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

By H. IKEDA,† E. STOCKERT, W. P. ROWE, F. A. BOYSE, F. LILLY, H. SATO,§ S. JACOBS, AND L. J. OLD

(From the Division of Immunology, Sloan-Kettering Institute for Cancer Research, New York 10021, the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014, and the Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461)

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The recent finding that the Fv-1 locus is in linkage group (LG) VIII 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-I strain) with different Fv-P mouse strains, we have found the expression of three murine leukemia virus (MuLV)-associated antigens—Gix (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-I 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-1+/Fv-1− (“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-1+/Fv-1− (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. I. Assignment of the Fv-1 locus to linkage group VIII of the mouse. J. Exp. Med. 137:850.
is probably not \textit{Fv-1} but is linked to \textit{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).

\textit{G_{ix} and GCSA Phenotypes}.—Both \textit{G_{ix}} and GCSA are cell-surface antigens not yet known to occur in the virion. Thymocytes are used for \textit{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 \textit{G_{ix}} (the \textit{G_{ix}} gene behaving as a semidominant), and the spleen has no detectable GCSA (the GCSA gene appearing recessive). Thus the phenotypes are \textit{G_{ix}++ GCSA+} (AKR), \textit{G_{ix}+ GCSA-} (hybrid), and \textit{G_{ix}- GCSA-} (B6). Altogether 163 mice of backcrosses to AKR from B6 have been typed for \textit{G_{ix}} and GCSA, giving 75 \textit{G_{ix}++ GCSA+} and 88 \textit{G_{ix}+ GCSA-} segregants (no \textit{G_{ix}-- GCSA-} or \textit{G_{ix}+ GCSA+} types). We concluded that \textit{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.

\textit{Correlation of \textit{G_{ix}} and gs Phenotypes, and Their Association with the LG VIII Markers \textit{Fv-1} and \textit{Gpd-1}}.—Table I is a summary of \textit{G_{ix}} and gs typing results for backcrosses to AKR from B6 and BALB/c (all NN X NB in various mating combinations) with separate tabulation for those segregants that were also typed for \textit{Fv-1} or \textit{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+.

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

Alternative (c) can essentially be eliminated by the lack of full concordance between \textit{G_{ix}} and gs (166 + 180 concordant; 64 + 59 discordant: Table I) and by the progeny testing of four gs++ \textit{G_{ix}+} segregants (Table I), which showed that the \textit{G_{ix}} and gs determinants were inherited independently (however, the reciprocal type \textit{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 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 $Fv-1$ ($Gpd-I$) region (calculated from the data in Table I, which indicate 25/129 presumed recombinants between $G_{IX}$ and the $Fv-1$ [$Gpd-I$] 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 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 $Fv-1$ ($Gpd-I$) region (calculated from the data in Table I, which indicate 25/129 presumed recombinants between $G_{IX}$ and the $Fv-1$ [$Gpd-I$] region).

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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 Gix 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 Gix.—The finding of a Gix gene in LG VIII of AKR mice is a surprise. The genetics of Gix 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 Gix 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 (Gix pos × GIx neg) × GIx pos, which reveal segregation of only Gv-1, the semidominant (or S) gene (1) required for expression of Gix 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 Gix expression (Gv-1) does indeed occupy different sites in 129 and AKR mice. (b) The association of Gix 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 Gix- 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 Gix (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 gs CSA 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.

REFERENCES
1. Stockert, E., L. J. Old, and E. A. Boyse. 1971. The $G_{IX}$ system. A cell surface alloantigen associated with murine leukemia virus; implications regarding chromosomal integration of the viral genome. *J. Exp. Med.* 133:1334.
2. Old, L. J., E. A. Boyse, and E. Stockert. 1965. The G (Gross) leukemia antigen. *Cancer Res.* 25:813.
3. Geering, G., L. J. Old, and E. A. Boyse. 1966. Antigens of leukemias induced by naturally occurring murine leukemia virus: their relation to the antigens of Gross virus and other murine leukemia viruses. *J. Exp. Med.* 124:753.
4. Pincus, T., J. W. Hartley, and W. P. Rowe. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies with naturally occurring viruses. *J. Exp. Med.* 133:1219.
5. Rowe, W. P. 1972. Studies of genetic transmission of murine leukemia virus by AKR mice. I. Crosses with $Fv-1^{a}$ strains of mice. *J. Exp. Med.* 136:1272.
6. Rowe, W. P., and J. W. Hartley. 1972. Studies of genetic transmission of murine leukemia virus by AKR mice. II. Crosses with $Fv-1^{b}$ strains of mice. *J. Exp. Med.* 136:1286.
7. Old, L. J., and E. A. Boyse. 1973. Current enigmas in cancer research. *Harvey Lect.* 67:273. In press.
8. Hilgers, J., M. Beya, G. Geering, E. A. Boyse, and L. J. Old. 1972. Expression of MuLV-gs antigen in mice of segregating populations. Evidence for mendelian inheritance. *In RNA Viruses and Host Genome in Oncogenesis.* P. Emmelot and P. Bentvelzen, editors. North-Holland Publishing Co., Amsterdam. 187.
9. Stockert, E., H. Sato, K. Itakura, E. A. Boyse, L. J. Old, and J. J. Hutton. 1972. Location of the second gene required for expression of the leukemia-associated mouse antigen $G_{IX}$. *Science (Wash. D. C.)* 178:862.
10. Robinson, R. 1972. Gene Mapping in Laboratory Mammals. Pt. B. Plenum Publishing Corp., New York.