EXPRESSION OF MURINE LEUKEMIA VIRUS ENVELOPE GLYCOPROTEIN gp69/71 ON MOUSE THYMOCYTES

Evidence for Two Structural Variants Distinguished by Presence Vs. Absence of G1X Antigen

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The G1X antigen is found on the thymocytes of some mouse strains (G1X+) and not of others (G1X-) (1). It is detected by the cytotoxicity assay in which G1X+ thymocytes are lysed by G1X antibody and complement. G1X belongs to the "T-cell differentiation antigens," so-called because they are predominantly or exclusively expressed on T cells, including thymocytes. These antigens,—including Thy-1, TL, and the Ly set (loci on chromosomes 6, 9, 17, 19, and elsewhere)—imply a group of genes selectively concerned with T-cell surface composition (2).

But G1X is further distinguished by an association with murine leukemia virus (MuLV) (1), although mice of the prototype G1X+ strain 129 are not life-long producers of demonstrable MuLV (as for example are AKR mice). Thus, productive infection with MuLV frequently causes cells of inherited G1X genotype and of non-T-differentiative pathways to express G1X. Therefore the presence of G1X on 129 thymocytes might be viewed as an MuLV-coded character whose expression is geared to the regulatory system for T-cell differentiation. In the progeny of crosses between 129 and mice of G1X- strains, the segregation of G1X is mendelian, and one outcome of such genetic studies was the establishment of two congenic strains, 129-G1X- and C57BL/6-G1X+ (B6-G1X+), which with their two respective partner strains 129 (G1X+ thymocytes) and B6 (G1X- thymocytes) form a valuable quartet for studies of G1X (3). The hypothesis that G1X thymocyte antigen is coded by MuLV was recently strengthened by evidence that G1X antigen is a feature of gp69/71, the major glycoprotein of the MuLV envelope. This conclusion rested on serological and biochemical data signifying that G1X antigen, which is found free in the serum as well as on the cell surface, was always inseparable from the intact gp69/71 macromolecule (4,5).

We report here that gp69/71 occurs on the thymocytes of many mice that do not express G1X. From this we conclude that MuLV gp69/71 is a surface constituent of thymocytes of most mouse strains, and that G1X antigen distinguishes a sub-set or allele of the mouse's inventory of genes for specifying this structural component of MuLV.

Technical considerations: (a) The well-known antiserum (W/Fu × BN)F1, anti-W/Fu(C58NT)D ("anti-NTD"), which has played a conspicuous part in MuLV studies since

*This work was supported by NIH grants AI-10976, AI-11851, CA-15297, CA-08748, and CA-16599.
its first description in 1966 (6), is produced by inbred rats immunized against a syngeneic rat leukemia induced by MuLV. When used in the cytotoxicity assay against intact viable 129 thymocytes it identifies the antigen G₁ₓ (named originally for its association with "Gross-type" [G] MuLV and with Linkage Group IX of the mouse) which we now regard as a feature of the gp69/71 envelope protein of MuLV (4, 5). In Ouchterlony and other tests with disrupted virions or extracts of MuLV-infected cells, anti-NTD recognizes a variety of MuLV antigens situated on the same or different components of MuLV (6). No method of producing anti-G₁ₓ serum free of antibodies to other MuLV components has yet been devised; so the serological specificity of the G₁ₓ system (cytotoxicity assay) depends on the use of anti-NTD with 129 thymocytes as the indicator cells, other cells being typed G₁ₓ⁺ or G₁ₓ⁻ according to their capacity to absorb cytotoxic activity in this test-system (1). A salient feature of the G₁ₓ typing method is that it relates exclusively to antigen that is available on the intact cell surface for reaction with antibody; antigen on some other part of the same gp69/71 molecule might be inaccessible to the respective antibody unless the plasma membrane were first disrupted. (b) The second technique that is crucial to the following study involves labeling the intact cell surface with radioactive iodine, followed by solution of the cell with the detergent Nonidet P-40 (NP-40). Reaction with the selected antibody is carried out afterwards, (the antigen-antibody complex then being precipitated by addition of anti-immunoglobulin serum). Thus, in this test system the detection of antigen is not limited by accessibility on the intact cell surface. To take a relevant example, the anti-NTD which detects G₁ₓ in the cytotoxicity assay may also contain antibody to other gp69/71 antigens that are so-placed that they are free to react in the second procedure (membrane disrupted) but not in the first (membrane intact). And similarly, according to this model, if the gp69/71 component in the plasma membrane of a particular cell lacked G₁ₓ then anti-NTD could give a positive reaction for gp69/71 in the second assay but not in the first. The iodine-labeling technique, like the cytotoxicity assay, detects only components of the cell surface, for only these are available for labeling on the intact cell.

These considerations are more than simply methodological, for they bear on how glycoproteins like gp69/71 are incorporated in the cell's outer membrane or in the virion envelope, and so have immunological and other implications.

Materials and Methods

Labeling of viable thymocytes with ¹²⁵I, followed by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and labeling of thymocytes and virus with [³⁵S]glucosamine are described elsewhere (5).

Goat anti-Rauscher-MuLV gp69/71, (anti-gp69/71), was prepared by immunizing a goat with the gp69/71 component of Rauscher-MuLV isolated by phosphocellulose chromatography and Sephadex gel filtration (this serum was generously supplied by M. Strand and J. T. August, Albert Einstein College of Medicine, Bronx, N. Y.). (7). Anti-NTD (6), pig antigoat Ig, and rabbit antirat Ig were also used.

Results and Discussion

The thymocytes of various mouse strains were assayed for presence of gp69/71 (envelope glycoprotein of MuLV) in the plasma membrane by ¹²⁵I labeling of the intact thymocyte surface, followed by: (a) NP-40 lysis and clearing by low-speed centrifugation, (b) reaction with anti-gp69/71, (c) precipitation with pig antigoat Ig, and (d) SDS-PAGE. The characteristic peak of gp69/71, which serves as the basis of the assay, was shown in Fig. 4 of reference 5. We cannot review here the evidence that anti-gp69/71, made by immunization with gp69/71 purified from virions, detects exclusively MuLV-gp69/71 and not any other cell surface
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### TABLE I

**Typing of Various Inbred and Congenic Mouse Strains for gp69/71 on the Surface of Thymocytes**

| Thymocytes of: | Known G₁X type (cytotoxicity assay with anti-NTD) | gp69/71 demonstrable† with: anti-gp69/71 | anti-NTD |
|----------------|---------------------------------------------|------------------------------------------|----------|
| The G₁X congenic strains |                               |                                          |          |
| 129             | G₁X+§                                      | +                                        | +        |
| 129-G₁X-        | G₁X-                                      | –                                        | -        |
| B6              | G₁X+                                      | +                                        | +        |
| B6-G₁X-         | G₁X-                                      | –                                        | -        |
| Other mouse strains |                                |                                          |          |
| ARK, C57, A, A-TL- § | G₁X+                                      | +                                        | +        |
| C57/Bl-G₁X-M, NOD, B6-G₁X-M § | G₁X+                                      | +                                        | +        |
| C57BL, B6-Ly-2.1, B6-H-2* | G₁X+                                      | +                                        | +        |
| B6-TL*†        | G₁X+                                      | +                                        | +        |
| BALB/c, NIH-Swiss (random bred) | G₁X-                                      | –                                        | -        |
| HSFS-Swiss (inbred) |                                            |                                          |          |

*All mice were about 2-mo old, and were bred in our Sloan-Kettering colonies.
† £-lactoperoxidase-label immunoprecipitation method: (+) indicates presence, and (−) absence, of the characteristic gp69/71 peak on SDS-PAGE (see e.g., Fig. I). For evidence that this is MuLV-gp69/71, and that both anti-gp69/71 and anti-NTD recognize the same macromolecule, see references 4 and 5.
§ Many G₁X+ mouse strains fall into three categories depending on how much G₁X antigen their thymocytes express, according to quantitative absorption data (1). G₁X strains express approximately one-third, and G₁X two-thirds, of the amount on G₁X (prototype 129) strains. G₁X-positive strains which do not fit these categories closely, or have not been adequately tested by quantitative absorption, are recorded as G₁X +.
† Congenic mouse strain produced and bred at Sloan-Kettering.
**glycoprotein; this rests particularly on lack of any reaction with uninfected control cells, even in the highly sensitive radioimmunoassay (7).**

The results are summarized in Table I, and our conclusions are: (a) gp69/71 is demonstrable, with anti-gp69/71, on the thymocytes of all strains whose thymocytes are known to be G₁X+, and also on several strains whose thymocytes are G₁X-. (b) When anti-NTD was substituted for anti-gp69/71 in the same procedure (final column of Table I) the results were invariably concordant with anti-gp69/71. Thus anti-NTD serum, which in the cytotoxicity assay identifies a restricted (G₁X+) set of mice with gp69/71+ thymocytes, identifies all strains with gp69/71+ thymocytes, when this antiserum is used in the iodine-labeling method. We infer that anti-NTD contains antibody to group specific (gs) antigen of gp69/71 as well as antibody to G₁X, the latter now appearing as a type specificity of gp69/71. We can account for the virtual lack of cytotoxicity of (rat) anti-NTD on G₁X−:gp69/71+ thymocytes, as well as of (goat) anti-gp69/71 on thymocytes of all mouse strains (4), by assuming that the anti-gs-gp69/71 activity of both antisera is directed to gs antigen sites that are relatively inaccessible in the intact plasma membrane (see introductory comments above). (It is immaterial in this context whether the two antisera recognize the same or different gs specificities of gp69/71.) This interpretation is favored in unpublished experiments performed by Obata and Tung in which anti-NTD, from which anti-G₁X activity (cytotoxicity assay) had been removed by absorption with intact G₁X+ thymocytes, was
still active in detecting gp69/71 in the 125I-immunoprecipitation assay; this we attribute to the disruption of the plasma membrane before reaction with gs antibody. (c) The phenotype of the B6 thymocyte is specially relevant to the postulate of two gp69/71 variants, one bearing G1X antigen and the other not, because the two congenic strains B6-G1X+ and 129-G1X− were both derived from a
cross between B6 and 129 mice. The gp69/71+ phenotype of B6 thymocytes tells us that if both \( G_{1x}^- \)gp69/71 and \( G_{1x}^+ \)gp69/71 molecules are coded by chromosomally integrated MuLV genomes, then these two gp69/71 genes belong to different loci that are not closely linked to each other. This follows from the fact that thymocytes of 129-G\( _{1x}^- \) mice are \( G_{1x}^- \)gp69/71 whereas B6 thymocytes are \( G_{1x}^+ \)gp69/71+. If the B6 \( G_{1x}^- \)gp69/71 locus were closely linked to the \( G_{1x}^+ \)gp69/71 locus it would have been introduced into the 129 stock during the serial backcrosses used to produce the 129-G\( _{1x}^- \) congenic stock, and the latter's thymocytes would be \( G_{1x}^- \)gp69/71+, which they are not; they are \( G_{1x}^- \)gp69/71-.

(d) The corollary is that thymocytes of the B6-G\( _{1x}^+ \) congenic stock should carry both species of gp69/71 molecule. Fig. 1 supports this by indicating that 129 and B6 thymocytes carry physically distinguishable gp69/71 components, and that thymocytes of congenic B6-G\( _{1x}^+ \) mice carry both. Fig. 1 A, B, and C shows SDS-PAGE patterns for thymocytes of 129, B6, and B6-G\( _{1x}^+ \) mice, respectively. The standard (solid line) in each case is \([\text{H}]\)-glucosamine-labeled Gross-MuLV-gp69/71 added to the \([\text{I}]\)-labeled thymocyte immunoprecipitates for coelectrophoresis. The gp69/71 component of 129 runs slightly in front of the standard (Fig. 1 A) and that of B6 somewhat behind (Fig. 1 B), whereas the gp69/71 peak of B6-G\( _{1x}^+ \) straddles the standard (Fig. 1 C), suggesting that it comprises both \( G_{1x}^+ \)gp69/71 (129) and \( G_{1x}^- \)gp69/71 (B6) components. (The experiment shown in Fig. 1 has been repeated twice, and the same differences in mobility were observed in both.) This accords with the higher titer of gp69/71 antigen recorded by Stockert et al. (3) for B6-G\( _{1x}^+ \) thymocytes as compared with either 129 or B6, and with radioimmunoassay measurements for spleen of these three strains (8, 9).

Two reports of close relevante to this study refer (a) to the presence of gp69/71 in spleen of three members of the “G\( _{1x} \) quartet,” and its absence from the fourth, 129-G\( _{1x}^- \) (8, 9), as we report here for thymocytes, and (b) to other evidence implying structural variation of MuLV-gp69/71 (10).

Summary

Thymocytes of several mouse strains were tested for expression of the gp69/71 envelope component of murine leukemia virus by surface iodination, followed by immunoprecipitation and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. These strains included two congenic lines differing from their partner stocks with respect to expression of G\( _{1x} \) antigen demonstrable in the cytotoxicity assay. We conclude that: (a) two structural variants of gp69/71 can be expressed on mouse thymocytes, (b) these are distinguishable by a small difference in mobility in SDS gels, (c) one carries G\( _{1x} \) antigen and the other not, (d) they are coded, or their expression is regulated, by different chromosomal loci that are not closely linked, and (e) both can be expressed together on the thymocytes of inbred mice. In the intact thymocyte plasma membrane, the sites of group-specific antigen shared by the two gp69/71 variants, unlike the G\( _{1x} \) type specificity carried by only one of them, are probably inaccessible to antibody.

We thank Dr. F. Lilly for a timely suggestion regarding the segregation genetics, now published (8), Dr. E. Stockert for the anti-NTD, Doctors M. Strand and J. T. August for anti-gp69/71, Dr. R.
Wilsnack for pig antigoat Ig, Dr. U. Hämmerling for rabbit antirat Ig, and Mrs. J. Seguin for valued technical assistance.

Received for publication 6 March 1975.

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