Determinants of Susceptibility and Resistance to Feline Leukemia Virus Infection. I. Role of Macrophages 1, 2, 3

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ABSTRACT—The role of autologous peritoneal feline macrophages (Mφ) in the age-related resistance of cats to feline leukemia virus (FeLV) was investigated by a study of the functional properties and FeLV susceptibility of Mφ from kittens and adult cats and the effect of hydrocortisone (HC) and silica on Mφ-FeLV interactions. Although the phagocytic functions of isolated Mφ from kittens and adults were equivalent, the mean FeLV susceptibility of Mφ from kittens was five times that of Mφ from adult cats, thus establishing a direct correlation between the age-related susceptibility of cats and Mφ from cats to FeLV. Mφ of viremic cats were found to be infected with FeLV in vivo; virus titers were slightly higher than those obtained after in vitro infection of Mφ. Mφ from cats that had experienced regressive FeLV infection in vitro were not significantly more resistant to FeLV infection in vivo than were Mφ from naive adult specific-pathogen-free cats. HC, which has been shown to enhance the in vivo FeLV susceptibility of cats, also enhanced the permissiveness of Mφ from cats to FeLV in vitro (600-fold for Mφ from adult cats and 200-fold for Mφ from kittens. Mφ permissiveness to FeLV was highly sensitive to HC and occurred in Mφ infected in vivo or in vitro. In parallel with the effect of HC on the natural resistance of cats to FeLV, administration of silica before virus inoculation also markedly enhanced the FeLV susceptibility of adult cats. Silica was toxic for isolated Mφ but not for lymphocytes in vitro, and silica produced monocytopenia and neutrophilia, delayed skin allograft rejection, and augmented feline oncovirus-associated cell membrane antigen antibody responses in vivo. These experiments indicate that Mφ were linked to the natural resistance of cats to FeLV and that the temporary elimination of Mφ functions (e.g., by silica) and/or the conversion of the Mφ-FeLV relationship from a nonpermissive to a permissive state (e.g., by corticosteroids) resulted in failure of early virus containment, in persistent virus amplification in hemolymphatic tissues, and in subsequent FeLV-related proliferative or antiproliferative disease.—JNCI 1981; 67: 889-898.

FeLV is transmitted by contact of susceptible cats with virus excreted in salivary and respiratory secretions of persistently infected cats (1-4). Whether lymphosarcoma, leukemia, or antiproliferative disease such as aplastic anemia occurs after virus exposure is determined by the cat's ability to contain FeLV replication in target hemolymphatic tissues in the first few weeks after FeLV exposure (5, 6). The capacity for early lymphoreticular restriction of FeLV is deficient in immature cats (5, 7) and can be abrogated in adult cats by treatment with adrenal corticosteroid (8) or by exposure to the immunosuppressive chemical carcinogen methyl nitrosourea (9). It is plausible that the interaction of Mφ with FeLV and with lcl determines whether progressive infection and leukemogenesis or regressive infection and elimination of FeLV-infected cells occur (5, 6). On the basis of this premise and observations in mice that demonstrate the obligatory role of mononuclear phagocytes in age-related resistance to viral infections (10-12) and to grafts of normal or neoplastic hemolymphatic cells (13-15), we have investigated the involvement of feline Mφ in the pathogenesis of FeLV infection.

MATERIALS AND METHODS

Cats.—All cats used were from a hysterectomy-derived (16) SPF breeding colony devoid of horizontally transmitted feline viruses.

Mφ collection and culture.—Peritoneal Mφ were collected from SPF cats by intraperitoneal lavage with 50-300 ml of saline 4 days after ip injection of 50-200 ml of 6% thiglycollate (Difco Laboratories, Detroit, Mich.). Mφ were washed in PBS and resuspended in growth medium consisting of McCoy's 5-A medium containing 10% horse serum, 2% glutamine, 2% essential amino acids, 1% nonessential amino acids, 1% sodium pyruvate, 0.1% gentamicin, and 0.05% Mycostatin. Mφ were counted with a hemocytometer and stained with 0.05% new methylene blue.

Mφ function assays.—Assays of phagocytic function were performed in glass slide-mounted, 8-well chambers (Lab-Tek Products, Naperville, III.) 24 hours after Mφ were seeded; each chamber contained 5×10⁵ Mφ (0.5 ml of 10⁶ Mφ/ml).

EA phagocytosis and rosetting.—SRBC were sensitized with rabbit IgG antibody at a subagglutinating

ABBREVIATIONS USED: Con A = concanavalin A; CrFK = Crandell feline kidney cells; dpm = disintegrations per minute; EA = erythrocyte-antibody; FeLV = feline leukemia virus; R-FeLV = Rickard strain of FeLV; FFLU = focus-forming units; FOCMA = feline oncovirus-associated cell membrane antigen; GSA = group-specific antigen; HC = hydrocortisone; [3H]dThd = triitated thymidine; lc = lymphocyte(s); Mφ = macrophage(s); MuLV = murine leukemia virus(es); F-MuLV = Friend MuLV; R-MuLV = Rauscher MuLV; PBS = phosphate-buffered saline; SPF = specific-pathogen-free; SRBC = sheep red blood cells; VI = viral infectivity.

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Phagocytic function of peritoneal Mφ from weanling kittens and adult cats.—Weanling (8-wk-old) SPF dilution of 1:10,000. A 0.2% suspension of sensitized, washed SRBC (10^7 cells/ml) in Mφ growth medium was incubated at 100 SRBC:1 Mφ in the culture chambers. For EA rosette determination, cultures were incubated at 37°C for 30 minutes, and nonadherent SRBC were removed by repeated washings with PBS before methanol fixation and Giemsa staining. For EA phagocytosis, cultures were incubated for 2 hours at 37°C, and nonphagocytized SRBC were lysed in 0.83% ammonium chloride in 0.01 M Tris buffer before the slides were fixed and stained. For each assay, 200 cells were examined microscopically, and the percent Mφ forming EA rosettes (at least 3 SRBC/Mφ), the number of Mφ phagocytizing EA, and the number of phagocytized EA per Mφ were determined.

Assays for phagocytosis of unopsonized heat-killed Candida pseudotropicalis were patterned after the assay technique of Cohen and Cline (17). Twenty-four-hour-old cultures of C. pseudotropicalis were grown in Sabouraud’s medium and killed by being boiled for 15 minutes. Suspensions of 100 heat-killed C. pseudotropicalis/Mφ were incubated in Mφ growth medium for 2 hours at 37°C. Cultures were then washed with PBS, fixed, stained, and counted as described above for EA phagocytosis.

Phagocytosis of 0.794-μm-diameter polystyrene latex beads (Sigma Chemical Co., St. Louis, Mo.) was determined in a similar manner. A 1:100 dilution of the commercial stock latex bead suspension containing 30% latex solids was used.

Virus.—R-FeLV (18) was used for all experiments. A virus stock consisting of a 20% homogenate of thymic lymphosarcoma tissue produced by passage of R-FeLV in SPF cats was used for in vivo inoculations. The inoculum contained 10^5 FFU infectious FeLV/ml as determined for the clone 81 assay system (19). This R-FeLV stock was toxic to cell cultures when diluted to less than 1:50; therefore, an R-FeLV pool of higher titer (10^6 FFU/ml) and lower cytotoxicity was prepared by inoculation of CrFK (20) as described in the accompanying report (21). For all in vitro Mφ inoculation experiments, the R-FeLV stock was prepared in CrFK.

FeLV infectivity assay.—The sarcoma-positive, leukemia-negative clone 81 of CrFK, originated by Fischinger et al. (19), was used for the assay of infectious FeLV, as described by Schaller and Olsen (22). The assays were performed in 16-mm-diameter, MultiWell culture dishes, as detailed in the accompanying report (21). We determined cell-associated or cell-free VI in FFU for each inoculated Mφ culture by assaying separately media and freeze-thaw lysates of Mφ collected by scraping adherent cells from the wells with a rubber policeman. The clone 81 indicator cells were inoculated with 1.0 ml of Mφ medium or with 10^5 Mφ. Cell-associated or cell-free VI was expressed per 10^6 Mφ recovered from the cultures at the time of harvest.

FeLV inoculation of Mφ.—All experiments involving FeLV infection of Mφ were done in 25-cm² flasks (Falcon Plastics, Oxnard, Calif.) in which 3.2×10^6 Mφ were seeded in 4 ml of Mφ medium. Polybrene (4 μg/ml) was incorporated into Mφ growth medium when the cells were seeded. Then 25-cm² flask cultures, 24 or 48 hours old, of feline peritoneal Mφ (3.2×10^6 Mφ seeded) were inoculated with 1 ml of R-FeLV containing 10^6 FFU/ml (multiplicity of infection=0.33). Cultures were incubated for 1 hour at 37°C, and Mφ growth medium with Polybrene was added. Culture medium was changed after 2 days, and cultures were harvested for the VI assay at 4 days. In some experiments, parallel FeLV-inoculated Mφ-containing flasks also were harvested at 7 days.

Inoculation and monitoring of cats for FeLV infection.—All cats were inoculated ip with 10^5 FFU of tissue origin R-FeLV. Blood samples were collected every 2 weeks for determination of FeLV GSA in blood cells (23) and of antibody titers to FOCMA (24).

Immunofluorescence assays for FeLV GSA and FOCMA antibody.—The procedure described by Hardy et al. (23), with minor modifications (25), was used for demonstration of FeLV GSA in blood leukocytes. The indirect membrane immunofluorescent procedure of Essex et al. (24) was used for determination of FOCMA antibody.

HC treatment of Mφ.—HC sodium succinate (Abbott Laboratories, North Chicago, Ill.), 10^4, 10^5, and 10^6 M, was added to the Mφ medium when the cultures were initiated.

[3H]dThd labeling of Mφ.— Cultures of peritoneal Mφ with and without HC were initiated in microtiter wells at a cell density of 10^5 Mφ/well. At the time of seeding, Mφ were labeled with 0.5 μCi of [3H]dThd (6.7 μCi/mmol; New England Nuclear Corp., Boston, Mass.) per 10^5 Mφ, and cell-associated [3H] incorporation was determined after 24, 48, and 72 hours by harvesting with a semiautomatic multiple sample processor and counting net dpm in a liquid scintillation spectrophotometer.

Silica—Microcrystalline silicon dioxide with a mean particle diameter of 1.1 μm was used (Minisil; Pennsylvania Glass Sand Corp., Pittsburgh, Pa.). Before use, the silica was washed in PBS, autoclaved, ultrasonicated, and incubated at 4°C for 24 hours in McCoy’s 5-A medium containing 5% fetal bovine serum. The silica particles were thus coated with protein, as described by Allison et al. (26), and their specificity for Mφ was enhanced by decreasing nonspecific cytotoxicity due to direct damage to cell membranes. The silica suspension was ultrasonicated again before 10 or 20 ml of a 200 mg/ml suspension was injected ip per adult cat. In vitro toxicity was determined by the exposure of Mφ cultured in 8-well, glass slide chambers to various concentrations of silica added to the Mφ medium 24 hours after the cultures were established. We evaluated subsequent morphologic changes by staining and microscopically examining Mφ at 24, 48, and 72 hours after silica exposure.

RESULTS

Phagocytic function of peritoneal Mφ from weanling kittens and adult cats.—Weanling (8-wk-old) SPF
kittens are highly susceptible to experimental FeLV infection, whereas adult SPF cats (≤4 mo of age) have substantial resistance to the same virus dose (7). To determine whether the greater susceptibility of immature cats to FeLV might correlate with an age-related deficit in Mθ function, we evaluated the phagocytic capacity of thioglycollate-elicited peritoneal Mθ collected from kittens and adult cats.

The phagocytic indices of Mθ from weanling kittens and adult cats were not significantly different (P<0.10, paired t-statistic) (table 1). Thus no evidence was obtained of an age-related general deficit in Mθ function.

Susceptibility of Mθ from kittens versus adult cats to FeLV.—To determine whether age-related differences in the susceptibility of cats to FeLV might correlate with specific differences in Mθ susceptibility to FeLV (if not to general differences in Mθ function), we inoculated thioglycollate-induced peritoneal Mθ from 19 weanling and 18 adult SPF cats with R-FeLV in vitro. The mean permissiveness of Mθ from kittens to productive FeLV infection was five times greater than that of Mθ from adult cats (P<0.01, paired t-statistic) (table 2). The maximum incidence of FeLV infection in Mθ from kittens was approximately 1% (0.01 FFU/Mθ inoculated); that of Mθ from adult cats was 0.2% (table 2).

Effect of HC on the susceptibility of feline Mθ to FeLV.—On the basis of the correlation between the age-related susceptibility of cats and Mθ from cats to FeLV and of the previous observation (6) that treatment of adult cats with adrenal corticosteroid markedly enhanced their susceptibility to FeLV by impairing early containment of viral replication in lymphoreticular tissues, we examined the tenet that the glucocorticoid augmentation of the FeLV susceptibility of cats may correlate with glucocorticoid enhancement of Mθ permissiveness to FeLV. Therefore, peritoneal Mθ from kittens and adult cats were exposed to various concentrations of HC sodium succinate before they were inoculated with R-FeLV.

HC markedly enhanced the susceptibility of feline Mθ to FeLV infection (text-fig. 1); the permissiveness of Mθ from adult cats was increased over 600-fold and that of Mθ from kittens, nearly 200-fold (table 3). The mean increase in total (cell-associated plus cell-free) infectious virus in HC-treated Mθ from all cats tested was 300-fold (table 4). An average of 1.6 FFU of FeLV/Mθ was produced by HC-treated Mθ cultures as compared with 0.005 FFU of FeLV/Mθ in non-HC-treated Mθ. Mθ permissiveness to FeLV was quite HC-sensitive; 10⁻⁸ M HC, the lowest concentration tested, still enhanced FeLV production eightfold (text-fig. 1). HC treatment increased Mθ DNA synthesis twofold, as measured by [³H]dThd uptake, whereas FeLV replication increased 270-fold (text-fig. 2). HC treatment, therefore, markedly enhanced FeLV replication in Mθ, rendered Mθ from adult cats and kittens equally permissive, and elicited virus titers equal to those obtained with feline cells considered highly permissive for FeLV replication (e.g., CrFK or feline embryo fibroblasts).

FeLV replication in peritoneal Mθ from viremic versus FeLV-exposed, nonviremic cats.—The objectives of these experiments were threefold: 1) to compare the magnitude of FeLV infection in peritoneal Mθ of persistently viremic cats (in which FeLV replicates extensively in bone marrow myelomonocytic progenitor

### Table 1.—Comparison of phagocytic function of peritoneal Mθ from kittens and from adult cats

| Age of cat, mo | No. of cats | Phagocytosis* | Fe receptor, EA rosettes (%) |
|---------------|-------------|---------------|------------------------------|
|               |             | EA | C. pseudotropicalis | Latex |                              |
| 2             | 4           | 46 (4.3) | 75 (6.5) | 84 (13) | 56 (24) |
| 6             | 4           | 32 (6.7) | 52 (7)   | 82 (11)  | 80 (15) |

* Values are mean percents as No. phagocytized/Mθ (standard errors).

### Table 2.—Susceptibility of peritoneal Mθ from kittens versus adult cats to R-FeLV infection in vitro

| Mean age, mo | No. of cats | Cell-associ- | Cell-free | Total |
|--------------|-------------|--------------|-----------|-------|
|              |             | associated  |           |       |
| 2            | 19          | 301 (92)    | 814 (517)| 1,140 (580) |
| 9            | 18          | 109 (49)    | 92 (41)  | 202 (88)    |

* Infectivity was determined for the clone 81 assay. Cells were harvested 4 days after inoculation.

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![Text-Figure 1.—HC-induced dose-related enhancement of permissiveness of feline Mθ to FeLV infection.](original_document)
cells) with that obtained after in vitro FeLV inoculation of MØ from normal cats, 2) to determine whether MØ from cats that had experienced self-limiting FeLV infection in vivo would be resistant to FeLV infection in vitro, and 3) to assess whether the FeLV permissiveness of MØ from either group of cats could be augmented by HC treatment in vitro. Accordingly, thioglycollate-induced peritoneal MØ were collected from persistently viremic, preleukemic cats and cultured as in previous experiments but without in vitro FeLV inoculation and in the presence or absence of HC. Peritoneal MØ were collected also from adult cats that had been inoculated with R-FeLV 6-10 weeks previously and that had remained nonviremic and developed FOCMA titers of 1:16 by 4 weeks post inoculation. These MØ were inoculated with FeLV in vitro with and without HC as in previous experiments.

MØ from viremic cats were found to be infected in vivo and produced infectivity titers similar to (P>0.10) those produced by MØ from kittens infected with R-FeLV in vitro (table 5). The susceptibility of MØ from FeLV-exposed nonviremic adult cats to FeLV infection in vitro was similar to that observed previously for MØ from naive adult cats (table 5). In both instances, FeLV infection was enhanced by HC treatment in vitro (table 5). Thus we concluded that the level of productive FeLV in peritoneal MØ derived from viremic cats approximated that obtained after in vitro infection and that previous self-limiting FeLV infection did not render MØ resistant to reinfection.

**Effect of systemic MØ impairment on resistance of cats to FeLV.**—Because the above in vitro experiments and previous in vivo experiments suggested that the age-related resistance of cats to FeLV correlated with early viral replication versus containment of FeLV by MØ, we investigated whether temporary elimination of MØ in vivo would impair FeLV resistance. Microcrystalline silica was administered to adult cats as a single 2- or 4-g dose, (prorated by weight from studies in mice (27-33), 4 days before virus inoculation.

Silica pretreatment abrogated the natural resistance of cats to FeLV. Persistent viremia developed in 92% of cats treated with 4 g of silica as compared with 9% of non-silica-treated controls (table 6). The enhancement of FeLV susceptibility by silica was dose related: A 50% reduction in the silica dose produced an 80% reduction in the incidence of progressive FeLV infection (table 6). In contrast to the minimal FOCMA antibody responses of viremic cats infected with R-FeLV in previous studies (2), 54% (5/11) of silica-pretreated viremic cats developed FOCMA antibody titers of ≥1:16 (mean peak titer= 1:59) (table 7). Antibody titers occurred at a mean interval of 2.9 weeks after inoculation; titers peaked at a mean interval of 6.5 weeks and declined thereafter.

Silica treatment of cats produced monocytopenia, transient lymphopenia, and neutrophilia that were independent of R-FeLV inoculation. In silica-treated FeLV-exposed viremic cats, lymphopenia persisted and neutropenia developed by 4 weeks when all of the cats had become viremic (text-fig. 3). Lymphopenia and neutropenia were previously observed consequences of FeLV infection of bone marrow cells and of the onset of viremia (5).

Two indices of cell-mediated immunity in silica-treated cats were moderately suppressed, independent of R-FeLV inoculation. The survival of cutaneous allografts was prolonged in 6 silica-treated cats compared with control cats grafted with skin from the same unrelated donor cat (table 8), and the blastogenic

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### Table 3.—Effect of HC on the permissiveness of MØ from adult cats and kittens to R-FeLV infection in vitro

| HC, M       | Mean age of cats, mo | No. of cats | FeLV infectivity, FFU/10^6 MØ (SE)^a | Enhancement index^b |
|-------------|----------------------|-------------|---------------------------------------|---------------------|
|             |                      |             | Cell-associated | Cell-free | Total |                          |
| 0           | 2                    | 12          | 324 (78)        | 417 (104) | 721 (173) | 184                     |
| 10^a        | 2                    | 12          | 46.184 (12,811) | 85.666 (19,011) | 132.742 (35,415) | 2                     |
| 0           | 9                    | 12          | 159 (69)        | 141 (57) | 330 (127) | 20                     |
| 10^a        | 9                    | 12          | 104.133 (55,558) | 81.212 (16,239) | 176.346 (65,568) | 534                   |

* Infectivity was determined for the clone 81 assay. Cells were harvested 4 days post inoculation.

### Table 4.—HC enhancement of the susceptibility of feline MØ to productive R-FeLV infection

| HC, M       | Mean age of cats, mo | No. of cats | FeLV infectivity, mean FFU/10^6 MØ (SE)^a | Enhancement index^b |
|-------------|----------------------|-------------|--------------------------------------------|---------------------|
|             |                      |             | Cell-associated | Cell-free | Total |                          |
| 0           | 5                    | 24          | 241 (61)        | 279 (66) | 520 (117) |                          |
| 10^a        | 5                    | 24          | 74,000 (28,739) | 83,439 (12,257) | 157,439 (38,125) |                          |

* Infectivity was determined for the clone 81 assay. Cells were harvested 4 days post inoculation.

* Total FeLV infectivity (cell-associated plus cell-free) in HC-treated MØ/total infectivity in control MØ.
that MΦ are central to the mechanism of age-related resistance to viral infection and that the resistance of MΦ to productive viral infection correlates with subsequent progression versus containment of viral infection (10-12, 22). Although the role of MΦ in host resistance to leukemogenic retrovirus infection has received less attention, it has been shown that impairment of MΦ function increases the susceptibility of mice to F- and R-MuLV (36-38), that splenic MΦ are among the earliest cells infected in mice susceptible to Friend virus disease (39), and that MΦ are involved in

response of blood Ie to Con A was suppressed only transiently in silica-treated cats, despite the presence of lymphopenia (text-fig. 4).

Exposure of isolated feline MΦ to silica in vitro produced dose-related degeneration and death of MΦ but not of Ic (tables 9, 10). Neither was the blastogenic response of feline Ic impaired by incubation with concentrations of silica highly toxic to MΦ (table 10).

We concluded from these experiments that silica was an effective MΦ toxin in cats and that the silica enhancement of FeLV susceptibility in cats implicated MΦ in the resistance of cats to FeLV.

DISCUSSION

Investigations of experimental poxvirus, herpesvirus, and coronavirus infections in mice have established

| Table 6.—Effect of silica on the susceptibility of adult cats to FeLV |
|-------------------------|-----------------|--------------------|
| Silica dose (g) | R-FeLV challenge (FFU) | No. of cats | Incidence of persistent viremia (%) |
|----------------|------------------|--------------|-------------------------------|
| 4              | 10^4             | 12           | 92                            |
| 2              | 10^5             | 6            | 17                            |
| 0              | 10^4             | 6            | 0                             |
| 4              | 0                | 6            | 0                             |
| 2              | 0                | 6            | 0                             |

| Table 7.—Correlation between the induction of viremia and FOCMA antibody in adult SPF silica-treated cats |
|---------------------------------------------------------------|
| Cat No. | R-FeLV viremia status | Wk of viremia conversion | Peak FOCMA titer | Wk of peak titer |
|---------|-----------------------|--------------------------|-----------------|----------------|
| 1       | Positive              | 2                        | <4              | ---            |
| 2       | Positive              | 3                        | <4              | ---            |
| 3       | Positive              | 2                        | <4              | ---            |
| 4       | Positive              | 2                        | <4              | ---            |
| 5       | Positive              | 2                        | <4              | ---            |
| 6       | Positive              | 5                        | 16              | 6              |
| 7       | Positive              | 5                        | 32              | 5              |
| 8       | Positive              | 5                        | 64              | 5              |
| 9       | Positive              | 3                        | 64              | 7              |
| 10      | Positive              | 2                        | 128             | 7              |
| 11      | Positive              | 1                        | 128             | 9              |
| Mean value | 2.9                           | 59                       | 6.5             | 8              |

a Cats were treated with 4 g silica 4 days before R-FeLV inoculation.
b Value is reciprocal of highest serum dilution.
c --- no peak occurred.

d.

| Table 5.—FeLV replication in peritoneal MΦ of viremic cats and in peritoneal MΦ from R-FeLV-exposed nonviremic cats inoculated in vitro |
|---------------------------------------------------------------|
| No. of cats | FeLV status of MΦ of cats | FeLV infectivity, mean FFU/10^6 MΦ (SE)^a |
|--------------|---------------------------|----------------------------------|
|               | Cell-associated | Cell-free | Total           |
|               | No HC | 10^-6 M HC | No HC | 10^-6 M HC | No HC | 10^-6 M HC |
| 4            | Viremic cats, in vivo infection of MΦ | 572 (117) | 3,065 (1,172) | 1,282 (507) | 7,075 (1,195) | 8,187 (797) | 13,905 (2,460) |
| 4            | Regressor cats, in vitro infection of MΦ | 41 (9) | 3,065 (1,172) | 43 (19) | 7,538 (1,791) | 84 (23) | 10,632 (2,805) |

a Infectivity was determined in the clone 81 assay. Cells were harvested 5 days post inoculation.

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the regression of Friend virus disease in resistant mice (40-43). In vitro, MØ are relatively resistant to infection by F-MuLV (43) and, similarly, avian MØ resist infection by the A and D-G subgroups of avian leukemia virus (44). We report that, although isolated feline MØ also are relatively inhospitable hosts for FeLV, the age-related susceptibility of cats to FeLV (7) correlates with greater permissiveness of MØ from kittens versus those from adult cats to FeLV infection in vitro. Moreover, agents that impair MØ function in vitro and in vivo, i.e., HC and silica, also impair the FeLV resistance of adult cats. These findings support the tenet that the

**Table 8.** Effect of silica administration on skin allograft rejection times of R-FeLV-inoculated adult cats

| Cat group | No. of cats | Mean rejection time, days (range) |
|-----------|-------------|----------------------------------|
| Silica    | 6           | 25.0 (21-30)                     |
| Control   | 4           | 16.2 (15-17)                     |

* Value is time when graft was judged ≤10% viable by clinical examination, as described by Perryman et al. (35).

**Table 9.** Effect of silica on viability of feline peritoneal MØ

| Silica, mg/ml medium | 1.0 | 0.5 | 0.10 | 0.05 |
|----------------------|-----|-----|------|------|
| Hr after exposure    |     |     |      |      |
|                      |      |     |      |      |
| 0                    | 100 (7) | 100 (6) | 100 (9) | 100 (8) |
| 2                    | 83 (88) | 72 (83) | 65 (74) | 73 (35) |
| 24                   | 34 (84) | 30 (73) | 45 (65) | 38 (38) |
| 48                   | 31 (100)| 23 (100)| 50 (83) | 53 (80) |
| 72                   | 31 (100)| 26 (100)| 35 (95) | 57 (85) |

* Values are percent MØ still attached to glass as compared with non-silica-treated control cultures (percent attached MØ with morphologic evidence of degeneration).

**Table 10.** Effect of silica on viability and blastogenic response of feline le

| No. of cats | Con A blastogenic response: | Viability: |
|-------------|------------------------------|------------|
|             | Silica, mg/ml medium         | Silica, mg/ml medium |
| 0           | 45,682 (6,804)               | 90 (4)     |
| 0.5         | 40,273 (4,325)               | 88 (3)     |

* Mean cpm [H]Thd (SE) is reported after 72-hr culture.

**Text-Figure 4.** Effect of treatment of adult SPF cats with silica (Si) only or silica plus FeLV on blood le blastogenic response to Con A.
autogenous resistance of cats to their leukemogenic retrovirus is M0-dependent.

Cells that appear most important in the early phases of retrovirus infections in vivo are neither those most sensitive to viral infection nor those most readily propagated and studied in vitro. The MuLV replicate extensively in lymphoid cells in vivo; however, cultures of murine lc are relatively resistant to infection in vitro and require mitogenic stimulation to initiate infection of minority populations of cells (45, 46). Feline M0, although relatively refractory to in vitro FeLV infection as compared with sensitive (but more artificial) host cells such as fibroblasts, still are more permissive than are feline lc (21). In the Friend virus system, Marcelletti and Furmanski (43) found that resident and thioglycollate-induced adult mouse peritoneal M0 were highly resistant to viral infection either in vitro or after transfer to the peritoneal cavity of viremic progressors mice. The proportion of M0 capable of entering DNA synthesis in a population greatly influenced permissiveness to F-MuLV infection (41). Addition of M0 colony-stimulating factor was required for the induction of productive infection in 0.08-0.1% of mouse peritoneal M0 (41). In our present studies with thioglycollate-elicited feline M0, no specific growth-stimulating factors were added, yet cell-associated FeLV infectivity was induced in up to 0.1-0.3% of the inoculated M0. In our earlier experiments (47), less favorable culture conditions produced lower indices of M0 infection; however, a greater divergence was observed between virus susceptibility of M0 from kittens versus those from adult cats, which suggests that suboptimal culture conditions are more sensitive in discriminating age-related differences in M0 virus susceptibility.

The magnitude of FeLV infection in peritoneal M0 from viremic cats was only slightly higher than that found after in vitro inoculation of M0 from kittens. This finding was unexpected in view of the extensive FeLV replication that occurs in myeloid progenitor cells of viremic cats (3, 5). Similar observations, however, were reported for M0 from F-MuLV viremic mice and suggest that most mature peritoneal M0 either are derived from uninfected myeloid clones or become refractory to or abort FeLV infection during the process of differentiation in vivo (44). Although our experiments with silica indicate that, as with F-MuLV, M0 are essential to the regression of FeLV infection in cats, peritoneal M0 of regressor cats are not rendered significantly more resistant to FeLV infection than are M0 of naive adult cats.

HC enhancement of M0 FeLV permissiveness was the most striking finding in this study. This phenomenon has not been described previously in retrovirus infections. HC enhanced M0 FeLV susceptibility a hundredfold to a thousandfold and resulted in virus titers in M0 from both kittens and adult cats comparable to those observed in sensitive embryonic cell cultures used to propagate FeLV in vitro (i.e., 1-2 FFU/M0). Moreover, the presence of high-affinity binding sites for glucocorticoids on thioglycollate-elicited peritoneal M0 in mice (48) is consistent with the premise that corticosteroids modulate the FeLV susceptibility of cats principally through corticosteroid action on M0. DNA synthesis in feline M0 was doubled by HC treatment, whereas viral replication increased 250-fold. This finding implies that enhanced FeLV production was related not merely to increased numbers of cells entering the mitotic cycle, but probably also to an increase in number of M0 infected or an increased production of viral progeny by infected M0. Because feline M0 were quite sensitive to HC (concentrations as low as 10^{-4} M being effective), cortisol levels obtainable under physiologic conditions could conceivably alter M0 FeLV susceptibility sufficiently to be responsible for the empirically observed effect (Hoover EA: Unpublished observations) of stress on the FeLV resistance of cats. Studies in mice have shown that both physiologic stress and administration of cortisone produce comparable impairment of M0 tumoricidal capacity (49).

Data concerning corticosteroids and retroviruses in other in vitro cell systems indicate that glucocorticoids exert their most dramatic effects in naturally nonpermissive cell populations and that both induction of viral receptors and induction of viral synthesis in cells nonproductively infected with retrovirus are plausible mechanisms. The epigenetic resistance of chicken M0 to avian leukosis viruses appears to reflect lack of the cellular receptor function responsible for facilitating viral penetration because the adsorption of restricted (A, D, G) and unrestricted (B, C) subgroup viruses to M0 is equivalent (44). Whether the virus envelope-cell receptor interactions responsible for restriction of avian leukosis virus by M0 could be abolished by glucocorticoid treatment of M0 would be of interest. Corticosteroids appear to produce relatively little effect on the replication of ecotropic MuLV in permissive mouse cells (50-52) but to enhance substantially the replication of 1) ecotropic MuLV in nonproductively infected mouse embryo cells (51-54), 2) ecotropic FeLV in nonproductively infected human embryo cells (55), and 3) xenotropic MuLV (53, 54) and FeLV (19) in heterologous cells. Cell transformation by defective murine and feline sarcoma viruses also is enhanced by corticosteroids, possibly through augmented helper leukemia virus replication in target cells (19, 55, 57). Thus available information concerning glucocorticoids and retrovirus expression supports the tenet that HC enhances the FeLV susceptibility of cats by converting the M0-FeLV relationship from a nonpermissive state that leads to early virus containment and initiation of immune response to a permissive state that results in early viral amplification, virus-mediated immunosuppression, persistent viral infection, and leukemogenesis.

Although glucocorticoid receptors on M0 appear abundant (46), information concerning the effect of glucocorticoids on isolated M0 is relatively limited. In contrast to the effects of HC on feline M0, corticosteroid treatment of mouse peritoneal M0 has been reported to inhibit M0 proliferation and protein synthesis (58) and to have variable effects on bacterial
phagocytosis and killing (59, 60). The proliferative capacity for long-term survival, lipogenesis, and sus-
tenance of hematopoiesis by murine bone marrow Mθ, however, is increased by HC (61, 62). Likewise, we have
found that proliferation, survival, lipogenesis, and FeLV susceptibility of feline bone marrow Mθ are
augmented by HC (Hoover EA, Rojko JL: Unpublished data).

The abrogation of FeLV resistance by silica implicates Mθ in the initial lymphoreticular containment of
FeLV in cats. Likewise, Mθ have been implicated in the control of MuLV leukemogenesis. Pretreatment
of mice with silica or carrageenan enhances susceptibility to F-MuLV and R-MuLV infection, respectively (36,
37). Both spontaneous and statolon-induced regression of Friend erythroleukemia is dependent on Mθ func-
tion and inhibited by systemic Mθ toxins (37, 39, 63). Therefore, Mθ can plausibly act in cats both as effector
cells against FeLV-replicating cells and as initiators of the immune response through antigen presentation
and other lc cooperation mechanisms (Rojko JL, Hoover EA, Finn BL, Olsen RE: Unpublished data).

The incidence (55%) and magnitude (mean titer = 1:59) of FOCMA antibody titers that accompanied the
induction of persistent viremia in silica-treated, FeLV-
exposed, viremic cats were more than tenfold greater than those observed in viremic cats in previous
experiments by us and others (1-3, 7, 24, 64, 65). This finding suggests that short-term Mθ removal by silica
facilitated the induction of persistent viremia and that the subsequent adjuvant effect of silica (66) may have
amplified the capacity for FOCMA antibody produc-
tion, which has been shown to be present at least transiently in many viremic cats. In rodents, silica
administration has been shown to enhance antibody response concomitant with regenerative hyperplasia of
the mononuclear phagocytes after silica-induced deple-
tion (66-68). A similar, though less dramatic, pattern in
FOCMA antibody responses occurred in 29% of viremic
adult cats given low doses of methylprednisolone before FeLV inoculation (8). The feasibility exists,
therefore, that either reduction of the silica dose below
that used in our present studies or prolongation of the interval between silica and FeLV administration
would augment rather than impair FeLV resistance. In
this regard, experiments examining the effect of the
administration of Mθ-stimulating substances, such as
glucan or pyran copolymer, to kittens before R-FeLV
challenge are in progress.

In agreement with studies on murine Mθ (29, 69,
70), silica particles were rapidly toxic to isolated feline Mθ, but neither cytotoxic effects nor impairment of
blastogenic responses occurred in cultures of isolated feline lc exposed to concentrations of silica highly
toxic to Mθ (Rojko JL, Hoover EA, Finn BL, Olsen
RG: Unpublished data). Also, in agreement with ob-
servations in mice (27), administration of silica to cats
resulted in moderate prolongation of skin graft survival.

In effecting systemic Mθ depletion in mice, silica
also has been shown to deplete the 89Sr-sensitive
mononuclear cell population associated with genera-
tion of natural killer cells (71) and with the age-related,
thymus-independent resistance of mice to grafts of
normal or malignant hematopoietic cells (13, 14).
Likewise, the mechanism of genetically determined
resistance of mice to F-MuLV has been related to the
89Sr-sensitive marrow cell population (41, 42). If one
considers these observations in mice and that elimina-
tion versus amplification of FeLV-infected cells in cats
occurs in the bone marrow and lymphoid tissues soon
after virus inoculation (5), the role of both marrow
silica-sensitive cells and natural killer cells in the
mechanism of FeLV resistance in cats should be
investigated.

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