SPONTANEOUS AUTOIMMUNIZATION TO G\text{IX} CELL SURFACE ANTIGEN IN HYBRID MICE*

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The major envelope glycoprotein of murine leukemia virus (MuLV), gp70, occurs on the thymocytes of several mouse strains which are not overt virus producers (1–3). G\text{IX}-gp70 is a type-specific variant demonstrable by the cytotoxicity assay on thymocytes of the prototype G\text{IX}+ strain 129, and a number of other mouse strains (4). Two congenic mouse stocks have been derived, B6-G\text{IX} (allele donor, 129) and 129-G\text{IX}- (allele donor, B6), which differ from B6 (G\text{IX}–) and 129 (G\text{IX}+), respectively, in regard to expression of G\text{IX}-gp70 on their thymocytes (5). We call these four strains "the G\text{IX} quartet." B6-G\text{IX}+ will be abbreviated as B6+, and 129-G\text{IX}– as 129–.

On thymocytes, G\text{IX} is an accessible antigen, hence its demonstrability in the cytotoxicity assay. Group-specific (gs) antigens elsewhere on the gp70 molecule are relatively or wholly inaccessible unless the membrane is first disrupted (2). Accessibility may be important in determining the consequences of autoimmunization involving gp70 antigens. But so far, G\text{IX} antibody has never been found in normal mouse serum, nor has it been possible to produce it by immunization of mice. Description of the G\text{IX} system has depended on the well-known antiserum "anti-NTD" prepared in inbred rats (4).

We now report that a certain hybrid, the (B6+ × 129)F1 mouse, spontaneously produces G\text{IX} antibody. We shall use the abbreviation "F1 serum" in reference to any pool of normal sera from these hybrids, selected for high titer against B6+ thymocytes with little or no titer against B6 thymocytes.

Materials and Methods

The "two-step" cytotoxicity assay for G\text{IX} (1) was used for all tests with the F1 serum, and the usual "one-step" test (4), in which the cells are not presensitized and washed before adding complement (C), was used for all other purposes. The cytotoxicity index (CI) = (a - b)/(100 - b); where a = % cells lysed by antiserum and C, and b = % cells lysed in the controls with antiserum omitted. Results are expressed either as CI, or as "cells lysed %" (= CI × 100).

The labeling of viable thymocytes with 125I by lactoperoxidase, followed by lysis with Nonidet P-40 (Shell Chemical Co., New York) immunoprecipitation, and electrophoresis in sodium dodecyl

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Abbreviations used in this paper: a, anti; BR, C57BR mouse strain; B6, C57BL/6 mouse strain; CI, cytotoxicity index; GCSA, Gross cell surface antigen; gs, group-specific; Ir, immune response (locus); MuLV, murine leukemia virus; PAGE, polyacrylamide gel electrophoresis; RIP, radio-immunoprecipitation; SDS, sodium dodecyl sulfate; TL, thymus leukemia.
sulfate polyacrylamide gel (SDS-PAGE) is described elsewhere (2). We use the abbreviation "radio-immunoprecipitation (RIP)-lactoperoxidase method" in the text.

The Ig class of the naturally occurring Gxx antibody in F_i serum was determined by affinity chromatography (6). An ammonium sulfate precipitate (38% final saturation) of rabbit anti-mouse IgM (Litton Bionetics, Inc., Kensington, Md.) was conjugated to Sepharose® 4B activated at pH 11.2 with CNBr. F_i serum was applied to the column and the column was washed with phosphate-buffered saline until the OD_{280} of the effluent was <0.025. Class specificity was confirmed by control tests in which anti(α)-H-2 antibody of the IgG class was not retained.

Results and Discussion

1. G_{IX} Specificity of Antibody in the F_i Serum. This is indicated by the positive reaction with B6^{+} thymocytes in the cytotoxicity assay, as compared with the negative or low reaction with B6 thymocytes (Table I). This specificity has been confirmed by segregation tests in the backcross (B6 × B6^{+}) × B6^{+}. Thymocytes of these backcross mice were typed for G_{IX} conventionally with αNTD, and also with F_i serum; the results were entirely concordant. The incidence and titer of G_{IX} antibody rise with age (Fig. 1).

Because the G_{IX} congenic strains differ from their partner strains in expression of at least one other virus-coded protein, p30, as well as gp70 (5, 9), possibly the specificity of the cytotoxic F_i serum might be related to a viral component other than gp70. It is also possible that the antigen recognized by the F_i serum is a feature of the G_{IX}-gp70 molecule but is not identical to G_{IX}. Neither the serological distinctions between the G_{IX} congenic lines (Table I) nor the concordant segregation data exclude these two possibilities, but the following evidence makes them unlikely: (a) It is highly characteristic of G_{IX} identified by the standard rat typing serum αNTD that cytotoxic reactions with the thymocytes of G_{IX}^{+} homozygotes are much higher than with heterozygotes although absorption shows precisely 50% expression on the latter (4); the same is true of the F_i serum. (b) Of 18 various mouse stocks whose G_{IX} phenotypes have already been established conventionally with αNTD (4) all give the same typing reactions with the F_i serum, by both direct tests and absorption. (c) 14 transplanted leukemias and 3 other tumors, 9 G_{IX}^{+} and 8 G_{IX}^{-} (4), were tested for their ability to absorb cytotoxic activity from the F_i serum, the absorbed serum being tested against B6^{+} thymocytes. All the G_{IX}^{+} tumors removed cytotoxic activity from F_i serum; none of the G_{IX}^{-} tumors did so. (d) It is typical of G_{IX} that the thymocytes of different inbred strains display uniformly different amounts of G_{IX} antigen, which greatly influence their sensitivity to αNTD in the cytotoxicity assay (4). Similar differences are seen in sensitivity to the F_i serum, corresponding to published data for the "G_{IX}^{3}, G_{IX}^{2}, and G_{IX}^{1}" categories of G_{IX}^{+} mouse strains (4). (e) The tissue representation of the antigen recognized by the F_i serum is the same as that of G_{IX} (4); i.e. it is demonstrable on thymocytes but not on spleen cells of G_{IX}^{+} low-virus mice, and on thymocytes, spleen, and lymph node cells of high-virus mice like AKR. (f) The serum of 129 mice contains free G_{IX}-gp70 that neutralizes the cytotoxic activity of αNTD against G_{IX}^{+} thymocytes (1). The F_i serum is also neutralized by 129 serum but not by 129^{-} serum. Thus by these several criteria the F_i serum specificity is identical to G_{IX} in cytotoxicity assays.
Table I

Cytotoxicity Assays of Sera from 18 (B6+ × 129) Hybrid Male Mice,*
Selected for High Activity against B6+ Thymocytes with Negligible Activity against B6 Thymocytes

| Mouse strain | Thymocytes lysed† by F1 serum diluted 1/ |
|--------------|----------------------------------------|
|              | 4 | 8 | 16 | 32 | 64 | 128 | 256 |
| B6*          | 94% | 94% | 82% | 65% | 59% | 31% | 9% |
| B6           | 12% | 13% | 7%  | 4%  | 0%  | 0%  | 0%  |

* The data are mean readings for the 18 separate titrations.
† CI × 100 (see Materials and Methods).

![Graph showing incidence and titer of natural cytotoxic GIX antibody in the serum of 144 (B6+ × 129) hybrid mice aged 1-6 mo.](image)

**Fig. 1.** Incidence and titer of natural cytotoxic GIX antibody in the serum of 144 (B6+ × 129) hybrid mice aged 1-6 mo. The serum of each mouse was graded by subtracting the control, "B6 thymocytes lysed %" (CI × 100), from "B6+ thymocytes lysed %" (CI × 100); this allows for the variable and generally low background of cytotoxicity for B6 thymocytes attributable to thymocyte autoantibody of unknown specificity (7, 8).

below) produces appreciable amounts of αGIX. Control by immune response (Ir) genes is a possible explanation. To explain the "nonresponder" status of the parental B6+ and 129 stocks, two dominant Ir loci can be postulated, affecting B and T cells, respectively, or two functionally different T-cell subclasses. Both would be required for the "GIX responder" phenotype, and each parent would contribute one of the two genes to the hybrid. Thus GIX autoimmunization may constitute a natural example of experiments in which matings between mice that are nonresponsive to certain antigens yield responsive progeny (10, 11).

The control hybrid (B6 × 129−) is genetically identical to (B6+ × 129) except for the region of Gv-1, which governs the GIX phenotype, yet it produces no αGIX.
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**Table II**
Assay for G$_{IX}$ Antibody in the Serum of Mice of Various Inbred Strains and Hybrid Stocks*

| Inbred and hybrid mouse stocks | No. of mice (♀ ♂ ages 4-6 mo) with natural G$_{IX}$ antibody: |
|-------------------------------|------------------------------------------------------------|
|                               | - | + | ++ | +++ |
| **The G$_{IX}$ Quartet**      |   |   |    |     |
| 129                          | 16 |   |    |     |
| 129$^-$                      | 9  |   |    |     |
| B6                           | 9  |   |    |     |
| B6$^+$                       | 20 |   |    |     |
| **Quartet hybrids**          |   |   |    |     |
| B6$^+$ × 129                 | 6  | 13 | 12 | 45  |
| B6 × 129                     | 15 | 6  | 3  | 3   |
| B6 × 129$^-$                 | 24 |    |    |     |
| 129 × 129$^-$                | 10 |    |    |     |
| **G$_{IX}$ mutant and companion strains** |   |   |    |     |
| B6 (see above)               | 9  |   |    |     |
| B6-G$_{IX}^+$ M              | 1  | 5  | 5  | 1   |
| BR                           | 16 |    |    |     |
| BR-G$_{IX}^+$ M              | 6  | 3  | 2  |     |
| Other hybrids with 129       |   |   |    |     |
| BALB/c × 129                 | 9  |   |    |     |
| **Other inbred G$_{IX}^+$ strains** |   |   |    |     |
| AKR, AKR-H-2$^a$, C58, A, SJL/J, DBA/2, C3H/An | 31$^\S$ |
| **Other Inbred G$_{IX}^-$ Strains** |   |   |    |     |
| C57L, BALB/c, GR             | 12$^\S$ |   |    |     |
| **Hybrids with AKR**         |   |   |    |     |
| B6 × AKR                     | 6  |   |    |     |
| C57L × AKR                   | 5  |   |    |     |

* Most G$_{IX}^+$ strains fall into three categories (G$_{IX}^+$, G$_{IX}^2$, and G$_{IX}^4$) according to the amount of G$_{IX}$ their thymocytes express (see Table II of reference 4); for example, 129 is G$_{IX}^4$, AKR is G$_{IX}^2$, and C3H/An is G$_{IX}^1$.

† For calculation of (-) to (+++) grades, see legend to Fig. 1. The grades indicate G$_{IX}$-specific lysis of thymocytes, at 1:8 dilution, as follows: -, <15%; +, 15–50%; ++, 50–75%; ++++, >75% (50% end point titer 1:32 to 1:64).

$^\S$ Minimum four mice, maximum six mice, of each strain.

antibody. Therefore the essential immunogen causing autoimmunization is endogenous G$_{IX}$ antigen. The hybrid (B6 × 129) should carry the same complement of Ir alleles, and it expresses G$_{IX}$, though in half the amount because only the 129 parent is G$_{IX}^+$. Autoimmunization might therefore be expected, and it occurs (Table II), although somewhat later than in Gv-l-homozygous (B6$^+$ × 129) hybrids, presumably because the amount of G$_{IX}$ autoantigen is halved. Ultimately the levels of αG$_{IX}$ antibody are as high (data not shown). The expectation for the hybrid (B6$^+$ × 129$^-$) is that it should resemble the hybrid (B6 × 129) in αG$_{IX}$ antibody production. Our data so far indicate that this is so. Finally, a basic genetic interpretation requires that the reciprocal hybrid (129 × B6$^+$) should resemble the (B6$^+$ × 129) hybrid on which most of the study was based. This too is the trend of data now being collected.
So far we have found only two other stocks that spontaneously produce αG\textsubscript{IX} antibody, B6-G\textsubscript{IX}\textsuperscript{+}M and BR-G\textsubscript{IX}\textsuperscript{+}M, which originated as spontaneous mutations from G\textsubscript{IX}\textsuperscript{−} to G\textsubscript{IX}\textsuperscript{+} in B6 and C57BR (BR), respectively (5). There is no ready explanation of why these two stocks should produce αG\textsubscript{IX}, especially since the B6\textsuperscript{+} congenic mouse does not make αG\textsubscript{IX}. Evidently B6 is not a total nonresponder, despite the lack of autoimmunization in B6\textsuperscript{+} congenic mice.

II. Group-specific (gs) Specificity of the F\textsubscript{1} Serum in Immunoprecipitation Tests with Lysed Cells. Antibody to gs antigen of gp70 occurs in some mouse sera (12, 13), but has been tested only against viral gp70, not against gp70 occurring in the thymocyte plasma membrane without production of virus. Such αgs-gp70 antiserum, produced by rabbits or goats immunized with purified gp70 from mouse virus of the FMR type, has little activity against G\textsubscript{IX}\textsuperscript{+} thymocytes in the cytotoxicity assay because the reactive gs antigen is relatively inaccessible (2, 14). Such antisera can partially block the reaction of αG\textsubscript{IX} with G\textsubscript{IX}\textsuperscript{+} thymocytes and so presumably react to some extent with gp70 in the plasma membrane (1). No doubt "gs antigen" comprises a set of determinants, some partially buried in the membrane, and others which are completely inaccessible unless the membrane is first disrupted as in the RIP-lactoperoxidase method.

The G\textsubscript{IX} quartet is ideal for detecting αgs-gp70 antibody by this method, because the four members differ in regard to two gp70 molecules, 0-gp70 and G\textsubscript{IX}-gp70, where expression is governed by separate unlinked genes. The four phenotypes are 0-gp70 [B6]; 0-gp70 and G\textsubscript{IX}-gp70 [B6\textsuperscript{+}]; G\textsubscript{IX}-gp70 [129]; and neither [129\textsuperscript{−}] (14). We have tested the F\textsubscript{1} serum with the thymocytes of all four strains by the RIP-lactoperoxidase method. All except 129\textsuperscript{−} gave the characteristic gp70 peak in SDS-PAGE. The positive reaction of B6 signifies that the F\textsubscript{1} serum recognizes 0-gp70, and so must include a second antibody (αgs) with broader specificity than αG\textsubscript{IX}.

Pooled sera from the two parental strains, B6\textsuperscript{+} and 129 (donors aged >6 mo), gave no reaction for gp70 in the same test system, nor did pooled sera from B6, 129\textsuperscript{−}, or (B6 × 129\textsuperscript{−}) mice. This does not exclude that a few individual mice of these genotypes might have αgs-gp70 antibody which was too diluted by pooling to be detectable, but unquestionably the high gs antibody of the hybrid is not typical of either parent stock.

III. Spontaneous Antigenic Modulation In Vivo. Ever since antigenic modulation was first discovered in the thymus leukemia (TL) system (15, 16), there has been much interest in its possible role in disease. Preimmunization against TL does not protect TL\textsuperscript{−} mice from challenge with syngeneic TL\textsuperscript{+} leukemias. This is the classical instance in which antigenic modulation allows malignant cells to escape destruction by an immune response.

Spontaneous immunization to Gross cell surface antigen (GCSA) occurs naturally in the B6 strain (17), and high levels of αGCSA antibody can be induced by deliberate immunization of B6 mice (18). But this confers little if any protection against GCSA\textsuperscript{−} leukemic transplants, evidently because GCSA is modulated by αGCSA antibody (19).

On the other hand, administration of specific antiserum can confer protection against transplants of leukemias induced by Gross virus (20) and against transplants of X.1\textsuperscript{+} leukemias (21). In these instances malignant cells are not protected by antigenic modulation.
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**Table III**

*Spontaneous Antigenic Modulation of G\textsubscript{tx} on Thymocytes of Autoimmune (B6\textsuperscript{+} × 129) F\textsubscript{1} Mice In Vivo*

| Mice† | Age | Expression of antigens on thymocytes‡ | αG\textsubscript{tx} antibody in serum|| | End point | Lysis |
|-------|-----|--------------------------------------|---------------------------------|---|----------------|-----|
|       |     | G\textsubscript{tx} (αNTD) | G\textsubscript{tx} (F\textsubscript{1} serum) | TL | H-2 | |
|       | mo | % |
| Group 1 (B6\textsuperscript{+} × 129) | 1: | 1 | 1.0 | 1.0 | 1.1 | 0.4 | 0 | 0 |
|       | 2: | 1 | 1.0 | 0.9 | 0.9 | 0.9 | 0 | 0 |
|       | 3: | 1 | 1.0 | 1.0 | 1.1 | 0.8 | 0 | 0 |
|       | 4: | 1 | 1.0 | 0.9 | 1.1 | 0.9 | 0 | 0 |
|       | 5: | 1 | 1.0 | 1.0 | 1.0 | 0 | 0 | 0 |
|       | 6: | 1 | 0.9 | 1.1 | 0.9 | 0 | 0 | 0 |
|       | 7: | 1 | 0.9 | 1.1 | 0.9 | 0 | 0 | 0 |
|       | 8: | 2 | 0.9 | 0.9 | 0.9 | 0 | 0 | 0 |
|       | 9: | 2 | 0.7 | 0.7 | 1.0 | 1.2 | 0 | 0 | 0 |
|       | 10: | 7 | 0.6 | 0.4 | 1.1 | 1.3 | 0 | 0 | 0 |
|       | 11: | 2 | 0.4 | 0.2 | 1.1 | 1.0 | 0 | 0 | 0 |
|       | 12: | 8 | 0.4 | 0.1 | 1.1 | 1.3 | 0 | 0 | 0 |
|       | 13: | 7 | 0.4 | 0.0 | 1.1 | 1.3 | 0 | 0 | 0 |
|       | 14: | 8 | 0.4 | 0.0 | 1.1 | 1.2 | >64 | >64 | 0 |
|       | 15: | 10 | 0.3 | 0.0 | 1.1 | 1.5 | 32 | 32 | 0 |
|       | 16: | 14 | 0.2 | 0.1 | 1.2 | 1.3 | 16 | 16 | 0 |
|       | 17: | 4 | 0.2 | 0.0 | 1.1 | 0.9 | 32 | 32 | 0 |
|       | 18: | 6 | 0.0 | 0.0 | 1.1 | 1.0 | 64 | 64 | 0 |
|       | 19: | 10 | 0.0 | 0.0 | 1.2 | 1.5 | >64 | >64 | 0 |
|       | 20: | 14 | 0.0 | 0.0 | 1.2 | 1.3 | 8 | 8 | 0 |
|       | 21: | 14 | 0.0 | 0.0 | 1.2 | 1.3 | >64 | >64 | 0 |
| Group 2 (control) 129 | 1: | 6 | 1.1 | 1.1 | 1.1 | 1.2 | 0 | 0 |
|       | 2: | 9 | 1.1 | 1.1 | 1.1 | 1.3 | 0 | 0 |
|       | 3: | 11 | 1.1 | 1.1 | 0.9 | 1.4 | 0 | 0 |
|       | 4: | 6 | 1.0 | 1.1 | 1.0 | 1.1 | 0 | 0 |
|       | 5: | 1 | 1.0 | 1.0 | 1.2 | 1.4 | 0 | 0 |
|       | 6: | 9 | 1.0 | 1.0 | 1.2 | 1.2 | 0 | 0 |
|       | 7: | 10 | 1.0 | 1.0 | 0.7 | 1.6 | 0 | 0 |
| Group 3 (control) B6\textsuperscript{+} | 1: | 1 | 1.0 | 1.0 | 1.0 | 1.0 | 0 | 0 |
|       | 2: | 3 | 1.0 | 1.0 | 1.0 | 1.0 | 0 | 0 |
|       | 3: | 4 | 1.0 | 1.0 | 1.0 | 1.0 | 0 | 0 |
|       | 4: | 11 | 1.0 | 1.0 | 0.9 | 0 | 0 |
|       | 5: | 12 | 1.0 | 1.0 | 1.1 | 0 | 0 |
|       | 6: | 10 | 1.0 | 0.9 | 1.1 | 0 | 0 |
|       | 7: | 11 | 1.0 | 0.9 | 1.0 | 0 | 0 |

* A few tested mice were excluded because their thymocytes were abnormally sensitive to C, suggesting that the cells had been sensitized by the autoantibody, either in vivo or during removal of the thymus and preparation of the thymocyte suspension.

† Untreated, individual mice; (all except numbers 3, 15, and 19 of group 1); listed in order of the sensitivity of their thymocytes to G\textsubscript{tx} (αNTD) antibody (3rd heading); group 1 comprised ♀♀ and ♂♂ that had never been mated, groups 2 and 3 include some virgin ♀♂ and some ♀♂ breeders from inbred matings of the respective breeding colonies.
Clearly the extent and effects of antigenic modulation vary in different tumor-associated systems. Regarding cancer, antigenic modulation can only be harmful to the host, and the examples of TL and GCSA suggest that indeed it may well be detrimental under natural conditions. But the situation is different in the case of immune responses that are potentially pathological rather than protective, as in diseases caused by or involving autoimmunization. Here antigenic modulation should be beneficial, and the possibility of antigenic modulation of G\text{IX} in autoimmune hybrids can be viewed in that light. We have studied the thymocytes of the (B6\′ × 129) hybrids, at ages from 1 to 14 mo, and have found that the progressive rise in spontaneous G\text{IX} antibody with age is accompanied by decreased expression of G\text{IX} antigen (Table III). In general, the more aG\text{IX} antibody there is in the serum, the lower the quantity of G\text{IX} demonstrable on thymocytes. The thymocytes of four hybrids, ages 6, 10, 14, and 14 mo, were completely negative for G\text{IX} antigen (Table III).

An alternative explanation for loss of the G\text{IX} phenotype from thymocytes of hybrids making G\text{IX} antibody is that G\text{IX} ÷ cells were destroyed, leaving only medullary thymocytes which characteristically have little or no G\text{IX} antigen. There was no obvious change in the size or cellularity of the thymus, but the more direct evidence against elimination of G\text{IX} ÷ cells is that the thymocytes of the hybrids showed no significant deviation in expression of TL and H-2 antigens (Table III) nor of Thy-1 and Ly antigens (data not given). Thus the thymic cell population of autoimmune hybrids has the usual antigen profile of the major cortical population, not that of the minor medullary population which has no TL, much less Thy-1, and much more H-2.

IV. Other Autoimmune Phenomena in the Hybrid. From section III we infer that antigenic modulation may prevent the destruction or impaired function of G\text{IX} ÷ thymocytes. This raises the question to what extent antigenic modulation may be beneficial in autoimmune states generally: We have some evidence that the hybrids do not escape unscathed. We have observed pronounced splenomegaly, evidently nonleukemic because syngeneic passage of cells from the enlarged spleen does not yield transplantable leukemia, and also histological lesions in the male reproductive tract where large amounts of G\text{IX}-gp70 are normally secreted (reference 22, and personal unpublished observations). Neither sign has so far been seen in age-matched (B6 × 129-) controls. Thus the hybrids are liable to a pathological autoimmune syndrome and are not completely protected by antigenic modulation.

§ Ratio of CI for thymocytes of mouse being tested to CI for control thymocytes. The control thymocytes in each system were from comparable mice of strains not exhibiting spontaneous G\text{IX} antibody production, e.g., B6\′ in the case of the G\text{IX} system. The variation in readings for TL and H-2 is not more than is to be expected from fluctuations in the relative proportions of TL ÷:H-2-low (cortical) and TL ÷:H-2-high (medullary) members of the thymocyte population (16).

¶ Not tested.
At least a part of the florid autoimmune syndrome of the NZB mouse and its hybrids has been ascribed to reactions against the C-type RNA virus which these mice produce in abundance (23). The autoimmune (B6\(+\times129\)) hybrid, on the other hand, expresses only certain virus components, notably G\(_{\text{GIX}}\)-gp70. For this reason, future details of the autoimmune syndrome of the hybrid should be of special interest in revealing the consequences of autoimmunization against a single C-type virus component (perhaps more than one, but not the complete viral set), in a mouse with no underlying genetic abnormality that would predispose to such disease in the absence of that antigen; the latter follows from the fact that the control (B6\(\times129\)-) hybrids have so far shown no signs of disease. It is true that electron microscopy of the (B6\(+\times129\)) hybrid shows small amounts of virus, but not more than are found in B6\(+\) (electron microscope study kindly conducted by Dr. Gloria Koo, Memorial Sloan-Kettering Cancer Center, New York) and in several other mouse strains (5). So there is no obvious reason to think that the autoimmunity we describe depends on the production of complete virus.

V. Ig Class of the G\(_{\text{GIX}}\) Autoantibody. To determine the Ig class of the G\(_{\text{GIX}}\) autoantibody, a pool of F\(_1\) serum was collected from >20 hybrids selected for high \(\alpha\)G\(_{\text{GIX}}\) activity in the cytotoxicity assay. Filtration of this serum pool through Sephadex G200 suggested that the \(\alpha\)G\(_{\text{GIX}}\) activity was in the macroglobulin fraction (mol wt >600,000) with no demonstrable activity in the fractions with low molecular weight (mol wt <200,000). Selective elimination of IgM by affinity chromatography confirmed this; \(\alpha\)G\(_{\text{GIX}}\) activity was thereby reduced to a negligible level. Evidently, the G\(_{\text{GIX}}\) autoantibody belongs mainly to the IgM class.

Summary

The G\(_{\text{GIX}}\) antigen expressed on the thymocytes of G\(_{\text{GIX}}^+\) mice is a type-specific constituent of glycoprotein gp70, which forms the major envelope component of murine leukemia virus. In the prototype G\(_{\text{GIX}}^+\) mouse strain 129, this glycoprotein is a Mendelian character expressed independently of virus production. In the intact thymocyte plasma membrane, part of this glycoprotein, bearing group-specific (gs) antigen, is inaccessible to antibody. The moiety bearing the type-specific G\(_{\text{GIX}}\) determinant is accessible to G\(_{\text{GIX}}\) antibody, which may be an important factor in determining the consequences of autoimmune responses involving G\(_{\text{GIX}}\).

Previously, all attempts to induce G\(_{\text{GIX}}\) antibody in mice had failed. We now find that the hybrid mouse (B6-G\(_{\text{GIX}}^+\times129\)) spontaneously produces substantial amounts of G\(_{\text{GIX}}\) antibody, presumably of the IgM class appearing as early as 2 mo of age. The specificity of the G\(_{\text{GIX}}\) natural mouse antibody is the same as that recognized by the conventional G\(_{\text{GIX}}\) typing serum produced in rats ("anti-NTD"). As neither parent strain produces appreciable G\(_{\text{GIX}}\) antibody, we surmise that this autoimmune response requires two dominant genes, each parent contributing a high-response allele to the hybrid. These can be envisaged as two immune response loci, controlling different immunocompetent cells which must cooperate to produce G\(_{\text{GIX}}\) antibody.
Production of G\(_{x}\) antibody by the hybrids increases progressively with age. This is accompanied by decreased expression of G\(_{x}\) antigen on their thymocytes. We attribute this to antigenic modulation.

Antibody to gs antigen of gp70 is also found in autoimmune (B6-G\(_{x}\) \(\times\) 129) hybrids but not in either parent strain.

We are investigating evidence of a pathological autoimmune syndrome in these hybrids. The special interest of this syndrome is that it presumably signifies the consequences of autoimmunization to a single C-type virus component, expressed without significant virus production, in a mouse with no evident genetic predisposition to such disease in the absence of that antigen.

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