IMMUNOPATHOGENICITY AND ONCOGENICITY OF MURINE LEUKEMIA VIRUSES

I. Induction of Immunologic Disease and Lymphoma in (BALB/c \( \times \) NZB)\( F_1 \) Mice by Scripps Leukemia Virus\*; \( \dagger \)

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These studies were undertaken to help define a possible viral role in the etiology of the lupus like disease of NZ mice. This disease, characterized by antinuclear antibodies (ANA)\(^1\) and immune complex type glomerulonephritis, is found in (NZB \( \times \) NZW)\( F_1 \) and NZB mice (1) and closely resembles systemic lupus erythematosus (SLE) of man. In addition, Coombs’ type antibodies and associated hemolytic anemia occur in the NZB mice. The ANA appear central in murine lupus since they arise early and are of primary importance in the production of glomerulonephritis (2). Of the various possible sources of immunogenic material which might initiate an ANA response, oncornaviruses which infect most mice and present an unusually wide spectrum of foreign nucleic acids to the host would seem to be ideal candidates. The immunogenicity of oncornaviruses is evidenced by the fact that (NZB \( \times \) NZW)\( F_1 \) mice spontaneously make antibody to every recognizable macromolecule synthesized during their replication (3, 4).

The presence of murine leukemia viruses (MuLV) in NZ mice is well established, and an immunologic hyperresponsiveness of NZB and (NZB \( \times \) NZW)\( F_1 \) mice to these agents has been described (5–7). Both NZB and (NZB \( \times \) NZW)\( F_1 \) mice exhibit seropositivity for Gross murine leukemia virus antigen (GSA) early in life, and this is followed by rising

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\(^1\) Abbreviations used in this paper: ANA, antinuclear antibodies; \( \beta \)PL, \( \beta \)-propiolactone; FITC, fluorescein isothiocyanate; GSA, Gross murine leukemia virus antigen; H \& E, hematoxylin and eosin; IFA, indirect fluorescent antibody technique; MuLV, murine leukemia viruses; SLE, systemic lupus erythematosus; SLV, Scripps leukemia virus.
titers of free GSA antibody which suggest immune elimination of GSA with associated circulating immune complexes preceding and during the development of glomerulonephritis (5, 6). In further support of a role for antigen-antibody complexes in this glomerulonephritis, fluorescent antibody studies of glomerulonephritic kidneys have demonstrated MuLV group antigen in NZB mice (5) and DNA-anti-DNA complexes in (NZB × NZW)F1 mice (7). In addition, elution of renal antibody has demonstrated concentration of GSA antibody in the kidneys of NZB mice (6) and antibody to nucleoprotein and to Gross virus antigens (7) in the kidneys of (NZB × NZW)F1 mice.

If in fact MuLV is the cause of murine lupus, it might be possible to mimic the spontaneous disease by infecting susceptible but otherwise normal strains of mice with such an agent. However, attempts to produce a similar disease in healthy mice by transfer of cell-free filtrates from NZ mice have yielded conflicting and inconclusive results. Two positive reports by Mellors and Huang describe limited success in producing glomerulonephritis and hemolytic anemia with transfers of cell-free filtrates of NZB lymphoma or aged normal spleens (8, 9). In contrast, Holmes et al. (10), East and Prosser (11), Howie and Heyer (1) and East et al. (12) were unable to produce any immunologic abnormalities with transfers of NZB cell-free material, although whole spleen cells would transfer Coombs' positivity and lymphoma in certain situations. In this report we show that purified virus obtained from a NZB thymoblastoid line (13) induced ANA, glomerulonephritis, and thymoblastic lymphoma when injected into newborn (BALB/c × NZB)F1 mice.

**Materials and Methods**

**Cells and Viruses.** All cells were grown in Eagle's MEM supplemented with sodium pyruvate, nonessential amino acids, 10% fetal calf serum, and antibiotics (Flow Laboratories, Inc., Rockville, Md.). NIH Swiss 3T3, BALB/c 3T3, and XC cells were obtained from Dr. V. Klement, Children's Hospital, Los Angeles, Calif. Secondary (BALB/c × NZB)F1 embryo cultures were prepared as needed in our laboratory. The isolation and properties of Scripps Clinic Research Foundation (SCRF) 60A NZB lymphoblasts have been previously described (13). The SCRF 60A cells were grown from a density of $2 \times 10^4$ to $2 \times 10^6$ to a maximum density of $2 \times 10^6$ cells/ml.

Small volumes of cells (20 ml) were grown as stationary cultures in Falcon flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Large volumes of cells (200 ml to 1 liter) were grown in Erlenmeyer flasks on gyrotory shakers as previously described (13).

Scripps leukemia virus (SLV 60A) was purified from supernatant fluids of SCRF 60A cells as previously described (13) and stored at −195°C in liquid nitrogen until use. AKR MuLV, which had been purified by sedimentation in sucrose gradients to its isopropic density (1.16 g/cm³), was prepared by Electo-Nucleonics, Inc., Bethesda, Md. (no. 221-79) and supplied to us by the National Cancer Institute, Bethesda, Md. For some experiments, virus was inactivated by addition of β-propiolactone (βPL) (Sigma Chemical Co., St. Louis, Mo.) to a concentration of 10 mM at 4°C for 2 h and overnight incubation at 37°C (SLV 60A βPL).

Infectious virus titers were determined by a modification (14) of the XC test (15) in Linbro dispo trays (Linbro Chemical Co., New Haven, Conn.) or in 60-mm petri dishes. Linbro trays were seeded with $2 \times 10^4$ substrate cells/well. The next day the substrate cells were treated with DEAE-cellulose dextran (Sigma Chemical Co.) and 0.2 ml of each virus dilution was inoculated into four wells. Fresh media was added after 2–3 days, and after an additional 2–3 days the substrate cells were UV irradiated and overlaid with $10^5$ XC cells. 2–3 days later the cells were fixed and the number of syncytia counted. The virus titer was calculated by the method of Reed and Muench (16) and expressed as tissue culture infective dose (TCID₅₀)/ml.
**Inoculated Animals.**

**Virus inoculation.** BALB/c and C3H mice were obtained from Strong Laboratories, San Diego, Calif. NZB mice were from the Scripps colony. (BALB/c × NZB)F1 hybrids were bred in the Scripps vivarium. AKR/J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Animals were provided water and lab chow.

(BALB/c x NZB)F1 mice were inoculated within 24 h of birth or at 6 wk of age with the designated dose of virus (prepared by dilution of a standard virus pool). Experimental animals were divided into the groups as indicated in Table I. All mice were weighed and bled from the retro-orbital plexus at 4-wk intervals.

**Serologic studies.** Since the emphasis of this study was on the immunologic consequences of SLV infection, we chose to follow the infection by measuring the serum Mu gs-1 levels. This protein, the major structural component of the virion, should best represent the antigenic load resulting from the infection and not be affected by variations in the production of infectious virus vs. noninfectious viral products.

| Group | Inoculum | Dose | Age of recipient | No. male/ no. female |
|-------|----------|------|------------------|---------------------|
| 10³   | SLV 60A virus | 10³ TCID₅₀ | Birth | 8/7 |
| 10²   | " " " " | " " | " | 8/12 |
| 10¹   | " " " " | 10¹ " " | " | 10/12 |
| 10⁰   | " " " " | 10⁰ " " | " | 14/8 |
| 10⁻¹  | " " " " | 10⁻¹ " " | " | 10/8 |
| 10⁻²  | " " " " | 10⁻² " " | " | 4/9 |
| A     | AKR virus | 10³ " " | " | 7/12 |
| I     | SLV 60A βPL* | 10³ TCID₅₀‡ | " | 1/7 |
| N     | None | — | — | 5/10 |
| DV    | SLV 60A virus | 10³ TCID₅₀ | 6 wk | 8/7 |

Virus doses are based on dilution of aliquots from a standard pool; titers are expressed as infectivity for Swiss 3T3 cells.

* SLV 60A virus which had been inactivated with βPL.
‡ Equivalent titer before inactivation.

Sera were analyzed for Mu gs-1 by a radioimmunoassay that is a modification of the procedures described by Oroszlan et al. (17) and Parks and Scolnick (18). ANA titers were determined by the indirect fluorescent antibody technique (IFA); sections of mouse kidney were used as the target tissue. ANA titers were determined by endpoint dilution, and the geometric mean titer was calculated. Mice were examined for anti-RBC antibodies by the direct Coombs' test with anti-Mu Ig which had been absorbed with RBCs before use. For the Coombs' test, RBCs from 8- to 12-mo old NZB mice were used as positive controls and RBCs from 2-mo old C3H/St mice were used as negative controls.

**Histologic studies.** Moribund mice were anesthetized with ether and exsanguinated. WBC and RBC counts were determined on a Coulter counter (Coulter Electronics, Inc., Fine Particle Group, Hialeah, Fla.) and blood smears were prepared for morphologic examination. Spleen weights were determined and histologic sections of heart, lung, liver, kidney, bowel, spleen, thymus, lymph node, and gonad were stained with hematoxylin and eosin (H & E) or periodic acid-Schiff (PAS) before microscopic examination.

Rabbit antibody against the 3rd component of murine complement (C₃) was prepared by using a zymosan C complex as antigen (19). Only those rabbit sera that produced one precipitin line (against whole mouse serum) when analyzed by immunoelectrophoresis and Ouchterlony gel diffusion were used. Antisera were partially purified and concentrated by precipitation with 50% saturated
ammonium sulfate (SAS), pH 7.4. Conjugation of globulin with fluorescein isothiocyanate (FITC) was performed by the dialysis method of Clark and Shepard (20); the FITC-labeled globulin was fractionated on DEAE-cellulose, and the material eluting at 0.05 M NaCl, 0.0175 M potassium phosphate, pH 7.4 was used. Goat antimurine Fab was provided by Dr. James C. Clagett (Department of Microbiology and Immunology, University of Washington School of Medicine, Seattle, Wash.).

Guinea pig and rabbit anti-Mu gs-1 were provided by Dr. Raymond Gilden of Flow Laboratories, Inc., Bethesda, Md. Before IFA tissue staining, anti-Mu gs-1 antisera, and FITC anti-lg sera were absorbed with whole murine sera which had been concentrated by precipitation with SAS and coupled to Sepharose 4B by cyanogen bromide (21). Cells and tissues for electron microscopy were fixed in phosphate-buffered 2% glutaraldehyde, post-fixed in osmic acid, dehydrated, embedded in Vestopal (Henley & Co., New York), sectioned, and stained with uranyl acetate and lead citrate.

Pools of AKR anti-C3H/St thymocyte and the reciprocal C3H/St anti-AKR/J thymocyte sera were prepared by weekly intraperitoneal injections of 10^7 thymus cells (22). Pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.), 0.1 ml, was used as adjuvant for the first three injections. Mice were bled every other week after the 6th wk, and sera from 15 to 20 animals were pooled. As determined by IFA staining, these unabsorbed antisera bound only to the surfaces of viable thymocytes from the strain used as immunogen.

Single cell suspensions of lymphoma tissues were stained for C3H thymocyte and AKR thymocyte antigens by the IFA. The number of stained cells was determined with a Zeiss fluorescence microscope (Carl Zeiss, Inc., New York) adapted for dark-field fluorescence and standard or dark-field phase. This microscope allowed the same field to be examined by fluorescence and phase-contrast microscopy, so that the fluorescent to total cell ratio could easily be determined.

**Results**

**Virus.** SLV 60A virus had a titer of 10^5 TCID_{50} on Swiss 3T3 cells and 5 x 10^4 on (BALB/c x NZB)F1 secondary embryo cells. AKR MuLV had a titer of 10^6 TCID_{50} on Swiss 3T3 cells and no demonstrable infectivity on the (BALB/c x NZB)F1 cells. SLV 60A βPL-inactivated gave no detectable infectivity for Swiss 3T3 cells. MuLV titers throughout the paper are based on infectivity of Swiss 3T3 cells.

**Inoculated Animals.**

**Virologic studies.** Assays (XC test) for infectious MuLV were performed on 5% spleen suspensions of four (NZB x BALB/c)F1 mice which had received 10^5 TCID_{50} SLV 60A at birth. The animals were sacrificed at 10 wk, at which time they were clinically well but had elevated serum Mu gs-1 levels (see next section, Serological studies). One of these four animals had a histologically demonstrable lymphoma. The virus titers ranged from 2 x 10^2 to 2 x 10^8 TCID_{50}/mg spleen.

The relationship between virus dose and mortality is shown in Fig. 1. The 50% points for mortality and lymphoma are shown in Fig. 2 in which the linear relationship between the dose of virus, the onset of lymphoma, and mortality are demonstrated.

**Serological studies.** Mean serum Mu gs-1 values and SD were calculated for each group by month and subdivided by sex. The values for uninoculated animals (group N) ranged from 29 ± 31 ng/ml to 115 ± 110 ng/ml. Similar results were obtained for groups I (inactivated virus), A (AKR virus), and DV (virus injection delayed to 6 wk).

Serum Mu gs-1 values for groups of animals which received a dose of virus between 10^-2 and 10^3 TCID_{50} were plotted as a function of time in Fig. 3. 10-
100-fold elevations were evident by 4 wk. There was a linear dependence of the Mu gs-1 level on the virus dose as shown in Fig. 4. Groups of animals which received between $10^{-1}$ and $10^2$ TCID$_{50}$ of SLV 60A virus had peak Mu gs-1 responses at 3-4 mo that declined towards control levels afterwards. The subsequent fall in average Mu gs-1 reflected in part the early loss of animals with the highest levels of Mu gs-1.

All animals in the group which received $10^{-2}$ TCID$_{50}$ of SLV 60A virus maintained serum Mu gs-1 levels in the control range. There was no significant difference in serum Mu gs-1 between males and females in any group.

The groups which received no virus, inactivated virus, AKR virus, or SLV 60A virus as adults, had peak ANA titers averaging 1. The titers for ANA are given by sex in Table II for groups which received $10^{-2}$ to $10^4$ TCID$_{50}$ of SLV 60A virus. ANA titers were highest in groups receiving the highest virus doses ($10^3$, $10^2$, and $10^1$). Females of groups $10^2$ and $10^1$ had the highest peak ANA titers. ANA responses of virtually all infected animals peaked at 8-16 wk and then declined. The fall in average ANA titer at 20 and 24 wk was not from deaths of animals with the highest titers, because 24-wk survivors also had peak responses at 12-16 wk.
Fig. 3. Serum Mu gs-1 values for groups of animals which received a dose of virus from $10^{-2}$ to $10^3$ TCID$_{50}$. Group N was not injected.

Fig. 4. Relationship between serum Mu gs-1 and virus dose. The line shown was obtained by linear regression analysis of the 1-mo (4 wk) points ($r^2 = 0.64$, $P < 0.05$). The 3-mo line ($r^2 = 0.89$, $P < 0.005$) and the 5-mo line ($r^2 = 0.75$, $P < 0.01$) are not shown.
Table II

ANA Geometric Mean Titers in Virus-Inoculated and Control Mice

| Virus dose TCID<sub>50</sub> | Sex | 4 wk | 8 wk | 12 wk | 16 wk | 20 wk | 24 wk |
|-----------------------------|-----|------|------|-------|-------|-------|-------|
| 10<sup>1</sup> | M | 0 | 15 | 12 | -- | -- | -- |
| F | 0 | 17 | 12 | -- | -- | -- | -- |
| 10<sup>2</sup> | M | 0 | 4 | 10 | 16 | 12 | 25 |
| F | 3 | 43 | 18 | 6 | -- | -- | -- |
| 10<sup>3</sup> | M | 0 | 9 | 12 | 17 | 6 | 6 |
| F | 1 | 7 | 43 | 4 | 3 | 0 | -- |
| 10<sup>-1</sup> | M | 1 | 4 | 9 | 2 | 3 | 1 |
| F | 0 | 13 | 15 | 4 | 7 | 1 | -- |
| 10<sup>-2</sup> | M | 0 | 3 | 0 | 1 | 0 | 0 |
| F | 0 | 4 | 2 | 1 | 1 | 2 | -- |
| None | M | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 0 | 0 | 0 | 0 | 0 | 0 | -- |

Coombs' tests were performed at 4, 8, 12, 20, and 24 wk. Of the 64 animals tested throughout the period of observation from groups which had received no virus, inactivated virus, or virus as adults, a single positive result was observed at 4 wk. Of 113 animals in groups which received 10<sup>-2</sup>-10<sup>3</sup> TCID<sub>50</sub> of SLV 60A virus, 7 were positive at 4 wk and 3 of 11 females in the group which received 10<sup>3</sup> TCID<sub>50</sub> were positive at 12 wk; no other animals in these groups were Coombs' positive.

HISTOLOGIC STUDIES. At autopsy, tumor-bearing animals generally had grossly enlarged lymph nodes and thymuses. Spleen weights were 0.18–7.50 g in tumor-bearing animals and 0.05–0.29 g in control groups. In spite of palpable nodes and spleens and moribund appearance, less than 25% of the animals had more than 10 × 10<sup>3</sup> WBC/mm<sup>3</sup> (infected, 4–163 × 10<sup>3</sup> WBC/mm<sup>3</sup>; control, 2–10 × 10<sup>3</sup> WBC/mm<sup>3</sup>). Histologic examination of the tumors and involved organs showed primarily sheets of uniformly large round lymphoblasts with large nuclei and nucleoli. 5% of the tumors also had areas of reticulum cell sarcoma, with mixed infiltrates of plasma cells, eosinophiles, lymphocytes, and anaplastic reticulum cells. Tumor cells generally obliterated lymph node and thymic architecture and infiltrated the parenchyma of lung, liver, kidney, and ovaries. Testes were consistently free of lymphoma. In Fig. 5 a thymus with one involved and one uninvolved lobe is shown. Essentially, all animals had lymphomas at sacrifice (Fig. 2). Electron microscopy of the tumors confirmed the light microscopic appearance of the cells and demonstrated C-type virus in varying degrees of formation, budding from and present near tumor cells (Fig. 6).

10 separate cell suspensions from abdominal tumors were examined for C3H
and AKR thymic antigens by IFA staining. 98–100% of the cells in these suspensions stained positively for C3H thymic antigens, but there was no staining for AKR antigen. Tumor cells were approximately twice as large as normal thymus cells and had irregular rim staining (Fig. 7).

Light microscope study of renal lesions indicated a widespread eosinophilic, PAS-positive thickening of the capillary basement lamina (Fig. 8) in comparison to controls (Fig. 9). The PAS-positive material also extended into the mesangial areas and occasionally formed a confluent mass. In no case was there significant proliferation of endothelial or epithelial cells. Subendothelial electron dense deposits associated with fusion of the epithelial foot processes were seen when the kidneys were studied by electron microscopy (Fig. 10). Control glomeruli had smooth basement lamina and delicate foot processes (Fig. 11). IgG (Fig. 12) and C3 (Fig. 13) were seen within the glomerular capillary walls and mesangia. Mugs-1 was localized within glomeruli in a pattern similar to IgG and C3 and also was seen in the cytoplasm of leukemic infiltrates (Fig. 14). Only minimal mesangial localization of IgG was seen in glomeruli of control animals.
The incidence of glomerulonephritis in groups which received $10^{-2} - 10^{3}$ TCID$_{50}$ of virus is shown in Fig. 15. Since glomerulonephritis was diagnosed only by histologic examination of autopsied animals, its presence in living animals was not recognized; therefore, its recorded incidence is a minimum figure. In Fig. 16 the glomerulonephritis incidence in all groups is expressed as a function of the animal’s age at sacrifice; the younger the animal at sacrifice, the greater was the likelihood of glomerulonephritis.

Comparison of Fig. 16 and Table II indicates a coincidence between the peaks in ANA response and the greatest glomerulonephritis incidence. However, when
individual animals were scored for glomerulonephritis and ANA and analyzed by the chi-square test for correlation between ANA titer and glomerulonephritis, no significant statistical correlation was found. A greater percentage of females developed glomerulonephritis than did males. This sex difference was first evident at 12 wk and persisted throughout the period of observation with female to male ratios of up to 3:1.

Discussion

Inoculation of SLV 60A virus into immunologically normal, neonatal (BALB/c x NZB)F1 mice was followed within a few months by a dose-related development of high levels of serum Mu gs-1, high titers of ANA, glomerulonephritis, and lymphoma. In most respects, the immunologic disease consisting of ANA and an apparent immune complex glomerulonephritis was similar to the spontaneous glomerulonephritis of NZ mice except that its onset was much earlier, i.e., 2–3 mo vs. 5–6 mo. As in NZ mice, the incidence and titers of ANA and incidence of glomerulonephritis were greater in females. The peak ANA titers were also
temporally related to the peak incidence of glomerulonephritis. Since normal mice or mice injected with inactivated SLV or live AKR viruses did not develop these immunologic abnormalities and, since the infected mice developed them early and in proportion to the dose of virus given, it seems likely that the virus was their cause.

The linear relationship between virus dilution and subsequent serum Mu gs-1 suggests that the number of infectious particles determined the magnitude of the continuing infection. The spread of infection must have been constrained, since the increase in Mu gs-1 remained proportional to the initial dose for several months and did not reach a comparable maximum for all groups. One possible explanation is that the population of cells susceptible to infection was limited to the neonatal period, thus preventing spread of progeny virus to other cells. This is in contrast to other chronic viral infections such as lactate dehydrogenase virus. Within 4 days the marker serum protein, lactate dehydrogenase, reached constant levels independent of virus dose over six 10-fold dilutions (23).

The nature of the relationships between SLV infection on the one hand and ANA production and development of glomerulonephritis on the other hand are
not entirely clear but several things appear likely. First, the ANA formation seems to be a response to a dose-related immunogenic stimulus resulting from the viral infection. Whether the immunogen(s) is supplied directly by the virus or is a host nuclear substance released as a by-product of infection cannot be said, nor has any possible adjuvant action of the oncornavirus on the ANA response been evaluated to date. Second, the immune complexes causing the glomerulonephritis probably include both nuclear antigens—ANA and viral antigens—antiviral antibodies. Analysis of the Ig deposited in the glomeruli in this disease reveals that about half of it reacts with nucleoprotein and that an additional 10–20% reacts with oncornavirus antigens (17). Qualitative tests of Ig eluates from these kidneys show the presence of antibodies to gp 69-70 (S. J. Kennel, personal communication, reverse transcriptase (Raymond Gilden, personal communication), Mu gs-1 (P. McConahey and F. J. Dixon, unpublished observations), and SLV 60A neutralizing antibodies (F. C. Jensen, unpublished observations). Judging from the amount of Mu gs-1 antigen continuously present in the circulation, which should be a reasonable index of the antigenic load produced by the virus, the total amount of viral antigen to which the more heavily infected mice were exposed over a several month period should have amounted to many milligrams.

Fig. 9. Control, group N, 3 mo, showing normal appearance of glomerulus (PAS, × 500).
Thus, the size of the SLV infection could influence the degree of immune complex glomerulonephritis, both by determining the amount of ANA production and the amount of viral antigen available to form complexes with antiviral antibodies.
Our failure to induce a significant amount of Coombs' positivity in any of the groups of infected mice suggests that either the ANA-glomerulonephritis syndrome has an etiology quite different from the Coombs' antibody-hemolytic anemia or that (BALB/c × NZB)F₁ mice are particularly resistant to the formation of anti-RBC antibodies. In support of the former, work to be reported
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Fig. 12. Group 10³-glomerulus stained for IgG and showing circumferential staining of the glomerular capillaries (x 600).

elsewhere indicates this apparent etiologic separation of renal and hemolytic diseases in other murine strains.

The development of lymphomas in the mice injected with SLV at birth was directly related to both the size of the initial inoculum and the amount of continued viral replication as measured by serum Mu gs-1 levels. Those mice with the highest Mu gs-1 levels were the first to develop lymphoma in the high dose groups in which all mice became lymphomatous. In the intermediate groups having only some mice that developed lymphomas, high Mu gs-1 levels again were associated with tumor. The tumors were composed almost entirely of thymic antigen-positive virus-producing cells, suggesting that thymocytes or their precursor cells were among the SLV susceptible cells present in the newborn mice. Limitation of oncogenic infection to the neonatal period also suggests that recruitment of tumor cells by infection with progeny virus later in life was unlikely. An attractive explanation of these results is that a proportion of susceptible thymus cells was infected neonatally and that these same cells underwent malignant transformation and produced the tumors in the adult
animals. This hypothesis also explains the effect of virus dose on the latent period; the fewer infected cells present originally, the longer was the time before oncogenesis and the production of an observable tumor mass. A host permissive to viral infection was essential for oncogenesis as indicated by the failure of control groups of (BALB/c × NZB)F₁ mice receiving live AKR virus, killed SLV at birth, or live SLV at 6 wk of age to develop elevated Mu gs-1 titers or tumors.

The coexistence of strong pathogenic immune responses and the development of lymphomas as a result of SLV infection might not have been expected in view of the instances in which MuLV infection has been reported to suppress immune responsiveness to certain exogenous antigens (for review, 24). However, only in animals with advanced infections or large tumors were ANA levels seen to fall. Thus, these SLV neonatal infections induced ANA and antiviral antibody responses in spite of any immunosuppression they might have caused. Perhaps some time after infection, the mice would have responded poorly to other new antigens, but their levels of ANA increased for at least 3–4 mo. In this situation, the immunogenicity and/or adjuvanticity of the infection apparently outweighed any immunosuppression for at least several months.
It appears that infection with a number of murine oncornaviruses can cause the formation of ANA and the development of glomerulonephritis. We have observed that MuLV Moloney has immunopathologic effects generally similar to those of SLV. Cannat and Varet (25) have reported ANA induction but not glomerulonephritis by Friend-Moloney-Rauscher (FMR) group MuLV in (BALB/c × C57BL/6)F₁ and (C3H × C57BL/6)F₁ mice but not in the three parental strains. Glomerulonephritis has been reported in association with leukemia induced by the FMR group of viruses (26, 27). Further, it appears that the glomerulonephritis that develops spontaneously in aging mice of several strains is immune complex in type and associated with MuLV antigens (28-32). It should also be remembered that other virus infections such as lymphocytic choriomeningitis and polyoma may cause or enhance ANA and immune complex-type glomerulonephritis (33). Whether the different oncornaviruses differ significantly in their ability to cause immunologic disease or whether this ability is related only to the degree of virus production cannot be answered at present. It is tempting to anticipate that an

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oncornavirus isolated from a strain of mice with spontaneous immunologic disease (such as the NZ) might be particularly prone to induce such disorders. However, before such conjecture is warranted, rigid quantitative comparisons of the various oncornaviruses must be made in a number of murine strains. Thus, for the present our observations are best considered a demonstration of the immunopathologic potential of oncornaviruses and not an indication of any special properties of an oncornavirus isolated from NZB mice.

Summary

This report clearly demonstrates that a systemic lupus erythematosus (SLE)-like syndrome and lymphoma can be induced in immunologically normal (BALB/c × NZB)F1 mice by infection of neonates with a murine leukemia virus (MuLV) (Scripps leukemia virus [SLV] 60A) isolated from NZB lymphoblasts. SLV 60A was titered in vitro (XC test) and administered to newborn and adult (BALB/c × NZB)F1 mice over six log_{10} dilutions. Propagation of MuLV in the newborn recipients was indicated by greatly elevated serum Mu gs-1 levels which were proportional to the dose of virus given.
The SLE-like syndrome was characterized by antinuclear antibodies (ANA) and immune complex-type glomerulonephritis. ANA production was related to the dose of virus and reached the highest levels at 8–16 wk. The incidence of glomerulonephritis was also correlated with the dose of virus and reached nearly 50% in the animals given the highest virus dose. Both titers of ANA and incidence of glomerulonephritis were greater in females than in males, although the amounts of Mu gs-1 in sera of both sexes were equal. The incidence of direct Coombs' positivity was not significantly affected by inoculation of this virus.

The incidence and time of onset of thymocytic lymphoma were linearly related to the amount of virus inoculated. High serum Mu gs-1 levels predicted lymphoma development and reflected increases in the amount of infectious virus in the spleen. No induction of tumors, autoimmunity, or high serum Mu gs-1 levels followed administration of SLV 60A to 6-wk old (BALB/c × NZB)F1 mice or inactivated 60A or active AKR virus to newborns.

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