

ANTIBODY DIRECTED TO DETERMINANTS OF A
MOLONEY VIRUS DERIVED MCF GP70 RECOGNIZES A
THYMIC DIFFERENTIATION ANTIGEN*

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An extensive family of endogenous C-type murine leukemia viruses (MuLV) are present in all somatic and germ line cells of the mouse (1). Certain protein constituents of these retroviruses, most notably the surface glycoprotein gp70, can be expressed on lymphoid and epithelial cells, even in mouse strains such as 129 where no complete virus has ever been isolated (2). Furthermore, distinct gp70s are expressed at different sites (3–6), implying that gp70s represent a multigene family of differentiation antigens (7). In strains such as AKR and HRS, this seemingly compatible relationship between host and virus breaks down, resulting in virus-induced leukemia at around 6–12 months of age (8, 9). The onset of leukemia coincides with the appearance of leukemogenic recombinant viruses, derived via recombination between innocuous ecotropic and xenotropic retroviruses (10), primarily within the envelope gene encoding gp70 (11–13).

In the present study, we describe an antiserum that was raised against a chemically synthesized peptide derived from the nucleotide sequence of a Moloney MCF virus recently published by Bosselman et al. (13). We selected a region of the recombinant virus gp70 that was unique from the parental Moloney virus so as to provide a probe specific for the endogenous sequence involved in recombination. The results show that the antigenic determinants displayed by the above peptide are present specifically on an M_r 70,000 molecule produced by normal peanut agglutinin positive (PNA⁺) thymocytes.

Materials and Methods

Synthetic Peptides. We selected a 14-amino-acid-long region of Moloney MCF virus gp70 which resides 148 amino acids from the N-terminus of gp70, as published by Bosselman et al. (13). The sequence in this region is S-L-K-R-G-N-T-P-R-N-Q-G-P-C. This peptide was chemically synthesized as previously described (14) and conjugated to Keyhole Limpet Hemocyanin (KLH) through the cysteine residue as described for production of antisera (14).

Antisera. A broadly reactive goat antiserum to gp70 (G230, reference 15) was utilized as a positive control serum. R-47 (R₂06B08), a monoclonal antibody specific for Rauscher ecotropic virus, was also used as a check for specificity.

Viruses. Rauscher (R-MuLV), Moloney (M-MuLV), AKR (AKR-MuLV), Gross (G-

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MuLV), and Feline (FeLV) leukemia viruses were obtained from the Biological Carcinogenesis Branch of the National Institutes of Health. AKR 247 virus was grown in SC-1 cells as previously described (10). Cultures of pure ecotropic R-MuLV and Rauscher-MCF were the generous gift of Dr. M. Vogt of the Salk Institute.

Mice. Mice were obtained from the breeding colony at Scripps Clinic and Research Foundation, except for 129G_{1x}- and 129 strains, which were obtained from Dr. T. Boyse of the memorial Sloan-Kettering Cancer Center.

Preparation of Cell Extracts. Mice were sacrificed and tissues were immediately removed and macerated in phosphate-buffered saline (PBS) containing 2.0 mM phenylmethylsulfonyl fluoride (PMSF, Sigma Chemical Co., St. Louis, MO) at 4°C. Single cells were suspended, washed once, then extracted in PBS-PMSF containing 1.0% Nonidet P-40 (NP40, Sigma). After 15 min at room temperature, the extract was filtered (0.45 μ m) and stored at -20°C. Peanut agglutinin positive and negative cells were prepared as described (16) and treated as above for analysis.

Western Blot Analyses. Protein constituents of each tissue sample were separated by SDS-PAGE as previously described (17), then electrophoretically blotted onto nitrocellulose paper (18). Protein bands were stained with 0.01% amido black stain in 45% methanol/10% acetic acid to ensure transfer was adequate. The blots were then blocked and antibody labeled using a modification of standard procedures. Briefly, blots were exposed to a solution, designated BLOTTO, which contained 5% nonfat dry milk in PBS (pH 7.4) containing 0.01% Antifoam A emulsion (Sigma) and 0.001% merthiolate. The blots were allowed to block for 2-16 h at 4°C, then incubated with antibody in the presence of BLOTTO for 3 h. The blots were then washed three times in BLOTTO and incubated for 1 h at room temperature with ¹²⁵I-staphylococcus protein A at a concentration of 1 \times 10⁶ cpm per gel lane. They were then washed extensively as above, rinsed with water, dried, and subjected to autoradiography. Alternatively, blots were developed using a peroxidase staining technique described elsewhere (18).

Results

The results of Western blot analyses using anti-peptide 81 antibody vs. several virus preparations are shown in Fig. 1A. Reactivity was observed with AKR-247 (lane 3), with the lower of two gp70 components of Gross virus (lane 5) and with preparations of Moloney virus (lane 1) and Rauscher virus (lane 4). No reactivity was observed with AKR-MuLV gp70 (lane 2) or the predominant gp70 band of Gross virus (lane 5) which reacted strongly with G230 heteroserum (Fig. 1B, lane 5). All the virus preparations possessed gp70s strongly reactive with the broadly reactive goat anti-gp70 antiserum (Fig. 1B). Peroxidase staining of R-MuLV after treatment with G230 (Fig. 1C, lane 1) resolved two reactive bands at approximately M_r 70,000. Successive staining of one blot with anti-81 sera (Fig. 1C, lane 2), followed by reaction with R47 monoclonal antibody (Fig. 1C, lane 3) demonstrated that only the lower band was reactive with anti-81, while only the upper band was reactive with the ecotropic specific monoclonal. We conclude the RLV Lot JLS/V9 is contaminated with a Rauscher recombinant virus whose gp70 runs slightly lower than ecotropic gp70, a characteristic of MCF-type viruses. Additional confirmation of the nonreactivity of anti-81 with ecotropic R-MuLV was obtained using a known pure ecotropic R-MuLV (Fig. 1C, lanes 4 and 5). Extracts of SC-1 cells infected with this virus were positive with the goat anti-gp70 heteroserum (lane 4), but did not react with anti-81 (lane 5).

Treatment of 81-TAg with a new endoglycosidase, Endo F (19), which removes all N-linked glycans from glycoproteins reduced its molecular weight to M_r

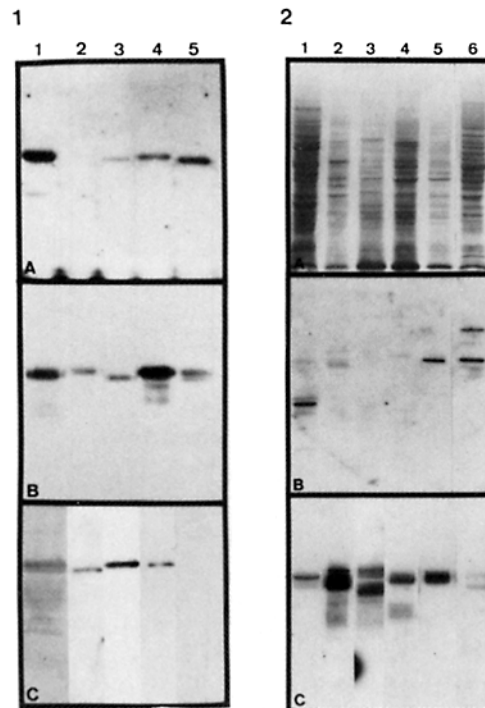


FIGURE 1. Reactivity of anti-peptide 81 antiserum with retrovirus preparations. Shown in panels *A* and *B* are Western blots of the following virus preparations: Moloney (M-MuLV) (lane 1), AKR-MuLV, (lane 2), AKR-247 (lane 3), Rauscher (R-MuLV) (lane 4), and Gross (G-MuLV) (lane 5). Panel *A* shows reactivity with anti-peptide 81 antiserum; panel *B*, reactivity with G230, a broadly reactive anti-gp70 heteroserum. Panel *C*, lane 1: peroxidase stained blot of R-MuLV versus G230, demonstrating the presence of two components not resolved using the ^{125}I -staphylococcal protein A technique. Lane 2 shows the reactivity of an identical blot with anti-81 peptide (^{125}I staphylococcal protein A labeled) and lane 3 shows the same blot labeled with R47, a monoclonal antibody specific for ecotropic R-MuLV. Lanes 4 and 5 contain a separate preparation of pure ecotropic R-MuLV demonstrating, respectively, the reactivity with G230 and lack of reactivity with anti-81 peptide. **FIGURE 2** Reactivity of anti-81 peptide antiserum with mouse tissues. Western blots of NP-40 extracts of the following tissues from a 2-month old NZB mouse are shown: testes (lane 1), genital tract (lane 2), bone marrow (lane 3), spleen (lane 4), thymus (lane 5), and brain (lane 6). Panel *A*, amido black stain of the preparations; panel *B*, reactivity of anti-81 peptide; panel *C*, reactivity with G230 heteroserum.

49,000 (not shown) comparable to viral gp70s (19).

We next evaluated the reactivity of anti-81 with detergent extracts of normal NZB mouse tissues. As can be seen on Fig. 2*A*, by amido black stain, a complex pattern of proteins was transferred to nitrocellulose from each tissue, including testes (lane 1), genital tract (lane 2), bone marrow (lane 3), spleen (lane 4), thymus (lane 5), and brain (lane 6). Reactivity with anti-81 antiserum (Fig. 2*B*), however, was very discrete. Most notably, M_r 70,000 bands were detected in thymus (lane 5) and brain (lane 6) and a low molecular weight component was also recognized in testes (lane 1). Brain also contains a reactive band at about M_r 150,000. Most significant was the lack of reactivity in bone marrow or spleen (lanes 3 and 4), although both tissues contained multiple components strongly reactive with G230 (panel *C*).

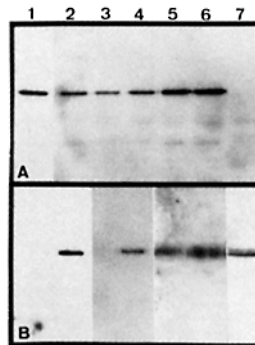


FIGURE 3. Expression of 81-TAg in thymus extracts. Thymus extracts of 1–3-mo-old male mice of the following strains are shown on panel A: 129 G_{IX} -/BoyScr (lane 1), 129/J (lane 2), HRS/J-*hr/hr* (lane 3), HRS/J-*hr/+* (lane 4), C57BL/6J (lane 5) and AKR/J (lane 6). Lane 7 contains AKR/J spleen extract. Panel B, anti-81-TAg reactivity with extracts from 18-mo-old (lane 1) and 2-mo-old (lane 2) AKR mice. Lanes 3 and 4, anti-81-TAg reactivity obtained with 2 month AKR thymocytes negative (lane 3) and positive (lane 4) for reactivity with peanut agglutinin (PNA). Lanes 5 and 6, reactivity with G230 heteroserum against PNA⁻ and PNA⁺ thymocytes, respectively. Lane 7, extract from a spontaneous AKR/J thymic leukemia indicating the positive reactivity of this tissue with anti-81TAg.

For the remainder of the studies reported here, we chose to focus on the thymus antigen, which we have termed 81-TAg. Fig. 3A, lanes 1–6 shows that 81-TAg is expressed in thymus tissues of all 1–3 mo-old mouse strains tested including 129 G_{IX} -/BoyScr, 129/J, HRS/J (*hr/hr*), HRS/J (*hr/+*), C57BL/6J and AKR/J, respectively. Lane 7 contains AKR/J spleen as a negative control. We next evaluated the expression of 81-TAg in thymus tissues of 18-mo-old mice, at which point thymus regression had occurred. Thymocyte extracts from pairs of 18-mo-old and 2-mo-old AKR/J, HRS/J (*hr/hr*), and C57BL/6J mice were tested by Western blotting as above. Identical results were obtained with all mouse strains. Results with AKR thymocytes are shown in Fig. 3B, lanes 1 and 2. 81-TAg is not expressed in the lymphoid cells of the older thymus (lane 1), but is expressed by thymocytes of 2-mo-old mice (lane 2). Thymocytes were further subdivided by agglutination with peanut agglutinin and subjected to Western blot analysis with anti-81-TAg (Fig. 3b, lanes 3 and 4) or G230 (Fig. 3b, lanes 5 and 6). PNA-negative thymocytes were negative for 81-TAg (lane 3) and PNA-positive thymocytes were positive (lane 4). Both PNA-negative (lane 5) and PNA-positive (lane 6) thymocytes were, however, positive for gp70, when reacted with G230 heteroserum. Finally, we examined spontaneous thymic leukemias from AKR/J mice. Fig. 3B, lane 7 is a Western blot of one AKR tumor showing that antigens related to 81-TAg appear in the transformed cells. All spontaneous AKR tumors examined expressed 81-TAg related molecules.

Discussion

In this report, we demonstrated the presence of an M_r 70,000 antigen (81-TAg) in PNA⁺ thymocytes, which is related to the endogenous retrovirus involved in MCF-type recombination. 81-TAg is a glycosylated protein present in 2–3-mo-old thymuses of all mouse strains tested, but not detectable in spleen, bone marrow, or lymphoid cells isolated from 18-mo-old thymuses. The results

indicate that this gp70 is not the same as the G_{IX} differentiation antigen (7), since 81-TAg was expressed equally by both G_{IX}^+ and G_{IX}^- mouse strains as well as all other strains tested.

Since the MCF virus from which the sequence for the peptide was taken causes a thymic leukemia (13), the expression of 81-TAg on thymocytes is particularly relevant. The restriction of 81-TAg expression to PNA⁺ thymocytes provides an interesting relationship to leukemic transformation. Certainly, if recombination resulted in constitutive expression of a differentiation antigen involved in cell maturation, it might lead to neoplastic transformation. Whether 81-TAg qualifies as such an antigen is a point of continued research.

Summary

We prepared an antiserum to a 14-amino-acid-long synthetic peptide derived from the published nucleotide sequence of a Moloney virus-derived MCF recombinant virus. This peptide is not found in the parental Moloney virus gp70 sequence or in other ecotropic viruses so far sequenced. When several tissues of the mouse were examined by Western blot analysis for related molecules, we found that thymocytes from 2–3-mo-old mice, but not splenic T cells or bone marrow cell extracts reacted with this probe. We found that the M_r 70,000 thymic glycoprotein recognized by the anti-peptide antiserum (81-TAg) was present at equivalent levels on peanut agglutinin positive thymocytes in all mouse strains tested. These results indicate that 81-TAg is a differentiation antigen that is related to the substituted portion of an MCF retrovirus.

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References

1. Jenkins, N. A., N. G. Copeland, B. A. Taylor, and B. K. Lee. 1982. Organization, distribution and stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of *Mus musculus*. *J. Virol.* 43(1):26.
2. Stockert, E., E. A. Boyse, Y. Obata, H. Ikeda, N. Sarkar, and H. Hoffman. 1975. New mutant and congenic mouse stocks expressing the murine leukemia virus-associated thymocyte surface antigen G_{IX} . *J. Exp. Med.* 142:512.
3. Tung, V-S., E. S. Vitetta, and E. Fleissner. 1975. Biochemical evidence linking the G_{IX} thymocyte surface antigen to the gp69/71 leukemia virus. *J. Exp. Med.* 141:198.
4. Strand, M., F. Lilly, and J. August. 1974. Host control of endogenous murine leukemia virus gene expression: concentration of viral proteins in high and low leukemia strains. *Proc. Natl. Acad. Sci. USA.* 71:3682.
5. Lerner, R. A., C. P. Wilson, B. C. Del Villano, P. J. McConahey, and F. J. Dixon. 1976. Endogenous oncornaviral gene expression in adult and fetal mice: quantitative, histological, and physiologic studies of the major viral glycoprotein gp70. *J. Exp. Med.* 143:151.
6. Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, T. M. Chused, H. C. Morse,

- J. W. Hartley, and W. P. Rowe. 1980. Differential expression of two distinct xenotropic viruses in NZB mice. *Clin. Immunol. Immunopathol.* 15:493.
7. Stockert, E., L. J. Old, and E. A. Boyse. 1971. The G_{IX} system. A cell surface allo-antigen associated with murine leukemia virus: implications regarding chromosomal integration of the virus genome. *J. Exp. Med.* 133:1334.
 8. Green, E. L. 1975. *Biology of the Laboratory Mouse*. E. L. Green, editor. 2nd edition. Dover Publication, Inc., New York.
 9. Meier, H., D. Myers, and R. J. Huebner. 1969. Genetic control by the *hr*-locus of susceptibility and resistance to leukemia. *Proc. Natl. Acad. Sci. USA.* 63:759.
 10. Hartley, V. W., N. K. Wolford, L. J. Old, and W. P. Rowe. 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc. Natl. Acad. Sci. USA.* 74:789.
 11. Elder, J. H., J. W. Gautsch, F. Jensen, R. A. Lerner, J. W. Hartley, and W. P. Rowe. 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (*env*) gene recombinants. *Proc. Natl. Acad. Sci. USA.* 74:4676.
 12. Rommelaere, J., D. V. Faller, and N. Hopkins. 1978. Characterization and mapping of RNase T1-resistant oligonucleotides derived from the genomes of AKV and MCF murine leukemia viruses. *Proc. Natl. Acad. Sci. USA.* 75:495.
 13. Bosselman, R. A., F. Von Straaten, C. Van Beveren, I. M. Verma, and M. Vogt. 1972. Analysis of the *env* gene of a molecularly cloned and biologically active Moloney mink cell focus-forming (MCF) proviral DNA. *J. Virol.* 44:19.
 14. Lerner, R. A., N. Green, H. Alexander, F-T. Liu, J. G. Sutcliffe, and T. M. Shinnick. 1981. Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles. *Proc. Natl. Acad. Sci. USA.* 78:3403.
 15. Elder, J. H., F. C. Jensen, M. L. Bryant, and R. A. Lerner. 1977. Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differentiation antigens encoded by a multi-gene family. *Nature (Lond.)*. 267:23.
 16. London, J., S. Berrih, and J-F. Bach. 1978. Peanut agglutinin I. A new tool for studying T-lymphocyte subpopulations. *J. Immunol.* 122(2):438.
 17. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680.
 18. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: Procedure and application. *Proc. Natl. Acad. Sci. USA.* 76(9):4350.
 19. Elder, J. H., and S. Alexander. 1982. Endo-B-N-Acetylglucosaminidase F: endoglycosidase from *Flavobacterium meningosepticum* that cleaves both high-mannose and complex glycoproteins. *Proc. Natl. Acad. Sci. USA.* 79:4540.