. A. Iritani, Miss L. Hubbard, and Miss G. Sarner in the conduct of this work was indispensable.

**APPENDIX**

These notes on determination of expected segregation ratios are appended at the suggestion of readers who offered comments on the manuscript.
The observed segregation ratios signify that expression of G1X antigen in 129 mice requires one fully dominant gene and one unlinked semidominant gene. The segregation ratios expected from this mode of determination are illustrated in these notes.

Let the two genes be D (dominant) and S (semidominant), their recessive (non-expression) alleles being indicated (−). Then the genotypes are:

129:DDSS,
C57BL/6:−−−−,
(129 × C57BL/6)F1:D−S−.

(a) For the backcross to 129 (D−S− × DDSS):

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Expected proportion of backcross mice of each phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDSS</td>
<td>G1X++ (100% expression)</td>
<td>1/2</td>
</tr>
<tr>
<td>D−SS</td>
<td>G1X+ (50% expression)</td>
<td>1/2</td>
</tr>
</tbody>
</table>

Only the segregation of S is evident in this cross, because DD gives the same phenotype as D− (100% G1X expression in both DD and D− mice); hence the single-gene segregation ratio 1:1.

(b) For the backcross to C57BL/6 (D−S− × −−−−):

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Expected proportion of backcross mice of each phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>D−S−</td>
<td>G1X+ (50% expression)</td>
<td>1/4</td>
</tr>
<tr>
<td>−−S−</td>
<td>G1X− (no expression)</td>
<td>3/4</td>
</tr>
</tbody>
</table>

In this cross the segregation of both genes is evident, hence the two-gene ratio 1:3.

(c) For the F2 generation (D−S− × D−S−):

<table>
<thead>
<tr>
<th>F1 gametes</th>
<th>F1 gametes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS</td>
<td>D−S−</td>
</tr>
<tr>
<td>D−S</td>
<td>D−S−</td>
</tr>
<tr>
<td>−−S−</td>
<td>−−S−</td>
</tr>
<tr>
<td>−−−S−</td>
<td>−−−S−</td>
</tr>
</tbody>
</table>

Genotypes of F2 generation
THE GIX SYSTEM

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Expected proportion of F2 mice of each phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>D D S S (1)</td>
<td>GIX++ (100% expression)</td>
<td>$\frac{9}{16}$</td>
</tr>
<tr>
<td>D -- S S (2)</td>
<td></td>
<td>$\frac{3}{16}$</td>
</tr>
<tr>
<td>D D S-- (2)</td>
<td>GIX+ (50% expression)</td>
<td>$\frac{3}{16}$</td>
</tr>
<tr>
<td>D -- S-- (4)</td>
<td></td>
<td>$\frac{1}{16}$</td>
</tr>
<tr>
<td>D D -- (1)</td>
<td></td>
<td>$\frac{1}{16}$</td>
</tr>
<tr>
<td>D -- -- (2)</td>
<td></td>
<td>$\frac{1}{16}$</td>
</tr>
<tr>
<td>-- S S (1)</td>
<td>GIX- (no expression)</td>
<td>$\frac{1}{16}$</td>
</tr>
<tr>
<td>-- S-- (2)</td>
<td></td>
<td>$\frac{1}{16}$</td>
</tr>
<tr>
<td>-- -- -- (1)</td>
<td></td>
<td>$\frac{1}{16}$</td>
</tr>
<tr>
<td>Total 16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The expected ratios of the three phenotypes are therefore 3:6:7.

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