Characterization of a Retrovirus That Cross-Reacts Serologically with Canine and Human Systemic Lupus Erythematosus (SLE)¹

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This report characterizes the SP104 virus, which was previously shown to contain an antigen that cross-reacts with an antigen present on surfaces of blood lymphocytes of human and canine patients with systemic lupus erythematosus (SLE). Morphologically, the virus was a Type C particle. By physicochemical characterization it was a typical retrovirus with a buoyant density of 1.15–1.17 g/cm³, high molecular weight RNA and RNA-dependent DNA polymerase. The virus had antigens that cross-reacted with p30, gp71, p12, and p15 of other murine retroviruses. Biologically, SP104 was characterized as a murine B-tropic virus that was only weakly oncogenic but highly efficient in eliciting the production of antinuclear antibody in mice. Nucleic acid hybridization experiments indicated that the RNA of SP104 virus had only partial identity with the other murine leukemia viruses tested. There was no evidence that the genetic sequences found in the SP104 virus were present in tissues from canine or human patients with SLE.

INTRODUCTION

The lesions of systemic lupus erythematosus (SLE) in mice, dogs, and humans may involve the participation of Type C RNA viruses. For example, a viral glycoprotein (gp69/71) of Type C viruses was found in the renal lesions of (NZB × NZW)F₁ mice (1), and proteins related to Type C RNA viruses were also found in the renal lesions of human SLE (2, 3). Furthermore, a fluorescein-conjugated antiserum against a murine Type C RNA virus, SP104, stained the membranes of lymphocytes from the blood of humans with SLE (4).

We now report studies that characterize the SP104 virus. In addition to a determination of the biochemical, immunological, and biological properties of SP104, we also used the method of nucleic acid hybridization to seek evidence of SP104 genes in tissue of mice, dogs, and humans with SLE. SP104 was isolated from an established cell line that originated from a murine plasmacytoma. The mouse that developed this tumor was injected at birth with a cell-free filtrate prepared from the spleen of a dog with SLE (5). The plasmacytoma produced a monoclonal IgA-κ protein that behaved like an antibody against double-stranded DNA (6). Such antibodies are characteristic of SLE (7).

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METHODS

Origin of SP104. This tumor developed in a 9-month-old (BALB/c × A/J)F1 mouse that was injected at birth with a cell-free filtrate prepared from the spleen of a dog, C-4. The dog was from a litter of seven produced by the mating of a normal male and a dam that had SLE. C-4 underwent splenectomy at the age of 6 years; at that time she had a positive LE preparation but lacked antinuclear antibody in her serum.

Establishment of the SP104 cell line. Cells were teased from the tumor, strained through No. 60 stainless-steel mesh, and suspended in sterile RPMI medium 1640 supplemented with 20% heat-inactivated fetal calf serum, glutamine, antibiotics, and vitamins. After several weeks the primary attached cells were trypsinized, washed, and placed into new plastic bottles with fresh medium. Cells were repeatedly passaged into the medium, selecting for adherent cells, until a continuous cell line was established at passage 15. The established cell line was maintained in RPMI medium 1640 containing 20% heat-inactivated fetal calf serum, 2 mm of glutamine, 33 μg/ml of amphotericin B, 120 U/ml of penicillin, and 120 μg/ml of streptomycin.

Electron microscopy. Fragments of tumor or pelleted tissue culture cells were fixed in 2.5% glutaraldehyde. After further washing in phosphate buffer, the pellet was postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. Ultrathin sections were mounted on uncoated grids, stained with uranyl acetate, and counterstained with lead citrate (8).

Preparation of [3H]uridine-labeled virus. SP104 cells were grown in 250-ml T flasks (Falcon Plastics, Oxnard, California, Catalog No. 3224) in RPMI medium. When the cells were about 50% confluent, 25 μCi of [3H]uridine was added to the medium. The radiolabeled virus was then harvested using a previously described method (9). Unlabeled SP104 virus was prepared in an identical manner, except for the omission of [3H]uridine.

Extraction and purification of viral RNA. RNA was extracted from purified [3H]uridine-labeled virus by the alkaline Tris–phenol–SDS method of Brawerman et al. (10). The [3H]uridine-labeled RNA was mixed with unlabeled polysomal RNA (28s and 18s) in buffer and submitted to rate zonal centrifugation. After centrifugation, 0.5-ml fractions were collected from the bottom of the gradient. Marker RNA was detected utilizing a Gilford uv spectrophotometer; 0.10 ml of purified yeast RNA (1 mg/ml) was added to each fraction, and the total RNA in each fraction was precipitated with 20% TCA. Acid-precipitable radioactivity was harvested on Whatman GSA glass fiber filters.

RNA-dependent DNA polymerase assay. A modification of the method of Ross et al. (11) was used. Band-purified, triton-disrupted virus was assayed for the enzyme in the presence of manganese acetate, poly rA (Miles Laboratories), oligo dT (Collaborative Research, Inc.), and [3H]TTP (New England Nuclear Corp.).

Hybridization experiments. Previously published procedures were used for the extraction of viral RNA, cellular RNA, and cellular DNA (12–14). [3H]DNA probes were prepared from band-purified SP104 virus, Rauscher leukemia virus, and the Harvey strain of murine sarcoma virus (MSV–MuLV), utilizing previously described procedures (12). The labeled substrate was [3H]dCTP (26 Ci/μmol, New
England Nuclear Corp.). Nucleic acid hybridizations were analyzed by either the $S_1$ nuclease technique (12), the CsSO$_4$ density gradient analysis (13, 14), or the hydroxyapatite column procedure (14, 15). The $S_1$ nuclease was purified as previously described (16). Additional details are given in the text and legends.

Radioimmunoassay for p30. The method of Parks and Scolnick (17) was used. Briefly, varying dilutions of antisera directed against disrupted SP104 virions (see below) were mixed with $10^4$ cpm $^{125}$I-labeled Rauscher virus p30. Controls included sera from the rabbit prior to inoculation and monospecific rabbit anti-Rauscher virus p30 (purified Rauscher virus p30 and the monospecific antiserum were a gift from Dr. David Livingston).

Analysis of immune complexes. The procedure for SDS gel electrophoresis of radiolabeled immune complexes was performed according to the method of Ihle et al. (18). Samples of immune complexes consisting of antiserum and $[^3]$H]labeled SP104 virus (see above) were disrupted in 1% SDS and 1% 2-mercaptoethanol in 0.01 M sodium phosphate buffer at pH 7.4, boiled for 30 min, and cooled at 4°C. SDS polyacrylamide gel electrophoresis was conducted as previously described by Laemmli (19) and modified to slab gels. Briefly, 20-μl aliquots of sample were run through 12% acrylamide-resolving gels for 3 hr at 100 V. Bromophenol blue was utilized as a tracking dye and ribonuclease, ovalbumin, and BSA were included as known molecular weight standards. Disrupted SP104 ($[^3]$H]leucine-labeled) virus and disrupted virus complexed with antisera with known specificities were included as controls.

Determination of viral tropism. SP104 virus was purified by isopycnic banding and assayed in the uv–XC procedure described by Rowe et al. (20). The assay cells were NIH Swiss and BALB/c mouse embryo cells prepared from 14- to 17-day-old embryos (21), which were trypsinized from primary lines. Positive and negative controls utilizing known stock suspensions of virus were included in each test. SP104 tissue culture supernatants were tested for xenotropic virus in the focus induction assay described by Fischinger et al. (22). This technique used cloned 8c cells (feline origin) transformed after single hit infections with murine sarcoma virus (81 cells). SP104 tissue culture cells were also assayed in an infectious center assay. The procedure just mentioned was used, but mitomycin-treated tissue culture cells were substituted for supernatant fluid from the tissue culture. This modification of the assay increased its sensitivity about 1000 times (23). In both assays, reference stocks of xenotropic NZB virus were utilized as positive controls.

Bioassay of SP104. Newborn (BALB/c × A/J)F₁ (hereafter called CAF₁) mice were inoculated with one of the following agents intraperitoneally: (a) 17–20 μg of protein of SP104 band-purified virus (tissue culture source), (b) varying numbers of viable SP104 tissue culture cells, or (c) $10^6$ cell equivalents of SP104 plasmacytoma cell-free filtrates. Controls included inoculation of unrelated plasmacytoma cell-free filtrates (MOPC 315) and 85 CAF₁ mice that did not receive any inoculation. Cell-free filtrates were prepared by freezing and thawing the cell suspension three times (30 min at −70°C and then 37°C, respectively) before passing it through a 0.45-μm Millipore filter (Millipore Corp., Bedford, Massachusetts). Filtrates not used immediately were stored at −70°C. Blood samples were collected from each mouse at monthly intervals and tested for antinuclear antibody (ANA). Postmortem
examination was conducted on all mice either at the time of spontaneous death or at 1 year of age.

*Fluorescent antinuclear antibody test.* A previously described method (5) was used. Rabbit anti-mouse immunoglobulin antisera were conjugated to fluorescein isothiocyanate at a FITC:protein ratio of 0.10:1 (w/w). The substrate was fresh cryostat sections of baby rat liver. All samples were coded and read independently by two observers.

*Fluorescent staining of plasmacytoma and tissue culture cells.* Tumor and tissue culture cells were washed twice in PBS (pH 7.2) with 5% BSA and centrifuged at 150g at 4°C for 8 min. The washed cells were smeared on coverslips and air dried. The smears were fixed in acetone, washed twice in PBS, and stained with FITC-conjugated antisera for 45 min at room temperature in a humid chamber. After washing in PBS, the coverslips were examined by fluorescent microscopy.

*Antisera.* Antiser to the isopycnic-banded SP104 virus were prepared by the subcutaneous inoculation into rabbits of 1 mg (total viral protein) of ether-disrupted virions in complete Freund’s adjuvant. The procedure was repeated four times at 20-day intervals. In gel diffusion analysis two sharp precipitating lines developed between antisera and disrupted virions and one sharp precipitin line (continuous with one of the previous lines) developed between the antisera and intact virus.

Antisera to the various murine immunoglobulin heavy and light chains were purchased from Gateway Immunosera, Cohaka, Illinois. These antisera were tested for monospecificity in both gel diffusion and immunoelectrophoresis against known myeloma proteins.

**RESULTS**

*SP104 Tumor*

Macroscopically the tumor consisted of lobulated masses that invaded both visceral and parietal peritoneum. Histologically, the tumor consisted almost entirely of plasma cells, many of which were binucleated. The ultrastructure of this tumor has been reported (5): typical plasmacytes and plasmablasts containing “A-type” virus particles were the main features. By direct immunofluorescent microscopy, the cytoplasm of most of the tumor cells stained with antisera against \( \alpha \) and \( \kappa \) chains. Antisera against \( \gamma \), \( \mu \), and \( \lambda \) chains did not stain the cells.

Both the ascitic fluids and sera of mice bearing the plasmacytoma contained a monoclonal immunoglobulin that was IgA-\( \kappa \). When an \((\text{NH}_4)_2\text{SO}_4\) precipitate of the ascitic fluid was chromatographed on Sephadex-G200, the \( \alpha \)-chain determinants localized to three regions with molecular weights of \( >200,000 \), \( 150,000-180,000 \), and \( 75,000-100,000 \) daltons. In each case, the \( \kappa \) light chain was present. This suggests that the IgA protein in the ascitic fluid was monomeric and dimeric plus a smaller unit with a molecular weight compatible with a 2-chain protein (or half-molecule IgA).

The urine of mice with large intraperitoneal tumors also contained a protein that reacted solely with monospecific anti-\( \alpha \) heavy chain antibody. No light chain antigens were detected in the urine. This urinary protein had no DNA-binding activity, in contrast to the DNA-binding property of the serum and the ascitic IgA protein (24).
Established Cell Line SP104

A continuous line was developed in tissue culture after the plasmacytoma had been passaged in vivo four times by intraperitoneal inoculation of tumor cells into newborn CAF<sub>1</sub> mice. The established culture (SP104) consisted of fibroblastic and epitheloid cells. Both types of cells were contact inhibited.

SP104 and two cloned sublines (CL4 and CL5) were examined by electron microscopy. All three lines consisted of mixtures of fibroblastic and epitheloid cells (25). Both types of cells displayed extracellular (juxtacellular, intravacuolar) immature and mature Type C particles (Fig. 1). Budding immature Type C particles were seen in great numbers even in shattered, apparently dying epitheloid cells. The number of Type C particles visualized in SP104 passage 95 was about 1/10 that observed in passage 5. Type A particles were not observed in any specimen.

SP104 Virus

Physicochemical characteristics. [3H]Uridine-labeled SP104 virus was subjected to ultracentrifugation on a linear 15–60% sucrose gradient. The density of the virions was 1.15–1.17 g/cm<sup>3</sup> (Fig. 6). RNA-dependent DNA polymerase activity was associated with virions that banded at that density (Fig. 2).

The sedimentation constant of the [3H]uridine-labeled RNA that was extracted

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**Fig. 1.** Portion of the cytoplasm of a "clear" epitheloid cell: Dilated rough endoplasmic reticulum cisterna (rer) and mitochondria (m) are seen; however, the ground substance is very scarce, the ribosome content is low, and only filaments (f) are prominent. Despite its paucity in organelles, this cell is able to replicate Type C particles, some of which are budding (arrows). ×40,000.
from purified virions was obtained by the method of Brawerman et al. (10). Figure 3 illustrates the results, which indicate a value of 59.1–63.3S.

**Biological Properties**

**Tropism of the SP104 virus.** Ecotropic virus was readily detected in supernatants of the SP104 cell line. Plaques were visualized only on BALB/c embryo cells indicating that the virus was B tropic. Assays for xenotropic virus were consistently negative.

**Oncogenicity.** The oncogenicity of band-purified SP104 virus was compared with that of cells from the SP104 line and with cell-free filtrates of the in vivo-passaged tumor. Recipients were newborn CAF1 mice, which were held for at least 1 year before autopsy. Both the purified virus and live SP104 cells were weakly oncogenic. Excluding pulmonary adenomas, a not uncommon neoplasm in CAF1 mice, and pooling the various groups, 2/19 recipients of purified virus, 2/23 recipients of SP104 cells, and 0/18 recipients of the cell-free filtrates from the SP104 tumor developed lymphomas. Two control groups were autopsied at the age of 1 year. None of 85 uninjected CAF1 mice and 0/12 animals injected with a filtrate prepared from an unrelated plasmacytoma (MOPC 315) had lymphomas.

**Production of antinuclear antibody (ANA).** Sera of the recipients described in the preceding experiment were obtained at monthly intervals and tested for the
presence of ANA by the immunofluorescent technique. Neither uninjected mice nor mice inoculated with filtrates from MOPC 315 developed ANA during the period of observation. By contrast, the recipients of SP104 cells, purified SP104 virus, or SP104 cell-free filtrates had ANA in their serum. Typical examples of results obtained from serial bleedings are shown in Table 1. The pattern of fluorescent staining at the last serial bleeding was diffuse in 14/56 tests, peripheral in 1/56 tests, and a mixture of the two in 40/56 tests.

Antigenicity of SP104 virus. An antiserum prepared in rabbits against ether-disrupted SP104 virions (see Methods) was assayed by radioimmunoassay for its ability to compete with purified p30. This serum contained a high titer of antibody against $^{125}$I-labeled p30 of Rauscher origin; 50% of the antigen was precipitated by an antiserum dilution of 1:3200. The IgG fraction of the serum was conjugated with fluorescein isothiocyanate and used to stain cells from the SP104 line as well as cells from the in vivo-passaged SP104 tumor. In both cases, a pattern of diffuse cytoplasmic stippling was observed.

TABLE 1

<table>
<thead>
<tr>
<th>Inoculum (n)</th>
<th>Percentage positive at given month</th>
<th>Mean titer$^2$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>SP104 virus (15)</td>
<td>33</td>
<td>66</td>
</tr>
<tr>
<td>SP104 culture cells (23)</td>
<td>20</td>
<td>66</td>
</tr>
<tr>
<td>SP104 myeloma cell-free filtrate (18)</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>MOPC 315 myeloma cell-free filtrate (12)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ n = total number of mice inoculated.
$^b$ Twelve-month geometric mean titer (1/log2).
**SDS polyacrylamide gel electrophoresis of radioimmune complexes.** Figure 4 illustrates the localization by molecular weight of various virus components precipitated by rabbit antisera produced with ether-disrupted SP104 virions. Major radioactive peaks were found in the 71,000, 30,000, 15,000, and 12,000 molecular weight regions. The virus core appears to have been completely disrupted since monospecific anti-feline leukemia virus (FeLV) p27 selectively precipitated the 30,000 molecular weight protein. In addition, an antisera directed against disrupted whole FeLV virus (not shown) precipitated radioactive counts in each of the four major peaks, suggesting that each has interspecies activity.

**Hybridization experiments.** To fixed amounts of a [³H]DNA probe complementary to the SP104 viral RNA, increasing amounts of either nonradioactive SP104 viral RNA, extracted from the virus, or total cellular RNA, extracted from SP104 cells, were added. The extent of hybrid formation was analyzed in terms of resistance to S₁ nuclease (12). Figure 5 illustrates that when sufficient quantities of either viral RNA or SP104 cellular RNA were added, the probe was almost completely protected from S₁ nuclease digestion. This indicated that both virus and SP104 cells contained nucleic acid sequences completely complementary to the probe.

The half-⁴Cᵢ value (⁴Cᵢ value which gave 50% protection to the probe) for viral RNA was 10⁻², whereas the half-⁴Cᵢ value for the total cellular RNA was 3. These values were similar to those reported by Fan and Baltimore (12) for the Moloney
leukemia virus and indicate that approximately one three hundredth of the total RNA extracted from SP104 cells contained SP104 virus-like sequences.

We next compared SP104 to a conventional murine leukemia virus. The percentage homology between SP104 viral RNA and RNA from Rauscher virus was determined. For this test a $[^3\text{H}]\text{DNA}$ probe synthesized with Rauscher viral RNA as template, or a $[^3\text{H}]\text{DNA}$ probe synthesized with SP104 viral RNA as template, was hybridized against increasing amounts of Rauscher viral RNA. Rauscher viral RNA afforded only 3.5% protection to the SP104 $[^3\text{H}]\text{DNA}$ probe, whereas it afforded the Rauscher $[^3\text{H}]\text{DNA}$ probe greater than 70% protection against degradation with $S_1$ nuclease. We obtained similar results when RNA derived from the Harvey strain of murine sarcoma virus (MuLV−H + MSV) was substituted for Rauscher RNA.

The lack of complete homology between SP104 viral nucleic acid sequences and those of a well-characterized murine leukemia virus, as well as the unusual origin of the cell line that produced the SP104 virus, raised questions as to the species of origin of this virus. We next investigated the possibility of homology between SP104 viral nucleic acids and cellular DNA from canine and other species. For this purpose the SP104 $[^3\text{H}]\text{DNA}$ probe was incubated with cellular DNA extracted from various species. Double-stranded DNA hybrids were detected utilizing a hydroxylapatite column (14). Under the conditions used, double-stranded DNAs were retained on the column whereas single-stranded DNA was not. By progressively raising the temperature during elution of the column, hybrid structures were "melted out" and eluted as a function of the extent of homology between the hybrid strands.

Figure 6 illustrates the results. Neither Escherichia coli nor human cellular DNA hybridized to the SP104 $[^3\text{H}]\text{DNA}$ probe. Only 5% of the canine DNA hybridized, whereas significant hybridization was seen (in order of increasing homology) with feline, rat, and murine DNA. In addition to the lower percentage
of hybridization between either rat, feline, or canine DNA and the SP104 DNA probe, the low temperature at which these hybrids melted indicated that a relatively unstable complex was formed with the DNA from these species.

The possibility that SP104 viral RNA contained sequences uniquely associated with tissue from animals or humans with SLE was tested by several methods. In the first, cellular RNA was extracted from tissues of CAF₁ and NZB mice and compared for its ability to protect the SP104 [³H]DNA probe from S₁ nuclease digestion. Hybridization of the probe was conducted with the total cellular RNAs at a high $C_{t,1}$ value ($3 \times 10^5$) to maximize detection of homologous sequences. Table 2 indicates that murine brain, liver, splenic, and renal tissue contained RNA with partial homology (5–33%) with the SP104 virus. However, no uniform differences in the extents of homology with the viral probe were found between CAF₁ and NZB tissues. The experiments were repeated with NZB tissue from either clinically normal 5-month-old animals or from 11-month-old diseased animals, and no differences could be demonstrated.

In another study, cellular RNAs extracted from dogs and humans with SLE were compared to normal tissues of both species for sequence homologies with the SP104 [³H]DNA probe. In all but one case, an insignificant amount of homology (<10%) was detected (Table 2). Nevertheless, it may be of interest that the highest value for hybridization of canine RNA to the SP104 [³H]DNA probe (13%) was found with RNA extracted from a tumor obtained from a dog with SLE (Table 2, RNA sample Fifi).

Since protection against S₁ nuclease digestion requires hybrids of high complementarity, another less stringent method was employed in order to detect the possibility of partial homology between the SP104 viral genome and certain cellu-
The experimental protocol utilized the S, nuclease method and a C, of $3 \times 10^3$. Tissues were obtained from the following sources: 5-month-old NZB mice and 3-month-old CAF, mice. B264, B16, and A179 were all offspring from SLE-afflicted parent dogs. B264 had positive antinuclear antibody (ANA) and LE tests. Both B16 and A179 had clinical and serological evidence of SLE. TD28 was a normal dog that was inoculated with the SP104 cell-free filtrate at birth and was positive for both ANA and LE tests at the age of 5 months. Fifi was the SLE-afflicted mother of C4. Her tumor was an adeno-carcinoma of the perianal gland and contained C-type particles by electron microscopy. Human splenic material was collected at autopsy from a patient with SLE and from another patient with an unrelated disorder.

Table 2

<table>
<thead>
<tr>
<th>Source of cellular RNA</th>
<th>Percentage hybridization</th>
</tr>
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<tbody>
<tr>
<td>NZB brain</td>
<td>18</td>
</tr>
<tr>
<td>CAF, brain</td>
<td>5</td>
</tr>
<tr>
<td>NZB liver</td>
<td>14</td>
</tr>
<tr>
<td>CAF, liver</td>
<td>33</td>
</tr>
<tr>
<td>NZB spleen</td>
<td>25</td>
</tr>
<tr>
<td>CAF, spleen</td>
<td>21</td>
</tr>
<tr>
<td>NZB kidney</td>
<td>6</td>
</tr>
<tr>
<td>CAF, kidney</td>
<td>24</td>
</tr>
<tr>
<td>Dog kidney, B264</td>
<td>6</td>
</tr>
<tr>
<td>Dog thymus, B264</td>
<td>5</td>
</tr>
<tr>
<td>Dog thymus, B16</td>
<td>5</td>
</tr>
<tr>
<td>Dog thymus, A179</td>
<td>0</td>
</tr>
<tr>
<td>Dog spleen, TD28</td>
<td>9</td>
</tr>
<tr>
<td>Dog tumor, Fifi</td>
<td>13</td>
</tr>
<tr>
<td>Dog spleen, normal</td>
<td>2</td>
</tr>
<tr>
<td>Human spleen, SLE</td>
<td>4</td>
</tr>
<tr>
<td>Human spleen, normal</td>
<td>2</td>
</tr>
</tbody>
</table>

The density of the [3H]DNA probe from 1.455 (Fig. 7A), the density of single-stranded DNA, to a density of 1.565, a density characteristic of DNA–RNA hybrids (13). Hybridization of the SP104 DNA probe with NZB cellular RNA led to a shift of about 10–20% of the probe to the hybrid region of the density gradient (Fig. 7C). These results are consistent with the partial homology between the probe and the NZB tissue RNA noted in the studies with S, nuclease (Table 2). Figure 7D represents the distribution of the [3H]DNA probe in the density gradient after incubation of the probe with RNA extracted from the spleen of a human patient with SLE. The density of 1.445 observed for the radioactive probe in this study suggests that no significant hybridization had taken place. Negative results were also obtained with another spleen from a human with SLE and with thymic tissue from three dogs with SLE.
DISCUSSION

The SP104 cell line was developed from a plasmacytoma with unique properties. This tumor arose in a mouse that was injected at birth with a filtrate prepared from the spleen of a dog with SLE. The tumor was associated with a monoclonal IgA protein, which behaved like an antibody to native DNA. An early passage of the original tumor was given to Dr. Norman Talal, who confirmed our finding that the monoclonal IgA it produced had specific serological reactions with native DNA (6). Since antibodies to native DNA are characteristic of SLE, it seemed unlikely that the neoplasm and its origin were merely coincidental. Therefore, we undertook to characterize the tumor and the virus it produces.

The first step in these experiments was to establish SP104 as a continuous line in tissue culture. This was achieved by selection for adherent cells. A similar method was used for the murine plasmacytoma MOPC 173 by Paraf et al. (26), whose results resemble ours. In both cases the original tumor consisted of immunoglobulin-producing plasma cells that contained many more Type A particles than Type C particles. And, as both MOPC 173 and SP104 were adapted in vitro, fibroblastic cells became dominant, immunoglobulin production ceased, and only Type C particles were produced. As judged by electron microscopy, the production of C-type virions diminished progressively as the number of passages in vitro increased; nevertheless, after more than 200 passages the SP104 line continues to produce Type C particles. The decreased production of Type C particles during in vitro passage and the shift from Type A to predominantly Type C particles after in vitro adaptation has been observed by others in cultured plasmacytomas (27). Schubert and Horibata (28) proposed that the production of immunoglobulins by cultured plasmacytomas is linked to their production of Type C viruses. In our opinion, the SP104 line stopped producing immunoglobulin because the plasma cells were replaced by fibroblastic and epithelioid cells. An attempt to “derepress” immunoglobulin synthesis in the established fibroblastic line by treatment with DMSO failed (unpublished observation).

We do not know if the virus particles produced by the established cell line were identical to those produced by the original tumor. Conceivably, the fibroblastic cells selected during in vitro passage of the tumor spontaneously “switched on” an unrelated, endogenous Type C RNA virus. Continuously cultured fibroblasts from several species may exhibit this property (29); however, we believe that this is unlikely because both the original tumor and the established line contained the same type of virus (e.g., B tropic).

The virus produced by the SP104 cell line had all the properties of a Type C RNA virus: It banded at a density of 1.15–1.17 g/cm³, contained high molecular weight RNA and RNA-dependent DNA polymerase, and serologically its p30 cross-reacted extensively with p30 from the Rauscher virus. In addition, we were able to demonstrate specific immune precipitation of radiolabeled SP104 proteins with molecular weights of 70,000, 30,000, 15,000, and 12,000, as detected by antisera both to SP104 and feline leukemia virus. Results with the latter reagent demonstrated the presence of interspecies reactivities. The XC test identified SP104 as a B-tropic virus: no xenotropic viruses could be detected in the culture.

Biologically, the virus was only weakly oncogenic, as judged by its ability to induce lymphomas or leukemias in newborn CA1 mice. The SP104 virus may be
Fig. 7. Detection of hybridization by CsSO₄ density gradient analysis. The SP104 [³H]DNA probe was hybridized with RNA from various sources at a Cₛ of $6 \times 10^3$ and analyzed on CsSO₄ as described under Methods. Values depict SP104 [³H]DNA alone (A), SP104 [³H]DNA plus SP104 viral RNA (B).
SP104 [³H]DNA plus total NZB spleen RNA (C), and SP104 [³H]DNA plus total RNA from spleen of a human patient with SLE (D).
more virulent in strains other than CAF1; this was not tested. The purified virus regularly triggered the production of ANA after inoculation into newborn mice. This may not, however, be a unique property of SP104. Isolates of Gross, Rauscher, and a Moloney-like virus can produce the same effect (30–32). Conceivably, many Type C RNA viruses can cause production of ANA, provided the genetic background of the infected host is permissive. In this regard, it is noteworthy that the characteristic virus of NZB mice, which spontaneously develop a disorder resembling SLE, is xenotropic (33). And in (NZB × NZW)F1 mice the glomerular lesions contain the principal glycoprotein of the viral envelope, gp69/71 (1), thus incriminating this class of virus in the lesions of the disease. However, it is important to acknowledge that there is as yet no unequivocal proof that Type C viruses cause SLE in NZB mice or their hybrids. Indeed, there is some evidence that the expression of xenotropic virus and autoimmunity may segregate as independent genetic traits in NZB mice (34).

Results of the nucleic acid hybridization experiments indicate that the SP104 virus is a murine virus that shares only partial nucleic acid sequence homology with the Rauscher or Harvey strains of murine leukemia virus. The DNA probe prepared from SP104 hybridized to a significant extent with cellular DNA from normal CAF1 mice, further suggesting that SP104 is an endogenous murine C-type RNA virus. The feeble oncogenicity of SP104 is consistent with this classification (35).

When hybridization between cellular RNA from NZB or CAF1 mice and the SP104 DNA probe was studied, no significant differences between the cellular RNAs from the two mouse strains were found. The results were consistent with what would be expected with a typical endogenous murine virus. We found no evidence that SP104 virus contains nucleic acid sequences unique to the NZB strain, even when tissues were obtained from NZB mice with overt autoimmune disease. Thus, we have no positive evidence that SP104 virus shares specific nucleic acid sequences with putative virus sequences involved in the production of autoimmune disease in NZB mice. This lack of evidence is complicated by the known pronounced differences between murine ecotropic and xenotropic viral RNA sequences (36) as well as known differences in homology between xenotropic viruses isolated from different strains of mice (37). We estimate that sequence homology between SP104, an ecotropic virus, and the NZB xenotropic virus does not exceed approximately 25%.

When canine cellular RNA was examined for sequences that hybridize to the SP104 virus probe, very little homology was demonstrated, regardless of whether or not the dog had SLE. However, an intriguing finding was that RNA extracted from a carcinoma of the perianal gland, obtained from a dog with SLE (the mother of C4), contained sequences which afforded higher protection of the SP104 probe than that obtained with RNA from other canine tissues. The 13% homology observed in this case is low enough to cause uncertainty, but it is of interest since C-type particles were found in this canine tumor by electron microscopy (unpublished observation). RNA from a human SLE spleen also failed to demonstrate significant sequence homology with the SP104 probe, as detected by either the S1 nuclease method or the CsSO4 density gradient method (Table 2, Fig. 7).

Assuming that the DNA probe accurately reflected the genetic constitution of
the SP104 viral RNA, then the sensitivity of our methods is such that the canine and human tissues cannot contain RNA sequences that are homologous to 5% or less of the SP104 virus. Although we cannot state with certainty that our probe represented a complete copy of the SP104, it was synthesized under conditions that enhance the synthesis of complete copies (12). No differences in extent of hybridization with the [3H]DNA SP104 probe were seen between cellular RNA from tissues of normal or SLE-afflicted dogs.

The hybridization results have not provided any direct evidence of the expression of SP104 genome in canine or human SLE. However, in both species, an antigen cross-reactive with the interspecies antigen of p30 seems to be present (4). Further serological analyses of tissues and cells from individuals with SLE need to be carried out. This approach has the advantage that it permits localization of viral antigens to the actual pathologic lesions of the disease (1-3). Thus, although the present studies fail to provide direct evidence that the SP104 virus (or a genetic region of that virus) is expressed in canine or human SLE, a possible role for C-type RNA viruses in the etiology of SLE has not been excluded.

REFERENCES