ENDOGENOUS ONCORNAVIRAL GENE EXPRESSION
IN ADULT AND FETAL MICE: QUANTITATIVE,
HISTOLOGIC, AND PHYSIOLOGIC STUDIES OF THE
MAJOR VIRAL GLYCOPROTEIN, gp70*

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In the past because of uncertainties concerning the molecular basis for persistence of
the RNA tumor virus genome, it was difficult to speculate as to how its expression might
be regulated (1–3). With the realization, however, that multiple copies of the viral
genome are integrated into the host cell chromosome two considerations become impor-
tant. First, because of the integrated state of the genome, it seems likely that its
expression is controlled by mechanisms similar to those implicated in control of other
regions of the chromosome (4–9). Second, it became possible to consider selective expres-
sion of viral gene segments which are integrated into chromosomal loci where induction
is linked to the differentiated state of the cell. Induction of the thymus leukemia antigen
by thymotrophic hormones may already be an example of linkage between induction of a
virus-related phenotype and the process of differentiation (10).

Recently, concepts which predicted a relationship between differentiation and viral
gene expression (11, 12) were strengthened by studies from our laboratory and others in
which it was demonstrated that the major oncornavirus glycoprotein, gp70, is a constitu-
tent of the surface of some normal thymocytes and shares immunologic and biochemical
properties with the thymocyte differentiation marker Gxx (references 13–16, and footnote
1). Since gp70 is coded for by the viral genome (17), the genetics of the Gxx system at once
became applicable to the major oncornavirus glycoprotein (18, 19). Thus, the Mendelian
inheritance of the Gxx was now applicable to a plasma membrane-associated viral protein,
the expression of which in some strains of mice accompanied only certain pathways of
differentiation.

Since the most important feature of the gp70(Gxx) system is the suggestion
that in at least some strains of mice expression of an endogenous viral genome is
linked to differentiation, we wanted to determine which cells of adult and

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developing mice express this gene. We now report our observations on expression of gp70 in the mouse. By using quantitative radioimmunoassays, immunofluorescence, and biochemical studies, we were able to define the major sites of expression of gp70 in adult and fetal mice. In all the mouse strains studied, in spite of considerable quantitative variation, the expression of this protein was largely restricted to lymphoid and epithelial cells.

Materials and Methods

**Mice.** NZB, NZW, and (NZB × NZW)F₁ mice were from our colony. AKR, BALB/c, Nu/Nu, NIH Swiss, C57Br, and C57BL/6J mice were purchased from L. C. Strong, San Diego, Calif. The 129 G-defense and the congenic 129 G-x (19) mice were kindly sent to us by Doctors Elisabeth Stockert and Edward Boyse of the Memorial Sloan-Kettering Cancer Center, New York.

**Timed Matings.** Female mice were placed with males for 1 h at 9:00 a.m. The day of mating was considered day 0. At days 10, 14, and 18, pregnant mice were sacrificed and the embryos removed for study.

**Radioimmunoassays for gp70 and p30.** The radioimmunoassay for gp70 was similar to that previously described (20). Rauscher gp70 was prepared according to the method of Strand and August (20). The radioimmunoassay for p30 was done using a goat anti-murine leukemia virus (MuLV) p30 and ¹²⁵I-MuLV (Scripps) p30 as an antigen.

**Fluorescent Microscopy.** Tissues were frozen in liquid nitrogen, sectioned, and fixed in ethanol:ether (1:1) for 10 min and in 95% ethanol for 20 min. The fixed sections were overlaid with appropriate dilutions of goat anti-Rauscher gp70 (20) or normal goat serum (as a control). After washing, the sections were stained with fluorescein isothiocyanate rabbit antigoat IgG which had been absorbed with mouse serum and spleen cells. Sections were viewed with a Zeiss RA fluorescence microscope (Carl Zeiss, Inc., New York) equipped with a Osram HBO 200 W mercury light source, a KP 500 exciter filter, and a 500 nM barrier filter.

**Radioiodination of Cell Surfaces.** Preparation of cells and radioiodination of the cell surface has been described in detail previously (13, 16, 21).

**Preparation of Purified Virus.** Purified MuLV (Scripps) was prepared from continuously growing suspension thymocytes which were grown as previously described (13).

**Radioiodination of Virus Proteins.** Approximately 100 μg of purified MuLV (Scripps) was disrupted with 0.2% nonidet P-40 and labeled with ¹²⁵I using 10 μg of chloramine T followed by 10 μg of sodium metabisulfite (22). After labeling, the virus was sonicated and used for immune precipitation.

**Immune Precipitation.** Indirect immune precipitations were carried out as described by Kennel and Lerner (23) using either goat anti-Rauscher gp70 (20) or rat antibody against MuLV (Mo-leone). The origins and properties of the latter antisera have been described (13, 16). Identical results were obtained with either antisera.

**Polyacrylamide Disc Gel Electrophoresis.** Immune precipitates were solubilized in 8 M urea, 1% SDS, and 2% β-mercaptoethanol, then ¹²⁵I-human IgG and IgM proteins were added as molecular size markers. This mixture was heated to 96°C for 30 min, then to 100°C for 3 min before electrophoresis. Samples were analyzed by electrophoresis as described by Weber and Osborn (24). Gels were frozen on Dry Ice, then cut into 1-mm slices with a gel slicer (Joyce, Loeb & Co., Inc., Burlington, Mass.), and counted in an automatic gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Data were corrected for background, ¹²⁵I crossover, and counting efficiency, and were plotted by a Hewlett-Packard system (Hewlett-Packard Co., Palo Alto, Calif.) (23).

**Metabolic Fate of gp70.** The persistence of passively transferred gp70 was measured in order to determine its rate of elimination. Eight C57BL/6J, one 129 G-x, and one 129 G-x adult male received 1 ml NZB serum containing 57.7 μg of gp70 intravenously (i.v.). Six additional C57BL/6J mice received 2 ml NZB serum containing 115 μg gp70 intraperitoneally. The i.v. mice were bled at 10 min, 1 h, 3 h, 8 h, and 24 h and the i.p. mice were bled at 3, 8, and 24 h, and the concentration of gp70 in each serum was determined.

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2 Abbreviations used in this paper: RIA, radioimmunoassay; MuLV, murine leukemia virus.
Next, the differences in rate of degradation of gp70 in vivo in NZW (high gp70) and C57BL/st (low gp70) mice were studied. One mouse of each strain received 150 ng Friend gp70 carrying a label of 5 μCi 125I. The 125I of this preparation was 85% precipitable in 10% TCA. 5 h later the mice were exsanguinated and their complete viscera made into a 10% homogenate. The amounts of TCA-precipitable 125I in serum and viscera were determined and considered to reflect the amount of gp70 still undegraded.

The third experiment undertook to determine whether gp70 might be removed from the circulation and concentrated in any of the tissues. A single 129 Gx⁻ male mouse received via combined i.v. and i.p. routes 6 ml of NZB serum containing 345 μg gp70 over a 4-h period. 6 h after the last injection he was exsanguinated and blood and tissue samples were taken for quantitative gp70 determinations and for immunofluorescence examination. In addition, one 129 Gx⁺ and one 129 Gx⁻ mouse received via i.v. and i.p. routes 10 ml of NZB serum containing 570 μg gp70 over a 4-day period. 18 h after the last injection, they were exsanguinated and studied for gp70 as above.

Results

**Distribution of gp70 in Tissues of Adult and Fetal Mice.** gp70 was demonstrated by immunofluorescence in epithelial, lymphoid, and hematopoietic cells in different intensities in various organs of 10 mouse strains studied. The NZW, NZB, and (NZB × NZW)F₁ had large amounts of GP70; the AKR and the 129 Gx⁺ intermediate amounts; and the C57BL/st, NIH Swiss, and nude mice small amounts. The 129 Gx⁻ mice had virtually no detectable gp70.

**Hematopoietic and Lymphoid Tissue.** Sections from the thymus, spleen, and bone marrow of the NZ mice contained faint staining for gp70, which accentuated the surface of most cells; in the spleen, the staining was most prominent in the periarterial tissue. In addition, clusters of rectangular cells (as many as 20% of the cells) which had bright cytoplasmic staining were present in the thymic medulla. Scattered cells with positive cytoplasm were also seen in the spleen and the bone marrow. Staining was also seen adjacent to cells in the central areas of the germinal vesicles of the spleen. Staining of decreasing intensity was present in the 129 Gx⁻, C57BL/st, and the NIH Swiss thymus and spleen and the nude spleen. The 129 Gx⁻ had no detectable gp70 in the thymus and only equivocal staining in the spleen.

**Reproductive Tract.** Intense staining for gp70 in the epithelium of the epididymis and vas deferens was found in the NZ mice (Figs. 1 A, 1 B, and 2 A). In the ductus epididymis the staining was largely confined to the stereocilia border of the columnar epithelium, with small amounts of positively staining material present in the luminal contents (Fig. 1 A). In the portions of the epididymis nearer the testis, staining was observed within the cytoplasm of the epididymal epithelium (Fig. 1 B). The stereocilia of the AKR mice also stained brightly; in areas cells were occasionally found with intense cytoplasmic staining correlating to cells with stereocilia deposits (Fig. 1 C). In the 129 Gx⁺, BALB/c, C57BL/st, NIH Swiss, and nude mice the stereocilia deposits were less intense, respectively, and involved only segments of the stereocilia (Figs. 1 D and 1 E). These latter strains had less intense staining of the vas deferens epithelium as well. The 129 Gx⁻ were essentially free of detectable deposits in both the epididymis and vas deferens (Fig. 1 F). The seminal vesicles and prostate of the NZW had staining of the luminal aspect of the lining epithelium, in the luminal contents and in the glandular epithelium, respectively (Figs. 2 A and 2 B). Small amounts of gp70 were present in the seminal vesicles of the
Fig. 1. (A) Sections of the ductus epididymis from a NZW mouse showing accumulations of gp70 along the ciliated luminal (stereocilia) border of the epididymal epithelium (joined arrows). gp70 was also present among the maturing sperm within the lumen of the epididymis (arrow). (B) Sections from an area of the epididymis near the testis from the mouse shown in Fig. 1 A showing gp70 concentrated in the luminal aspect of the epithelial cells (joined arrows). In this area of the epididymis staining was also present in the cytoplasm of the epididymal epithelium. (C) The epididymis of an AKR mouse showing scattered epithelial cells with faint gp70 staining (arrows) and striking accumulations of gp70 at the luminal aspect. (D) The epididymis from a 129 G
+ mouse showing gp70 in the area of the stereocilia; (joined arrows) but, in contrast to the NZ mice, the accumulation was of less magnitude and was not continuous around the circumference of the lumen. (E) The C57BL/6J epididymis showing small amounts of gp70 in the area of the stereocilia (arrows). The distribution was strikingly segmental. (F) The epididymis of the 129 G
− mouse did not contain detectable gp70. The stereocilia region of the epithelial cell appeared as an empty area (joined arrow). Indirect immunofluorescence using goat anti-Rauscher gp70 antisera and fluorescein isothiocyanate-conjugated rabbit antigoat IgG. Original magnification 160.
FIG. 2. (A) The vas deferens of NZB mouse showing diffuse cytoplasmic staining in the lining epithelium with heavy accumulations of gp70 at the luminal edge (joined arrows). (B) The prostate of a NZW mouse showing diffuse cytoplasmic staining (arrows). (C) The uterus of a gravid (10 days) NZB mouse showing diffuse staining in the uterine wall with scattered tubular structures having intense gp70 deposits (arrows). (D) The mammary gland of a gravid (10 days) NZB mouse showing islands of cells with cytoplasmic gp70 staining (joined arrows). The very bright spots (cross-hatched arrows) are autofluorescent granular cells. Indirect immunofluorescence using goat anti-Rauscher gp70 antisera and fluorescein isothiocyanate-conjugated rabbit antigoat IgG. Original magnification 160.

C57BL/st as well. No specific staining was identified in the testis of any of the mouse strains.

The uterine wall of nonpregnant NZB, NZW, and C57BL/st mice had slight staining for gp70. Staining seemed to increase in intensity when the uterus was gravid. Glandular structures in the gravid uterine wall of these three strains of mice stained intensely (Fig. 2 C). The ovaries of the NZW and NZB mice
occasionally had cells with cytoplasmic staining. The epithelium of the fallopian tubes of the NZB, NZW, and C57BL/st contained diffuse cytoplasmic staining. The mammary epithelium of the gravid NZB and NZW mice on days 7–18 of gestation stained intensely (Fig. 2 D).

**Digestive Tract.** Cells in the lamina propria of the villi and the epithelium of the crypts of Lieberkühn in the jejunum and ileum stained brightly for gp70 in the NZ mice (Fig. 3 A). Decreasing staining was present in the 129 Gx + (Fig. 3 B), C57BL/st, and NIH Swiss mice. No staining was seen in the 129 Gx− or nude mouse intestine. Lymphoid tissue in the intestinal wall stained brightly in the NZ and 129 Gx + mice. In the colon, cytoplasmic staining was observed in cells in the lamina propria and the epithelium of the glands of Lieberkühn, extending to the cells exposed to the lumen in the NZ and 129 Gx + mice. Very faint staining was present in the hepatic sinusoids and bright staining was found in the bile duct epithelium of the NZB and NZW mice. Less intense staining was noted in the bile duct epithelium of the NIH Swiss, C57BL/st, and 129 Gx + mice. No bile duct staining was observed in the nude or 129 Gx− mice. Intense staining for gp70 was observed in the gall bladder (Fig. 3 C) and the pancreatic and salivary gland epithelium of the NZ mice, and to a much lesser extent, in the C57BL/st and the 129 Gx + mice. No staining was present in these organs of the 129 Gx− mice.

**Other Organs.** Staining for gp70 was striking in the cytoplasm accentuating the luminal aspect of the epithelium of small diameter renal tubules in the NZ mice (Fig. 3 D). Similar but less intense staining was seen in the kidneys of NIH Swiss, C57BL/st and 129 Gx + mice, while the nude and 129 Gx− mice were negative. Skin and skeletal muscle of the NZ mice were negative for gp70.

**Fetal Tissues.** Expression of other virus markers in maternal and fetal tissues during murine and chick development has been documented (25–28) and we present here a similar analysis for gp70 in the mouse. The relatively large amount of this protein makes possible both histologic localization and quantitation. Serial sections of NZB, NZW, and C57BL/st embryos of known gestation were examined. On day 10, no staining for gp70 was observed in the fetuses of any of three strains. On day 14, comparable staining was observed in the fetal livers of the NZB, NZW, and C57BL/st mice in a pattern similar to that described for adult bone marrow (Fig. 4 A). By the 18th day of gestation, the distribution of gp70 was similar to that described in the adult mice, with a few exceptions. The liver retained areas in which staining resembled that of adult bone marrow (Fig. 4 B). Deposits were observed in the developing alveolar air sacs of the NZB and NZW mice (Fig. 4 B). This staining had not been noted in the adult NZW lung studied. Staining was present along the luminal aspect of the pancreatic acini (Fig. 4 C), in the cytoplasm of the cells of the crypts of Lieberkühn (Fig. 4 C), and in the lamina propria of the small intestine. Intense staining was also detected in clusters of cells in the medullary areas of the thymus (Fig. 4 D). Scattered cells with faint cytoplasmic staining were also seen in the 18-day fetal skin of NZB and NZW mice. The 18-day C57BL/st embryo contained staining in a distribution much like that described in the NZB and NZW embryos, only of less intensity. Tiny flecks of fluorescent material were present in the vascular intricacies of the placenta on days 10, 14, and 18 of gestation in the NZB and NZW mice.
Fig. 3. (A) The jejunum of a NZW mouse showing cytoplasmic gp70 staining in the crypts of Lieberkuhn (cross-hatched arrows). The lamina propria of the villi contained many cells with diffuse cytoplasmic staining (joined arrows). (B) The jejunum of a 129 G\textsubscript{ix}\textsuperscript{+} mouse contained very faint cytoplasmic staining in the crypt area with only a few scattered cells positive for gp70 in the lamina propria (joined arrows). (C) The gall bladder epithelium of a NZW mouse stained brightly for gp70 (joined arrows). (D) The kidney of a NZW mouse had bright cytoplasmic gp70 staining in scattered small diameter tubules (arrows). In some areas, the staining was most intense on the luminal aspect of the tubular epithelium. Indirect immunofluorescence using goat anti-Rauscher gp70 antisera and fluorescein isothiocyanate-conjugated rabbit antigoat IgG. Original magnification, (A) and (B) 160; and (C) and (D) 250.

Specificity of the Immunofluorescent Staining. In addition to the usual controls for immunofluorescence, the availability of 129 G\textsubscript{ix}\textsuperscript{+} and 129 G\textsubscript{ix}\textsuperscript{-} congenic mice provided both positive and negative controls for the specificity of our staining. We were also able to block the fluorescent staining of all the
Fig. 4. (A) The liver of a 14-day-old C57BL/6J fetus had diffuse cytoplasmic gp70 staining (arrows). (B) The lung, diaphragm, and liver of an 18-day-old NZB fetus are shown. The alveolar lumens stained for gp70 (hatched arrows) as did the cytoplasm of the hepatic cells (arrows). The diaphragm was free of staining. (C) The intestine and pancreas of an 18-day-old NZW fetus are shown. Faint cytoplasmic staining for gp70 is seen in the developing crypts of Lieberkuhn (single hatched arrows). The muscular wall of the intestine (opposed arrows) is free of staining. The luminal aspect of the pancreatic acini is brightly stained (double hatched arrows). (D) The thymus of an 18-day-old NZW mouse contained clusters of brightly staining cells in the medullary areas (arrows). Indirect immunofluorescence using goat anti-Rauscher gp70 antisera and fluorescein isothiocyanate-conjugated rabbit antigoat IgG. Original magnification 160.
organs of the NZW mouse by absorbing the goat anti-gp70 with purified MuLV (Scripps) before reacting it with tissue.

**Molecular Properties of gp70 in Tissues of Adult and Fetal Mice.** A study of the molecular properties of gp70 isolated from different tissues will be presented elsewhere. Some data are presented here to illustrate that fluorescent staining of a tissue was accompanied by the presence at that site of an antigen with molecular properties of gp70. When the surfaces of bone marrow cells, thymocytes, or sperm from fluorescence-positive mice were labeled with $^{125}$I and the proteins of the plasma membrane solubilized, gp70 could be shown to be present.

A comparative acrylamide gel analysis of the viral, thymocyte, bone marrow, and sperm proteins is shown in Fig. 5. From these studies, it is clear that in each case a molecule with the immunologic and biochemical properties of gp70 is present on the surface of thymocytes, bone marrow, and sperm cells of gp70-positive animals. During development (see above) a prominent site of expression of gp70 is the fetal liver. A molecule with a mol wt of 70,000 daltons could be shown to be present on the surfaces of embryonic liver cells (data not shown).

**Concentration of gp70 in Tissues of Adult and Fetal Mice.** Quantitation of gp70 in sera and spleens of groups of 3-mo-old mice of several strains (Table I) and in sera and several tissues of individual mice of various strains was done by radioimmunoassay (RIA) (Table II). The data in Table I shows the great differences in gp70 concentrations among murine strains. The two NZ strains and hybrid are significantly higher than other mice. The 129 G$_{IX}^+$ and AKR mice appear to occupy an intermediate position and the C57BL/6, C57Br, and 129 G$_{IX}^-$ strains have the lowest concentrations. For the most part, these quantitative data fit with the immunofluorescence observations described above and are consistent with an earlier report (29). There is, however, more gp70 found in the tissues of 129 G$_{IX}^-$ mice by RIA than might have been expected on the basis of low serum gp70 levels and the failure to find gp70 in most tissues by immunofluorescence.

In Table II, the gp70 concentrations in a variety of tissues of single mice of NZB, C57BL/st, 129 G$_{IX}^+$, and 129 G$_{IX}^-$ strains are presented. There were no consistent patterns of relative serum and tissue concentrations from strain to strain. The high values for epididymis in the NZB and 129 G$_{IX}^+$ mouse fit with the fluorescence observations of high concentrations of gp70 in the lining epithelia.

The study of tissues during pregnancy revealed a sharp differential in gp70 concentration between uterus and placenta (Table III). At both 14 and 18 days of gestation the uteri of all strains had a two- to fourfold higher gp70 concentration than did the placenta. The concentrations of gp70 in the whole fetus did not change consistently with progression of gestation. As suggested by fluorescence, the fetal liver had a significantly higher concentration of gp70 than the entire fetus, particularly at 14 days.

The p30 concentrations of the fetus did not change consistently throughout gestation but in the NZB and NZW mice appear to be higher at 10 days and 18 days than at 14 days (Table III). Fetal and placental levels of p30 are much lower than maternal levels in uterus and spleen at all stages of gestation. As in

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3 Del Villano, B. C., and R. A. Lerner. Manuscript in preparation.
maternal tissues, the levels of p30 in fetal tissues and placentae are much lower than gp70 levels.

Metabolic Fate of gp70 In Vivo. Transfer i.v. of NZB serum containing 57.7 µg of gp70 to C57BL/st recipients elevated preinjection gp70 levels of 1.5 µg/ml to 24 µg/ml 10 min postinjection. The serum gp70 levels fell at a constant rate for the first 8 h to 8.7 µg/ml and then 3.5 µg/ml over the next 16 h. Transfer i.p. of 115 µg gp70 resulted in serum gp levels of 28 µg/ml 3 h later followed by a fall to 15.7 µg at 8 h and 4.75 µg/ml at 24 h. The rate of loss of i.p.-injected gp70 from the serum of recipients was relatively constant with a half disappearance time of 5.2 h. There was no loss of immunologically detectable gp70 into the urine. Similar observations on the 129 Gix+ mouse and 129 Gix- mouse revealed a disappearance time from the circulation of 5.4 and 5.0 h, respectively.

No differences in degradation of gp70 were found when NZW (high gp70) and C57BL/st (low gp70) mice were injected with 125I-gp70. At 5 h, the amount of TCA-precipitable gp70 in the combined blood and viscera was: 23.3% for C57BL/st mice, and 22.9% for NZW mice. Thus, the rate of in vivo degradation of
## Table I

### Concentration of gp70 in Sera and Spleens of 3-Mo-Old Mice

| Strain          | No. of mice | Serum gp70 | No. of mice | Spleen gp70 |
|-----------------|-------------|------------|-------------|-------------|
| NZB             | 19          | 57         | 10          | 108         |
| NZW             | 26          | 57         | 10          | 63          |
| (NZB × NZW)F₁   | 17          | 35         | 12          | 97          |
| 129 Gₓ⁺⁺*       | 23          | 13         | 3           | 14          |
| AKR             | 19          | 10         | 9           | 32          |
| C₅₇Br           | 18          | 2.4        | 4           | 7           |
| C₅₇BL/st        | 26          | 1.9        | 5           | 7           |
| 129 Gₓ⁻⁻*       | 6           | 1.0        | 3           | 10          |

*Age undetermined.

## Table II

### Concentration of gp70 in Tissues of Adult Mice

| Mouse strain | Serum | Spleen | Liver | Thymus | Epididymis | Testis | Intestine |
|--------------|-------|--------|-------|--------|------------|--------|-----------|
| NZB          | 50    | 127    | 35    | 70     | 340        | 24     | 39        |
| 129 Gₓ⁺⁺*    | 13    | 22     | 30    | 12     | 36         | 13     | 18        |
| C₅₇BL/st     | 3     | 12     | 10    | 21     | 5          | 1.4    | 26        |
| 129 Gₓ⁻⁻*    | 1.4   | 10     | 7     | 7      | 5          | 12     | 10        |

## Table III

### Expression of p30 and gp70 in Maternal and Fetal Tissues

| Day of gestation | Mouse strain | Maternal serum | Maternal spleen | Uterus | Placenta | Fetus | Fetal liver |
|------------------|--------------|----------------|-----------------|--------|----------|-------|-------------|
|                  | p30          | gp70           | p30             | gp70   | p30      | gp70  | p30         |
|                  | µg/ml        | µg/g           | µg/g            | µg/g   | µg/g     | µg/g  | µg/g        |
| 10               | NZB          | 0.07           | 50              | 29     | 204      | 39    | 228*        |
|                  | NZW          | 0.04           | 60              | 32     | 108      | 25    | 73          |
|                  | C₅₇BL/st     | 0.03           | 2               | 8      | 42       | 4     | 30          |
| 14               | NZB          | 0.05           | 50              | 43     | 251      | 31    | 93          |
|                  | NZW          | 0.06           | 40              | 22     | 185      | 15    | 34          |
|                  | C₅₇BL/st     | 0.03           | 3               | 10     | 22       | 2     | 20          |
| 18               | NZB          | 0.06           | 32              | 25     | 270      | 34    | 148         |
|                  | NZW          | 0.09           | 41              | 12     | 61       | 12    | 25          |
|                  | C₅₇BL/st     | 0.03           | 2               | 7      | 24       | 2     | 17          |

* Uterus and placenta.
$^{125}$I-Friend gp70 as measured by this means bore no relationship to normal gp70 levels in the mice tested.

The quantitative and immunofluorescent observations on the 129 G$^{+}$ mouse infused with 345 $\mu$g gp70 indicated that circulating gp70 was not sequestered or concentrated at any anatomic site. As expected, the infusion increased the normally low serum gp70 to a concentration of 39 $\mu$g/ml of serum at time of sacrifice 6 h after the last injection. The tissue concentration of gp70 measured by radioimmunoassay in micrograms per gram was: vas-epididymis, 8.0; jejunum, 8.0; testis, 9.0; liver, 6.0; kidney, 10.0; spleen, 11.0; and thymus, 6.0; values similar to those found in untreated 129 G$^{+}$ mice (Table II). In spite of relatively high levels of circulating gp70, for 10 h there was no significant deposition or concentration of gp70 detectable by immunofluorescence in any tissue.

The 129 G$^{+}$ and 129 G$^{-}$ mice injected with 570 $\mu$g gp70 over 4 days showed, by immunofluorescence, at sacrifice (18 h after the last injection) a tissue distribution identical to that seen in untreated controls. Similarly, the amounts of gp70 in the tissues as measured by radioimmune assay were not elevated significantly in the G$^{+}$ mouse and only moderately elevated in the G$^{-}$ mouse.

Discussion

The major oncornavirus glycoprotein, gp70, appears to be present in relatively few cells of widely differing structure and function in a number of adult and fetal murine tissues. In view of the relationship of gp70 to thymocyte maturation, it is not surprising to find it in lymphoid tissue and bone marrow but its high concentration in a minority of medullary cells in the thymus might not have been expected. In nonlymphoid tissues, gp70 was concentrated in a variety of secretory and glandular epithelia of the gastrointestinal, genital, and urinary tracts. No function of gp70 at any of these sites of concentration is as yet recognized.

It is of considerable importance to realize that even in mice with high levels, production of gp70 does not predict the onset of neoplasia since it is expressed in a number of differentiated cells which rarely if ever become malignant. One reason for the fact that viral gene expression and oncogenesis are not coordinated may be that viral gene products have different oncogenic potential depending on the differentiated state of the cell. For example, insertion of a virion glycoprotein (i.e., gp70) into the plasma membrane of a thymocyte which must respond to environmental stimuli by controlled proliferation might be potentially more oncogenic than insertion of the same gene product into the membrane of a cell which under ordinary circumstances will divide very little. Alternatively, the differentiated state of the cell may determine if a virion glycoprotein gets incorporated into the plasma membrane at all.

One of the most important aspects of the gp70(G$^{+}$) system is the suggestion that expression of specific parts of the viral genome may be linked to differentiation. However, the present data show that control of expression of the gp70(G$^{+}$) marker is much more relaxed than previously suspected and expression is not confined to cells following the T-lymphocyte pathway of differentiation. Boyse and Bennett have already pointed out that the presence of G$^{+}$ on sperm was an
exception to the notion that $G_{\text{Ix}}$ was confined to T lymphocytes (30) and Fernandes et al. demonstrated the presence of viral particles in the reproductive organs of male NZB mice (31). Interestingly, Bentvelzen and his colleagues showed that the mouse epididymis was a major site of expression of mammary tumor virus antigens (32). With respect to control of expression of gp70, it is not unreasonable to assume that different regulatory or structural genes may be involved for different cells depending on their state of differentiation.

It is important to specify the exact relationship of the $G_{\text{Ix}}$ marker to the gp70 molecule. The evidence suggests that $G_{\text{Ix}}$ is a type-specific determinant on the gp70 molecule (33). Thus, depending on the types of endogenous viral genome(s) expressed, we could expect to detect $G_{\text{Ix}}^+(\text{gp70})$, or $G_{\text{Ix}}^-(\text{gp70})$, or both. In short, if a mouse is $G_{\text{Ix}}^+$, he must be gp70+ but if he is gp70+, he need not be $G_{\text{Ix}}^+$. This relationship is seen most clearly in the congenic C57BL/6($G_{\text{Ix}}^+$) and C57BL/6($G_{\text{Ix}}^-$) mice in which both $G_{\text{Ix}}^+$ and $G_{\text{Ix}}^-(\text{gp70})$ molecules are found (33). Of particular pertinence to the present discussion is the fact that in these mice, the $G_{\text{Ix}}^+(\text{gp70})$ and the $G_{\text{Ix}}^-(\text{gp70})$ molecules are coded for (or regulated) by unlinked genes. Thus, any measure of group (fluorescent microscopy) or interspecies determinants (as in our RIA procedures) in gp70 could score as equivalent, molecules potentially coded for by different genes. These facts become important when considering the quantitative studies for congenic pairs of mice such as 129 $G_{\text{Ix}}^+$ and 129 $G_{\text{Ix}}^-$. Here the apparent levels of gp70 in the sera (as determined by RIA of interspecies determinants) and the levels of gp70 in the genital tract (as determined by fluorescent microscopy) parallel the segregation of the $G_{\text{Ix}}$ phenotype. But, using the interspecies RIA, the amount of gp70 in the spleen, liver, thymus, and testis does not clearly reflect the $G_{\text{Ix}}$ phenotype. The simplest explanation for these data is that, as for the C57BL/6 mice, we are detecting by RIA two or more kinds of gp70 molecules coded for by different genes, only one of which carries the $G_{\text{Ix}}$-type specificity which we selectively detect by fluorescent microscopy. One reason for this may be that detection of a protein by fluorescent microscopy depends on point, not absolute concentration, a feature not distinguished by RIA. Thus, in the case of the 129 $G_{\text{Ix}}^+$ and $G_{\text{Ix}}^-$ congenic mice, we might be following genetic segregation of $G_{\text{Ix}}^+(\text{gp70})$ on a background of constitutive expression of $G_{\text{Ix}}^-(\text{gp70})$ in both strains. Studies are in progress to determine whether the genes which regulate gp70 expression at sites other than thymus show modes of inheritance similar to those operating in the thymus.

From the present observations, several conclusions concerning in vivo gp70 metabolism can be reached. First, in view of the comparable rate of degradation of $^{125}\text{I}$-gp70 in high and low gp70 strains of mice and the comparable rates of loss of transfused gp70 from the circulations of high and low gp70 strains of mice, the considerable differences among gp70 levels in various strains of mice are likely caused by different rates of synthesis. Second, there is apparently no significant uptake or sequestration of circulating gp70 by any tissue as evidenced by the failure to identify elevated gp70 levels in any tissue or even in small cellular foci of 129 $G_{\text{Ix}}^-$ mice in which very high serum gp70 levels were maintained for as long as 4 days. This suggests that the gp70 levels found in the tissues is at sites of synthesis. Third, the total amount of gp70 synthesis in the high gp70 strains is considerable if the half-life of circulating gp70 observed after passive transfer
is applicable. In some strains such as the NZ with 50 μg gp70/ml of serum, a half-life of 5.5 h for gp70 in the circulation would mean a synthesis of about 400 μg gp70/day for replacement of loss from serum and extravascular fluids alone. If in addition there were a degradation of gp70 in tissues or a direct excretory loss, the replacement of this amount would be added to that needed to sustain circulating levels.

We can compare the studies reported here for gp70 to the extensive studies of others in mice and chickens using p30(gs) as marker (25–28). As for gp70 the expression of p30 in chickens and mice is regulated by a dominant gene and its expression may or may not be coordinate with presence of infectious virus. In sharp distinction to gp70, expression of p30 correlates well with oncogenesis (29). In normal mice, there is approximately 100 times the amount of gp70 as p30 in serum. If all the gp70 and p30 were present as virus particles, the expected mass ratio of gp70 to p30 would be about 1:6. Thus, it is evident that gp70 is produced in great excess over the need to combine with p30 in virus particles. In fact, the amount of gp70 produced in 1 day by some strains of mice if used for virus production would be enough for about 10^{13} virus particles. Continued study of these two as well as other virus markers in parallel will be of importance so that we can segregate markers which are predictive of neoplasia in later life from those which are "normal" constituents of the mouse.

Summary

Endogenous expression of the murine leukemia virus (MuLV) genome has been studied in a number of strains of mice. Expression of the major envelope glycoprotein, gp70, is restricted to certain anatomical sites and cell types, prominent among which are lymphoid and epithelial cells. On a quantitative basis, the major site of gp70 expression is the male genital tract. During development, gp70 first appears in the hematopoietic liver of 14-day-old embryos and by day 18, it is already expressed at anatomical sites similar to those of the adult. In toto, these results show that control of expression of the MuLV genome in adult and developing mice is linked to differentiation.

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