RELATION OF CHROMOSOME 4 (LINKAGE GROUP VIII) TO MURINE LEUKEMIA VIRUS-ASSOCIATED ANTIGENS OF AKR MICE*

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The recent finding that the *Fv-1* locus is in linkage group (LG) VIII[1] and closely linked to the glucose phosphate dehydrogenase-1 [LG VIII biochemical marker] (*Gpd-1*) locus (W. P. Rowe and H. Sato, manuscript in preparation) prompts us to record that in segregating crosses of AKR (an $Fv\text{-}1^n$ strain) with different $Fv\text{-}1^b$ mouse strains, we have found the expression of three murine leukemia virus (MuLV)-associated antigens—G_{IX} (1), GCSA (Gross cell-surface antigen) (2), and gs (group specific, viral antigen) (3)—to be associated with genes in LG VIII. Alleles at *Fv-1* control levels of MuLV output of individual mice by determining the susceptibility of their cells to MuLV of N-tropic or B-tropic type (4). Thus the $Fv\text{-}1^n/Fv\text{-}1^n$ ("NN") genotype of AKR is "permissive" for N-tropic MuLV, which AKR mice produce, so that when MuLV is spontaneously induced in one or more cells of an AKR mouse, or of a hybrid between AKR and an NN mouse strain, the spread of infection is unchecked (5). By contrast, in the "restrictive" genotypes $Fv\text{-}1^n/Fv\text{-}1^b$ (NB) and BB, spread of infection is limited and total MuLV production is thus reduced (6). Consequently two explanations (stated here only in their simplest forms) may be proposed in associating any of the three antigens named with LG VIII: (*a*) expression of antigen reflects virus production and therefore is likely to be a secondary function of *Fv-1*, or (*b*) expression of antigen in AKR mice is mendelian and independent of virus production, in which case the gene responsible

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[1] Rowe, W. P., J. B. Humphrey, and F. Lilly. 1973. A major genetic locus affecting resistance to infection with murine leukemia viruses. III. Assignment of the *Fv-1* locus to linkage group VIII of the mouse. *J. Exp. Med.* **137**:850.

is probably not *Fv-1* but is linked to *Fv-1*. There is ample precedent for both mechanisms of antigen expression and they are not mutually exclusive. The study summarized below was conducted with young mice, where there is less chance of the mendelian phenotype, mechanism (*b*), being obscured by age-related antigenic changes associated with increased output of virus or onset of leukemia (reviewed in reference 7).

G_{IX} and GCSA Phenotypes.—Both G_{IX} and GCSA are cell-surface antigens not yet known to occur in the virion. Thymocytes are used for G_{IX} typing (1), and spleen cells for GCSA typing (2). AKR has both antigens, normal C57BL/6 (B6) mice neither. Thymocytes of the hybrid AKR × B6 (and reciprocal) have 50% expression of G_{IX} (the G_{IX} gene behaving as a semidominant), and the spleen has no detectable GCSA (the GCSA gene appearing recessive). Thus the phenotypes are G_{IX}^{++} $GCSA^+$ (AKR), G_{IX}^+ $GCSA^-$ (hybrid), and G_{IX}^- $GCSA^-$ (B6). Altogether 163 mice of backcrosses to AKR from B6 have been typed for G_{IX} and GCSA, giving 75 G_{IX}^{++} $GCSA^+$ and 88 G_{IX}^+ $GCSA^-$ segregants (no G_{IX}^{++} $GCSA^-$ or G_{IX}^+ $GCSA^+$ types). We concluded that G_{IX} and GCSA are coded or controlled by closely linked genes. Typing for GCSA was thereafter discontinued, allowing the spleen to be used for gs typing.

Correlation of G_{IX} and gs Phenotypes, and Their Association with the LG VIII Markers Fv-1 and Gpd-1.—Table I is a summary of G_{IX} and gs typing results for backcrosses to AKR from B6 and BALB/c (all NN × NB in various mating combinations) with separate tabulation for those segregants that were also typed for *Fv-1* or *Gpd-1*. The gs antigen is an internal component of the virion (3), and its expression in AKR hybrids with B6 or BALB/c is about half that of AKR (8); so the AKR backcross segregants are denoted gs^{++} vs. gs^+.

G_{IX} and gs are strongly correlated, but when analyzed in relation to the segregation of the *Fv-1* (*Gpd-1*) region, it is seen that they are only correlated with each other insofar as they are both correlated with the *Fv-1* (*Gpd-1*) marker. That is, in segregants of the same *Fv-1* (*Gpd-1*) type, G_{IX} and gs phenotypes show no clear-cut correlation. Among the NN or $Gpd\text{-}1^b$ mice 42 of 43 G_{IX}^{++} mice were gs^{++}, as compared with 10 of the 11 G_{IX}^+ mice, while in the NB ($Gpd\text{-}1^{ab}$) mice the corresponding numbers were 1 of 14 vs. 8 of 61. This could indicate (*a*) that G_{IX} and gs are separate loci on LG VIII, on opposite sides of the *Fv-1* (*Gpd-1*) region; (*b*) that one of the two antigens is coded by a locus on LG VIII, while the other is a reflection of virus titer, which is regulated by *Fv-1*; or (*c*) that both antigens result from high virus titers and are thereby correlated with *Fv-1*.

Alternative (*c*) can essentially be eliminated by the lack of full concordance between G_{IX} and gs (166 + 180 concordant; 64 + 59 discordant: Table I) and by the progeny testing of four gs^{++} G_{IX}^+ segregants (Table I), which showed that the G_{IX} and gs determinants were inherited independently (however, the reciprocal type G_{IX}^{++} gs^+ has not yet been confirmed by progeny testing).

With regard to alternative (*b*): of the two antigens, G_{IX} can more confidently be excluded from dependence on virus production on the grounds that after adjustment for gs type, G_{IX} does not correlate with virus titer (Fig. 1). In contrast, gs does correlate with virus titer, and this correlation is not influenced by G_{IX} type. Also, G_{IX} is less concordant than gs with *Fv-1* (48/62 concordant

TABLE I

*Correlation of G_{IX} and gs Antigens in Backcrosses to AKR of Fv-1^b Mice, and Their Association with LG VIII Markers**

Cross	no. of mice with phenotype				Total no. gs^{++}	Total no. G_{IX}^{++}
	gs^{++}** G_{IX}^{++}§	gs^{++} G_{IX}^{+}	gs^{+} G_{IX}^{++}	gs^{+} G_{IX}^{+}		
(BALB/c × AKR) × AKR						
Total	97	33	40	109	130/279 (47%)	137/279 (49%)
Fv-1^n (NN)‖	19	6	0	1	25/26 (96%)	19/26 (73%)
Fv-1^{nb} (NB)	1	5	6	24	6/36 (17%)	7/36 (19%)
(B6 × AKR) × AKR						
Total	69	31¶	19	71	100/190 (53%)	88/190 (46%)
Gpd-1^b	23	4	1	0	27/28 (96%)	24/28 (86%)
Gpd-1^{ab}	0	3	7	29	3/39 (8%)	7/39 (18%)
Grand total	166	64	59	180	230/469 (49%)	225/469 (48%)
NN or *Gpd-1^b*	42	10	1	1	52/54 (96%)	43/54 (80%)
NB or *Gpd-1^{ab}*	1	8	13	53	9/75 (12%)	14/75 (19%)

The mice for these experiments were derived from our own inbred colonies; the AKR and C57BL/6 (B6) colonies were originally established from Jackson breeders.

* For brevity, results with reciprocal hybrids and reciprocal backcrosses have been combined in this table.

** Phenotypes gs^{++} (AKR) and gs^{+} (B6 × AKR) are distinguished by titration of antigen (spleen sonicate) in the immunofluorescence-absorption (IFA) test (8) with E♂G2 (B6 passage A Gross leukemia) as the indicator cell: the gs^{+} phenotype represents about 50% less antigen than gs^{++}. Every mouse was typed with both anti-gs-1 (rabbit anti-MuLV) and anti-gs-3 (rabbit anti-FeLV). The gs-1 and gs-3 phenotypes were invariably concordant. Anti-gs specificity of the rabbit anti-MuLV (Rauscher) serum was confirmed by specific absorption with the major polypeptide peak obtained on GuHCl gel filtration of disrupted virus (kindly supplied by R. C. Nowinski); anti-gs-3 is present in this antiserum, but the specificity it monitors in the IFA test is gs-1 (i.e., the IFA reaction with E♂G2 cells was not neutralized by cat or hamster gs antigen, kindly provided by W. D. Hardy, although both neutralized the anti-gs-3 reaction of rabbit anti-FeLV with E♂G2).

§ G_{IX}^{+} = 50% expression of G_{IX} on thymocytes, as in F_1 hybrids; G_{IX}^{++} = full expression of G_{IX} on thymocytes, as in AKR: for G_{IX} typing see reference 1.

‖ In early tests, typing for NN vs. NB was performed by crossing each mouse with NN stock and challenging the progeny with Friend virus (NN susceptible; NB relatively resistant); this was necessary because the mice had been splenectomized for gs typing. In later tests, the mice were hemisplenectomized for gs typing and afterwards challenged with Friend virus: focus counting on the remaining half of the spleen is apparently adequate for NB typing.

¶ Progeny testing of four of these mice by crossing with AKR gave the following progeny phenotypes: gs^{++} G_{IX}^{++} (9); gs^{++} G_{IX}^{+} (12); gs^{+} G_{IX}^{++} (0); gs^{+} G_{IX}^{+} (0). This result indicates that expression of G_{IX} shows mendelian segregation in gs^{++} homozygotes.

for G_{IX} and *Fv-1*; 55/62 concordant for gs and *Fv-1*: Table I). We interpret these data as strongly indicating that a locus specifying G_{IX} is on LG VIII, about 19 map units from the *Fv-1* (*Gpd-1*) region (calculated from the data in Table I, which indicate 25/129 presumed recombinants between G_{IX} and the *Fv-1* [*Gpd-1*] region).

This leaves the problem whether the gs phenotype in this cross is an expression of virus titer regulated by *Fv-1* or an independent mendelian trait governed

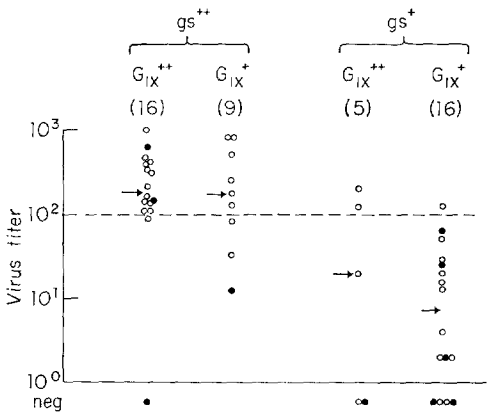

FIG. 1. Infectivity titer (5) (plaque-forming units per 0.4 ml of 2% extract) of tail extracts of AKR backcross segregants, in relation to gs and G_{IX} antigen expression. ○ = (B6 × AKR) × AKR; ● = (BALB/c × AKR) × AKR. Arrows indicate the median titer in each group, and the dashed line the median titer of all samples.

by a discrete locus in LG VIII. Present data do not suffice to decide this point; progeny testing and studies of crosses that do not segregate at *Fv-1* but use the *Gpd-1* marker are in progress, and these should resolve this question.

Location of the "S" Gene for G_{IX}.—The finding of a G_{IX} gene in LG VIII of AKR mice is a surprise. The genetics of G_{IX} thymocyte surface antigen have been studied extensively only in mice of the 129 strain, which characteristically lack all other MuLV-associated antigens and are not overt MuLV-producers like AKR. Expression of G_{IX} antigen on 129 thymocytes requires the presence of positive alleles at two loci: *Gv-1* in LG IX (1) and *Gv-2* in LG I (9). The latter is fully dominant and so need not be discussed here because we are concerned only with backcrosses to AKR of the type $(G_{IX}^{POS} \times G_{IX}^{NEG}) \times G_{IX}^{POS}$, which reveal segregation of only *Gv-1*, the semidominant (or S) gene (1) required for expression of G_{IX} on 129 thymocytes. It now appears that *Gv-1* is located in LG IX of 129 mice but in LG VIII of AKR mice. Two explanations are being considered: (*a*) The S gene for G_{IX} expression (*Gv-1*) does indeed occupy different sites in 129 and AKR mice. (*b*) The association of G_{IX} and H-2 types in backcrosses to 129 is an example of spurious linkage (10): this must be entertained especially in view of the considerable distance estimated between *H-2* and *Gv-1* in the 129 strain (our current figures for backcrosses to 129 from G_{IX}^- strains BALB, CBA, and C57BR are 362 nonrecombinant and 210 recombinant, giving a map distance of 36.7 ± 2.0 units). Genetic tests to discriminate between these two alternatives are in hand.

SUMMARY

Genes specifying or controlling the expression of G_{IX} (cell surface), GCSA (cell surface), and gs (internal viral) antigens are located in chromosome 4 (linkage group [LG] VIII) of the AKR mouse.

All three antigens may exhibit mendelian inheritance, mice being antigen positive or antigen negative, but each may also appear in leukemic cells of mice whose inherited genotype was antigen negative. The G_{IX}-determining gene in LG VIII of AKR mice apparently is equivalent to *Gv-1*, which determines expression of the same antigen in 129 strain mice, but which in the latter strain is located in LG IX. As the estimated distance of *Gv-1* from *H-2* in 129 mice is considerable (37 units) further tests are now indicated to assess the possibility of pseudolinkage in this case.

The *Fv-1* locus, also located in LG VIII, influences the mouse's titer of MuLV, and might thereby be thought to regulate the G_{IX} and gs phenotypes of AKR backcross segregants. But the data indicate a discrete LG VIII locus for G_{IX}, since expression of this antigen is mendelian and independent of infectious virus titer. Since the G_{IX} and GCSA phenotypes of AKR backcross segregants were invariably concordant, these two antigens must be specified or controlled by closely linked genes, and the latter also is presumably independent of virus titer. The question as to what extent expression of gs antigen in the segregants is secondary to virus production is undecided.

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