SPONTANEOUS AUTOIMMUNIZATION TO G_{IX} CELL SURFACE ANTIGEN IN HYBRID MICE*

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

On thymocytes, G_{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_{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_{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)F_1 mouse, spontaneously produces G_{IX} antibody. We shall use the abbreviation "F_1 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_{IX} (1) was used for all tests with the F_1 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 ^{125}I by lactoperoxidase, followed by lysis with Nonidet P-40 (Shell Chemical Co., New York) immunoprecipitation, and electrophoresis in sodium dodecyl

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[1] Abbreviations used in this paper: α, 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, radioimmunoprecipitation; 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 G_{IX} antibody in F_1 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_1 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

I. G_{IX} Specificity of Antibody in the F_1 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_1 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_1 serum might be related to a viral component other than gp70. It is also possible that the antigen recognized by the F_1 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_1 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_1 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_1 serum, the absorbed serum being tested against $B6^+$ thymocytes. All the G_{IX}^+ tumors removed cytotoxic activity from F_1 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_1 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_1 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_1 serum is also neutralized by 129 serum but not by 129^- serum. Thus by these several criteria the F_1 serum specificity is identical to G_{IX} in cytotoxicity assays.

Why is it that of many G_{IX}^+ mouse stocks we have tested (Table II), including the parents of the ($B6^+$ × 129) cross, only this hybrid (with exceptions noted

TABLE I

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

Mouse strain	Thymocytes lysed‡ by F_1 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).

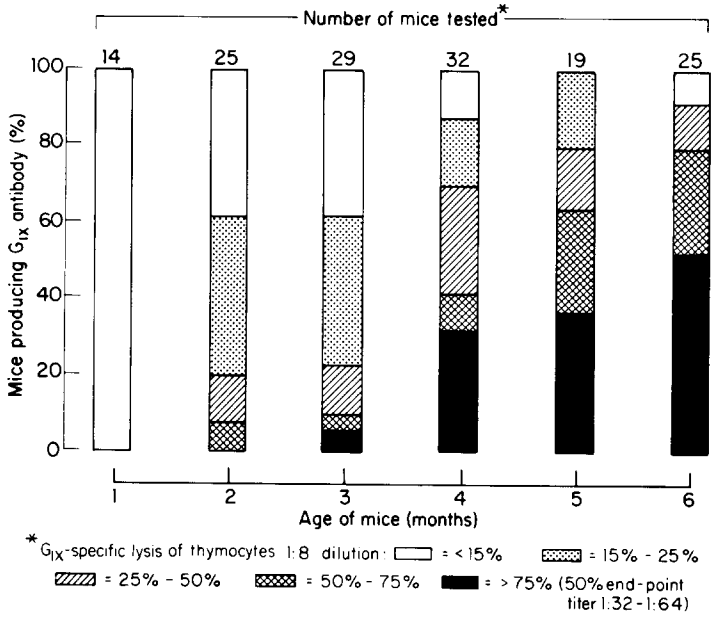

FIG. 1. Incidence and titer of natural cytotoxic G_{IX} antibody in the serum of 144 (B6$^+$ × 129) hybrid mice aged 1–6 mo. The serum of each mouse was graded by subtracting the control, "B6 thymocytes lysed %" (CI × 100), from "B6$^+$ thymocytes lysed %" (CI × 100); this allows for the variable and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

below) produces appreciable amounts of αG_{IX}? 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 "G_{IX} responder" phenotype, and each parent would contribute one of the two genes to the hybrid. Thus G_{IX} 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 G_{IX} phenotype, yet it produces no αG_{IX}

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				
$B6^+ \times 129$	6	13	12	45
B6 × 129	15	6	3	3
$B6 \times 129^-$	24			
$129 \times 129^-$	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*^{b}, C58, A, SJL/J, DBA/2, C3H/An	31§			
Other Inbred G_{IX}^- Strains				
C57L, BALB/c, GR	12§			
Hybrids with AKR				
B6 × AKR	6			
C57L × AKR	5			

* Most G_{IX}^+ strains fall into three categories (G_{IX}^3, G_{IX}^2, and G_{IX}^1) according to the amount of G_{IX} their thymocytes express (see Table II of reference 4); for example, 129 is G_{IX}^3, 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).

§ 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-1*-homozygous ($B6^+ \times 129$) 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 ($B6^+ \times 129^-$) 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 ($129 \times B6^+$) should resemble the ($B6^+ \times 129$) 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_{IX} antibody, B6-G_{IX}^+M and BR-G_{IX}^+M, which originated as spontaneous mutations from G_{IX}^- to G_{IX}^+ in B6 and C57BR (BR), respectively (5). There is no ready explanation of why these two stocks should produce αG_{IX}, especially since the $B6^+$ congenic mouse does not make αG_{IX}. Evidently B6 is not a total nonresponder, despite the lack of autoimmunization in $B6^+$ congenic mice.

II. Group-specific (gs) Specificity of the F_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_{IX}^+ thymocytes in the cytotoxicity assay because the reactive gs antigen is relatively inaccessible (2, 14). Such antisera can partially block the reaction of αG_{IX} with G_{IX}^+ 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_{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_{IX}-gp70, where expression is governed by separate unlinked genes. The four phenotypes are 0-gp70 [B6]; 0-gp70 and G_{IX}-gp70 [$B6^+$]; G_{IX}-gp70 [129]; and neither [129^-] (14). We have tested the F_1 serum with the thymocytes of all four strains by the RIP-lactoperoxidase method. All except 129^- gave the characteristic gp70 peak in SDS-PAGE. The positive reaction of B6 signifies that the F_1 serum recognizes 0-gp70, and so must include a second antibody (αgs) with broader specificity than αG_{IX}.

Pooled sera from the two parental strains, $B6^+$ and 129 (donors aged >6 mo), gave no reaction for gp70 in the same test system, nor did pooled sera from B6, 129^-, or (B6 × 129^-) 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^- mice from challenge with syngeneic TL^+ 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 $GCSA^+$ 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 $X.1^+$ leukemias (21). In these instances malignant cells are not protected by antigenic modulation.

TABLE III

*Spontaneous Antigenic Modulation of G_{IX} on Thymocytes of Autoimmune ($B6^+$ × 129)F_1 Mice In Vivo**

Mice‡		Age	Expression of antigens on thymocytes§				αG_{IX} antibody in serum‖	
			G_{IX} (αNTD)	G_{IX} (F_1 serum)	TL	H-2	End point	Lysis
		mo						%
Group 1 ($B6^+$ × 129)	1:	1	1.0	1.0	1.1	0.4	0	0
	2:	1	1.0	–¶	0.9	0.9	0	0
	3:	1	1.0	1.0	1.1	0.8	0	0
	4:	4	1.0	0.9	1.1	0.9	0	0
	5:	6	1.0	–	1.0	1.0	0	9
	6:	1	0.9	1.1	0.9	–	0	0
	7:	1	0.9	1.1	0.9	–	0	0
	8:	2	0.9	0.9	0.9	–	0	0
	9:	2	0.7	0.7	1.0	1.2	0	20
	10:	7	0.6	0.4	1.1	1.3	0	8
	11:	2	0.4	0.2	1.1	1.0	8	67
	12:	8	0.4	0.1	1.1	1.3	0	33
	13:	7	0.4	0.0	1.1	1.3	32	85
	14:	8	0.4	0.0	1.1	1.2	>64	80
	15:	10	0.3	0.0	1.1	1.5	32	76
	16:	14	0.2	0.1	1.2	1.3	16	72
	17:	4	0.2	0.0	1.1	0.9	32	78
	18:	6	0.0	–	1.1	1.0	64	89
	19:	10	0.0	0.0	1.2	1.5	>64	82
	20:	14	0.0	0.0	1.2	1.3	8	53
	21:	14	0.0	0.0	1.2	1.3	>64	89
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) $B6^+$	1:	1	1.0	1.0		1.0	0	0
	2:	3	1.0	1.0		1.0	0	0
	3:	4	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_{IX} (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_{IX} in autoimmune hybrids can be viewed in that light. We have studied the thymocytes of the ($B6^+ \times 129$) hybrids, at ages from 1 to 14 mo, and have found that the progressive rise in spontaneous G_{IX} antibody with age is accompanied by decreased expression of G_{IX} antigen (Table III). In general, the more αG_{IX} antibody there is in the serum, the lower the quantity of G_{IX} demonstrable on thymocytes. The thymocytes of four hybrids, ages 6, 10, 14, and 14 mo, were completely negative for G_{IX} antigen (Table III).

An alternative explanation for loss of the G_{IX} phenotype from thymocytes of hybrids making G_{IX} antibody is that G_{IX}^+ cells were destroyed, leaving only medullary thymocytes which characteristically have little or no G_{IX} antigen. There was no obvious change in the size or cellularity of the thymus, but the more direct evidence against elimination of G_{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_{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_{IX}-gp70 are normally secreted (reference 22, and personal unpublished observations). Neither sign has so far been seen in age-matched ($B6 \times 129^-$) 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_{IX} antibody production, e.g., $B6^+$ in the case of the G_{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).

‖ Procedure: Step 1; each mouse's serum was absorbed with BALB/c thymocytes to remove thymocyte autoantibody of unknown specificity (7, 8). Step 2; cytotoxicity assay (titration) of the absorbed serum on B6 and $B6^+$ thymocytes (in no case was there any reaction with B6). "End point" = dilution nearest to a 50% fall in CI below the CI of serum at 1:4 dilution ($0 = CI < 0.5$ at 1:4). "Lysis" = percentage of $B6^+$ thymocytes lysed by serum at 1:4 dilution ($CI \times 100$).

¶ 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 ($B6^+ \times 129$) hybrid, on the other hand, expresses only certain virus components, notably G_{IX}-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 ($B6 \times 129^-$) hybrids have so far shown no signs of disease. It is true that electron microscopy of the ($B6^+ \times 129$) 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_{IX} Autoantibody. To determine the Ig class of the G_{IX} autoantibody, a pool of F_1 serum was collected from >20 hybrids selected for high αG_{IX} activity in the cytotoxicity assay. Filtration of this serum pool through Sephadex G200 suggested that the αG_{IX} 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; αG_{IX} activity was thereby reduced to a negligible level. Evidently, the G_{IX} autoantibody belongs mainly to the IgM class.

Summary

The G_{IX} antigen expressed on the thymocytes of G_{IX}^+ mice is a type-specific constituent of glycoprotein gp70, which forms the major envelope component of murine leukemia virus. In the prototype G_{IX}^+ 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_{IX} determinant is accessible to G_{IX} antibody, which may be an important factor in determining the consequences of autoimmune responses involving G_{IX}.

Previously, all attempts to induce G_{IX} antibody in mice had failed. We now find that the hybrid mouse ($B6\text{-}G_{IX}^+ \times 129$) spontaneously produces substantial amounts of G_{IX} antibody, presumably of the IgM class appearing as early as 2 mo of age. The specificity of the G_{IX} natural mouse antibody is the same as that recognized by the conventional G_{IX} typing serum produced in rats ("anti-NTD"). As neither parent strain produces appreciable G_{IX} 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_{IX} antibody.

Production of G_{IX} antibody by the hybrids increases progressively with age. This is accompanied by decreased expression of G_{IX} antigen on their thymocytes. We attribute this to antigenic modulation.

Antibody to gs antigen of gp70 is also found in autoimmune (B6-G_{IX}^+ × 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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