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 autochthonous peritoneal feline macrophages (Mθ) 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 Mθ from kittens and adult cats and the effect of hydrocortisone (HC) and silica on Mθ-FeLV interactions. Although the phagocytic functions of isolated Mθ from kittens and adults were equivalent, the mean FeLV susceptibility of Mθ from kittens was five times that of Mθ from adult cats, thus establishing a direct correlation between the age-related susceptibility of cats and Mθ from cats to FeLV. Mθ 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 Mθ. Mθ from cats that had experienced regressive FeLV infection were not significantly more resistant to FeLV infection in vitro than were Mθ 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 Mθ from cats to FeLV in vitro (600-fold for Mθ from adult cats and 200-fold for Mθ) from kittens. Mθ permissiveness to FeLV was highly sensitive to HC and occurred in Mθ 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 Mθ 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 Mθ were linked to the natural resistance of cats to FeLV and that the temporary elimination of Mθ functions (e.g., by silica) and/or the conversion of the Mθ-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 methylnitrosourea (*9*). It is plausible that the interaction of Mθ with FeLV and with lc 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 Mθ 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.

Mθ collection and culture.—Peritoneal Mθ 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% thioglycollate (Difco Laboratories, Detroit, Mich.). Mθ were washed in PBS and resuspended in growth medium consisting of McCoy's 5-A medium containing 40% horse serum, 0.18% $NaHCO_3$, 2% glutamine, 2% essential amino acids, 1% nonessential amino acids, 1% sodium pyruvate, 0.1% gentamicin, and 0.05% Mycostatin. Mθ were counted with a hemacytometer and stained with 0.05% new methylene blue.

Mθ function assays.—Assays of phagocytic function were performed in glass slide-mounted, 8-well chambers (Lab-Tek Products, Naperville, Ill.) 24 hours after Mθ were seeded; each chamber contained 5×10^5 Mθ (0.5 ml of 10^6 Mθ/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; FFU = focus-forming units; FOCMA = feline oncovirus-associated cell membrane antigen; GSA = group-specific antigen; HC = hydrocortisone; [^{3}H]dThd = tritiated thymidine; lc = lymphocyte(s); Mθ = 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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dilution of 1:10,000. A 0.2% suspension of sensitized, washed SRBC (10^7 cells/ml) in Mθ growth medium was incubated at 100 SRBC:1 Mθ 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 Mθ forming EA rosettes (at least 3 SRBC/Mθ), the number of Mθ phagocytizing EA, and the number of phagocytized EA per Mθ 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*/Mθ were incubated in Mθ 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 Mθ 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 Mθ culture by assaying separately media and freeze-thaw lysates of Mθ collected by scraping adherent cells from the wells with a rubber policeman. The clone 81 indicator cells were inoculated with 1.0 ml of Mθ medium or with 10^6 Mθ. Cell-associated or cell-free VI was expressed per 10^5 Mθ recovered from the cultures at the time of harvest.

FeLV inoculation of Mθ.—All experiments involving FeLV infection of Mθ were done in 25-cm^2 flasks (Falcon Plastics, Oxnard, Calif.) in which 3.2×10^6 Mθ were seeded in 4 ml of Mθ medium. Polybrene (4 μg/ml) was incorporated into Mθ growth medium when the cells were seeded. Then 25-cm^2 flask cultures, 24 or 48 hours old, of feline peritoneal Mθ (3.2×10^6 Mθ 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 Mθ 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 Mθ-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 Mθ.—HC sodium succinate (Abbott Laboratories, North Chicago, Ill.), 10^{-4}, 10^{-6}, and 10^{-8} *M*, was added to the Mθ medium when the cultures were initiated.

[^{3}H]dThd labeling of Mθ.—Cultures of peritoneal Mθ with and without HC were initiated in microtiter wells at a cell density of 10^5 Mθ/well. At the time of seeding, Mθ were labeled with 0.5 μCi of [^{3}H]dThd (6.7 μCi/mmol; New England Nuclear Corp., Boston, Mass.) per 10^5 Mθ, and cell-associated ^{3}H 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 (Minusil; 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 Mθ 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 Mθ cultured in 8-well, glass slide chambers to various concentrations of silica added to the Mθ medium 24 hours after the cultures were established. We evaluated subsequent morphologic changes by staining and microscopically examining Mθ at 24, 48, and 72 hours after silica exposure.

RESULTS

Phagocytic function of peritoneal Mθ 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 (*8*) 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 eightyfold (text-fig. 1). HC treatment increased Mθ DNA synthesis twofold, as measured by [^{3}H]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*

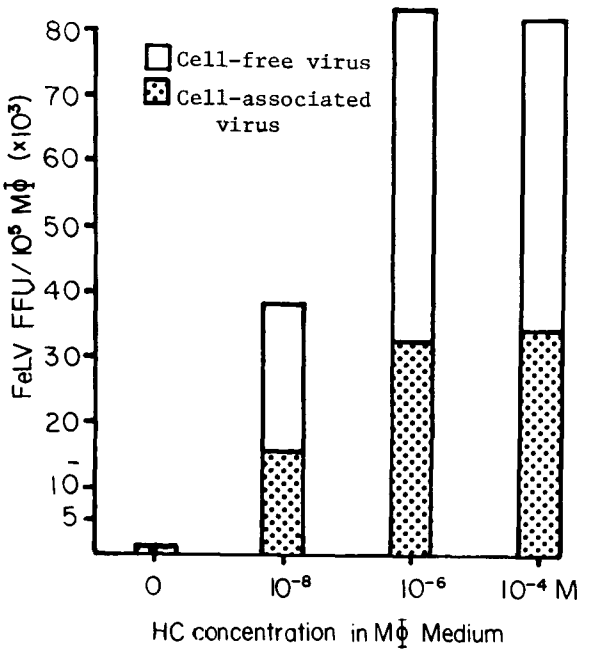
Age of cat, mo	No. of cats	Phagocytosis[a]			Fc receptor, EA rosettes (%)
		EA	*C. pseudotropicalis*	Latex	
2	4	46 (4.3)	75 (6.5)	84 (13)	56 (24)
6	4	32 (6.7)	52 (7)	82 (11)	80 (15)

[a] 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	FeLV infectivity, mean FFU/10^5 Mθ (SE)[a]		
		Cell-associated	Cell-free	Total
2	19	301 (92)	814 (517)	1,140 (530)
9	18	109 (49)	92 (41)	202 (88)

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



TEXT-FIGURE 1.—HC-induced dose-related enhancement of permissiveness of feline Mθ to FeLV infection.

TABLE 3.—*Effect of HC on the permissiveness of Mθ from adult cats and kittens to R-FeLV infection in vitro*

HC, *M*	Mean age of cats, mo	No. of cats	FeLV infectivity, FFU/10^5 Mθ (SE)[a]			Enhancement index[b]
			Cell-associated	Cell-free	Total	
0	2	12	324 (78)	417 (104)	721 (173)	184
10^{-6}	2	12	46,184 (12,811)	85,666 (19,011)	132,742 (35,415)	
0	9	12	159 (69)	141 (57)	330 (127)	534
10^{-6}	9	12	104,133 (55,558)	81,212 (16,239)	176,346 (65,568)	

[a] Infectivity was determined for the clone 81 assay. Cells were harvested 4 days post inoculation.
[b] Total FeLV infectivity (cell-associated plus cell-free) in HC-treated Mθ/total infectivity in control Mθ.

cells) with that obtained after in vitro FeLV inoculation of Mθ of normal cats, 2) to determine whether Mθ 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 Mθ from either group of cats could be augmented by HC treatment in vitro. Accordingly, thioglycollate-induced peritoneal Mθ 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 Mθ 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 Mθ were inoculated with FeLV in vitro with and without HC as in previous experiments.

Mθ from viremic cats were found to be infected in vivo and produced infectivity titers similar to ($P>0.10$) those produced by Mθ from kittens infected with R-FeLV in vitro (table 5). The susceptibility of Mθ from FeLV-exposed nonviremic adult cats to FeLV infection in vitro was similar to that observed previously for Mθ 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 Mθ derived from viremic cats approximated that obtained after in vitro infection and that previous self-limiting FeLV infection did not render Mθ resistant to reinfection.

Effect of systemic Mθ 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 Mθ, we investigated whether temporary elimination of Mθ 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 (*7*), 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.

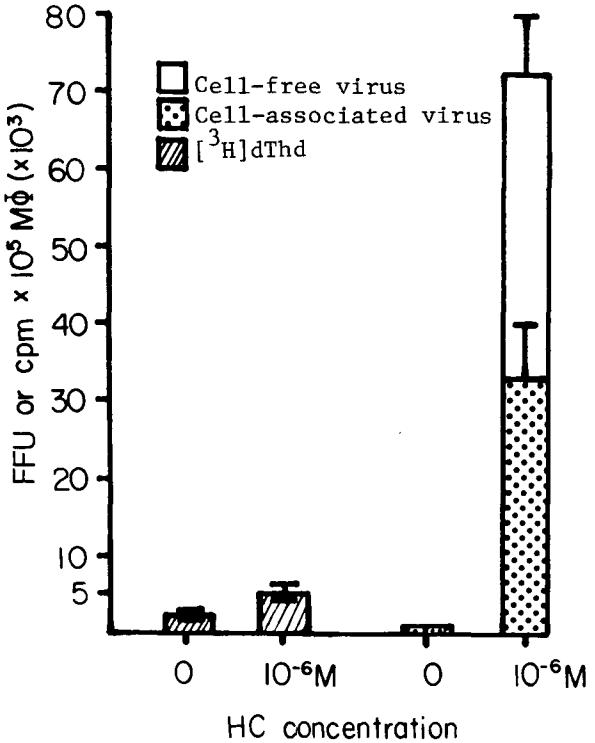
Silica treatment of cats produced monocytopenia, transient lymphopenia, and neutrophilia that were independent of R-FeLV inoculation. In silica-treated FeLV-exposed viremic cats, lymphopenia persisted and neutropenia developed by 4 weeks when all of the cats had become viremic (text-fig. 3). [Lymphopenia 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

TABLE 4.—*HC enhancement of the susceptibility of feline Mθ to productive R-FeLV infection*

HC, *M*	Mean age of cats, mo	No. of cats	FeLV infectivity, mean FFU/10^5 Mθ (SE)[a]		
			Cell-associated	Cell-free	Total
0	5	24	241 (61)	279 (66)	520 (117)
10^{-6}	5	24	74,000 (28,739)	83,439 (12,257)	157,439 (38,125)
Enhancement index[b]			307	299	303

[a] Infectivity was determined for the clone 81 assay. Cells were harvested 4 days post inoculation.
[b] Total FeLV infectivity (cell-associated plus cell-free) in HC-treated Mθ/total infectivity in control Mθ.



TEXT-FIGURE 2.—Comparison of the degree of enhancement of DNA synthesis vs. R-FeLV replication in feline Mθ treated with HC.

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

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

We concluded from these experiments that silica was an effective Mθ toxin in cats and that the silica enhancement of FeLV susceptibility in cats implicated Mθ 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 ip challenge, FFU	No. of cats	Incidence of persistent viremia, %
4	10^5	12	92
2	10^5	6	17
0	10^5	6	0
4	0	6	0
2	0	6	0

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

TABLE 7.—*Correlation between the induction of viremia and FOCMA antibody in adult SPF silica-treated cats*[a]

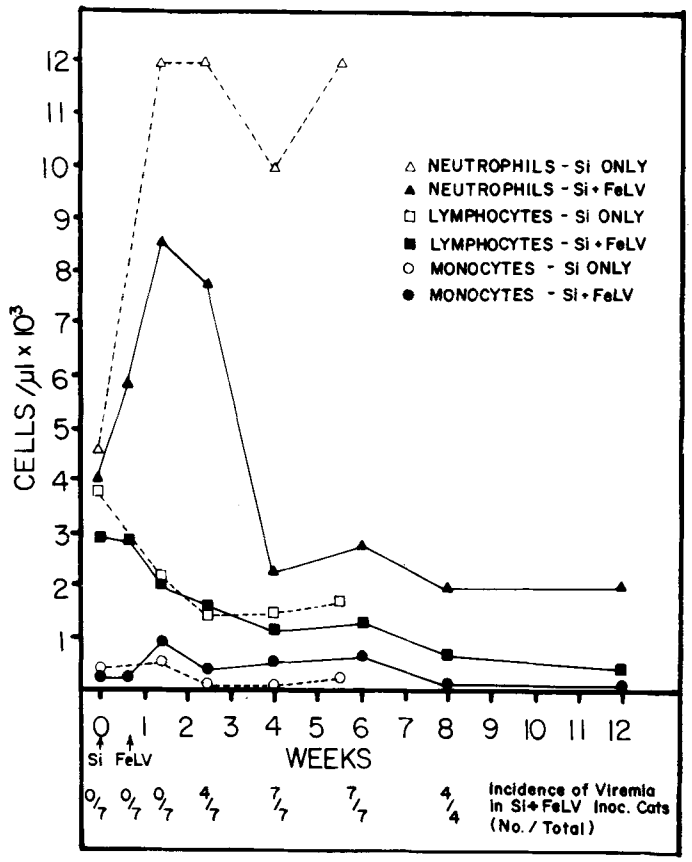
Cat No.	R-FeLV viremia status	Wk of viremia conversion	Peak FOCMA titer[b]	Wk of peak titer[c]
1	Positive	2	<4	—
2	Positive	3	<4	—
3	Positive	2	<4	—
4	Positive	2	<4	—
5	Positive	2	<4	—
6	Positive	5	16	6
7	Positive	5	32	5
8	Positive	5	64	5
9	Positive	3	64	7
10	Positive	2	128	7
11	Positive	1	128	9
Mean value		2.9	59	6.5
12	Negative		256	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.

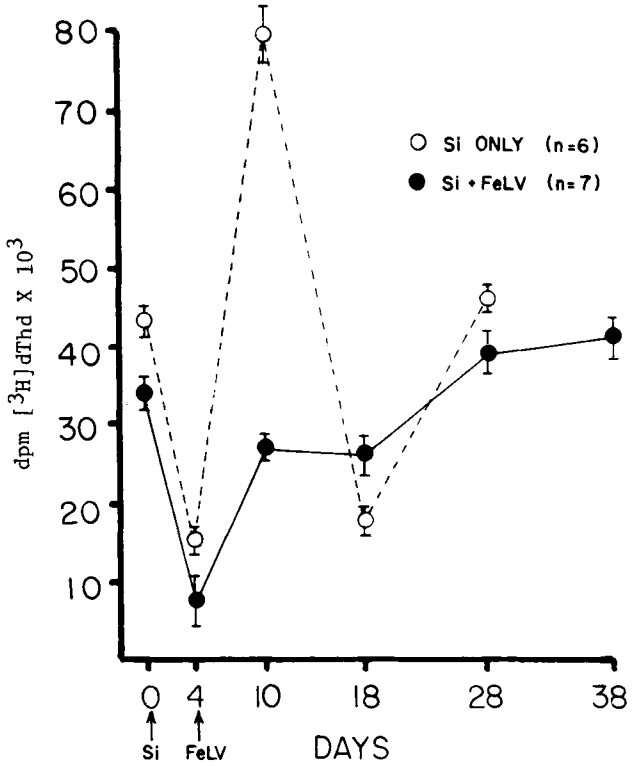
TABLE 5.—*FeLV replication in peritoneal Mθ of viremic cats and in peritoneal Mθ from R-FeLV-exposed nonviremic cats inoculated in vitro*

No. of cats	FeLV status of Mθ of cats	FeLV infectivity, mean FFU/10^5 Mθ (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 Mθ	572 (117)	7,075 (1,195)	1,282 (507)	8,187 (797)	1,885 (525)	13,905 (2,460)
4	Regressor cats, in vitro infection of Mθ	41 (9)	3,095 (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.



TEXT-FIGURE 3.—Effects of treatment of adult SPF cats with silica (Si) alone or silica plus FeLV on blood leukocytes.



TEXT-FIGURE 4.—Effect of treatment of adult SPF cats with silica (Si) only or silica plus FeLV on blood lc blastogenic response to Con A.

the regression of Friend virus disease in resistant mice (*40–43*). In vitro, Mθ are relatively resistant to infection by F-MuLV (*43*) and, similarly, avian Mθ resist infection by the A and D-G subgroups of avian leukosis virus (*44*). We report that, although isolated feline Mθ also are relatively inhospitable hosts for FeLV, the age-related susceptibility of cats to FeLV (*7*) correlates with greater permissiveness of Mθ from kittens versus those from adult cats to FeLV infection in vitro. Moreover, agents that impair Mθ 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)[a]
Silica[b]	6	25.0 (21–30)[c]
Control[d]	4	16.2 (15–17)

[a] Value is time when graft was judged ≤10% viable by clinical examination, as described by Perryman et al. (*34*).
[b] Dose was 2 g ip, 4 days before skin was grafted.
[c] $P<0.01$, paired t-statistic.
[d] Grafts were from same donor cat; control data were previously reported by Tarr et al. (*35*).

TABLE 9.—*Effect of silica on viability of feline peritoneal Mθ*

Hr after exposure	Silica, mg/ml medium[a]			
	1.0, attached (degenerated)	0.5, attached (degenerated)	0.10, attached (degenerated)	0.05, attached (degenerated)
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)

[a] Values are percent Mθ still attached to glass as compared with non-silica-treated control cultures (percent attached Mθ with morphologic evidence of degeneration).

TABLE 10.—*Effect of silica on viability and blastogenic response of feline lc*

No. of cats	Con A blastogenic response:[a] Silica, mg/ml medium		Viability:[b] Silica, mg/ml medium	
	0	0.5	0	0.05
4	45,682 (6,804)	40,273 (4,325)	90 (4)	88 (3)

[a] Mean cpm [³H]dThd (SE) is reported after 72-hr culture.
[b] Percent of cells excluding trypan blue (SE) is reported after 72-hr culture.

autogenous resistance of cats to their leukemogenic retrovirus is Mθ-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 Mθ, 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 Mθ were highly resistant to viral infection either in vitro or after transfer to the peritoneal cavity of viremic progressor mice. The proportion of Mθ capable of entering DNA synthesis in a population greatly influenced permissiveness to F-MuLV infection (*41*). Addition of Mθ colony-stimulating factor was required for the induction of productive infection in 0.03–0.1% of mouse peritoneal Mθ (*41*). In our present studies with thioglycollate-elicited feline Mθ, no specific growth-stimulating factors were added, yet cell-associated FeLV infectivity was induced in up to 0.1–0.3% of the inoculated Mθ. In our earlier experiments (*47*), less favorable culture conditions produced lower indices of Mθ infection; however, a greater divergence was observed between virus susceptibility of Mθ from kittens versus those from adult cats, which suggests that suboptimal culture conditions are more sensitive in discriminating age-related differences in Mθ viral susceptibility.

The magnitude of FeLV infection in peritoneal Mθ from viremic cats was only slightly higher than that found after in vitro inoculation of Mθ 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 Mθ from F-MuLV viremic mice and suggest that most mature peritoneal Mθ 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, Mθ are essential to the regression of FeLV infection in cats, peritoneal Mθ of regressor cats are not rendered significantly more resistant to FeLV infection than are Mθ of naive adult cats.

HC enhancement of Mθ FeLV permissiveness was the most striking finding in this study. This phenomenon has not been described previously in retrovirus infections. HC enhanced Mθ FeLV susceptibility a hundredfold to a thousandfold and resulted in virus titers in Mθ 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/Mθ). Moreover, the presence of high-affinity binding sites for glucocorticoids on thioglycollate-elicited peritoneal Mθ in mice (*48*) is consistent with the premise that corticosteroids modulate the FeLV susceptibility of cats principally through corticosteroid action on Mθ. DNA synthesis in feline Mθ 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 Mθ infected or an increased production of viral progeny by infected Mθ. Because feline Mθ were quite sensitive to HC (concentrations as low as 10^{-8} *M* being effective), cortisol levels obtainable under physiologic conditions could conceivably alter Mθ 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 Mθ 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 Mθ 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 Mθ is equivalent (*44*). Whether the virus envelope-cell receptor interactions responsible for restriction of avian leukosis virus by Mθ could be abolished by glucocorticoid treatment of Mθ 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 Mθ-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 Mθ appear abundant (*46*), information concerning the effect of glucocorticoids on isolated Mθ is relatively limited. In contrast to the effects of HC on feline Mθ, corticosteroid treatment of mouse peritoneal Mθ has been reported to inhibit Mθ 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 sustenance 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 function 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 Ic 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 production, 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 depletion (*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 observations 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 ^{89}Sr-sensitive mononuclear cell population associated with generation 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 ^{89}Sr-sensitive marrow cell population (*72*) and to the silica-sensitive systemic Mθ population (*41, 42*). If one considers these observations in mice and that elimination 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.

REFERENCES

(*1*) Hardy WD Jr, Old LJ, Hess M, Cotter S. Horizontal transmission of feline leukaemia virus. Nature 1973; 244:266-269.

(*2*) Jarrett W, Jarrett O, Mackey L, Laird H, Hardy W Jr, Essex M. Horizontal transmission of leukemia virus and leukemia in the cat. J Natl Cancer Inst 1973; 51:833-841.

(*3*) Hoover EA, Olsen RG, Mathes LE, Schaller JP. Relationship between feline leukemia virus antigen expression and viral infectivity in blood, bone marrow, and saliva of cats. Cancer Res 1977; 37:3707-3710.

(*4*) Francis DP, Essex M, Hardy WD Jr. Excretion of feline leukaemia virus by naturally infected pet cats. Nature 1977; 269:252-254.

(*5*) Rojko JL, Hoover EA, Mathes LE, Olsen RG, Schaller JP. Pathogenesis of experimental feline leukemia virus infection. JNCI 1979; 63:759-768.

(*6*) Hoover EA, Rojko JL, Olsen RG. Host-virus interactions in progressive vs. regressive feline leukemia virus infection in cats. In: Essex M, Todaro G, Zur Hausen K, eds. Viruses in naturally occurring cancer. Cold Spring Harbor conference on cell proliferation. Vol 7. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory, 1980:635-651.

(*7*) Hoover EA, Olsen RG, Hardy WD Jr, Schaller JP, Mathes LE. Feline leukemia virus infection: Age-related variation in response of cats to experimental infection. J Natl Cancer Inst 1976; 57:365-369.

(*8*) Rojko JL, Hoover EA, Mathes LE, Krakowka S, Olsen RG. Influence of adrenal corticosteroids on the susceptibility of cats to feline leukemia virus infection. Cancer Res 1979; 39:3789-3791.

(*9*) Schaller JP, Mathes LE, Hoover EA, Koestner A, Olsen RG. Increased susceptibility to feline leukemia virus infection in cats exposed to methylnitrosourea. Cancer Res 1978; 38: 996-998.

(*10*) Mims CA. Aspects of the pathogenesis of virus diseases. Bacteriol Rev 1964; 28:36-71.

(*11*) Allison AC. On the role of mononuclear phagocytes in immunity against viruses. Prog Med Virol 1974; 18:15-31.

(*12*) Mogensen SC. Role of macrophages in natural resistance to virus infections. Microbiol Rev 1979; 43:1-26.

(*13*) Bennett M. Prevention of marrow allograft rejection with radioactive strontium: Evidence for marrow-dependent effector cells. J Immunol 1973; 110:510-516.

(*14*) Kiessling R, Hochman PS, Haller O, Shearer GM, Wigzell H, Cudkowicz G. Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. Eur J Immunol 1977; 7:655-663.

(*15*) Bennett M, Baker EE, Eastcott JW, Kumar V, Yonkosky D. Selective elimination of marrow precursors with the bone-seeking isotope ^{89}Sr: Implications for hemopoiesis, lymphopoiesis, viral leukemogenesis and infection. J Reticuloendothel Soc 1976; 20:71-87.

(*16*) Rohovsky MW, Griesemer RA, Wolfe LG. The germfree cat. Lab Anim Care 1966; 16:52-59.

(*17*) Cohen AB, Cline MJ. The human alveolar macrophage: Iso-

lation, cultivation in vitro, and studies of morphologic and functional characteristics. J Clin Invest 1971; 50:1390-1397.
(18) RICKARD CG, POST JE, NORONHA F, BARR LM. A transmissible virus-induced lymphocytic leukemia of the cat. J Natl Cancer Inst 1969; 42:987-1014.
(19) FISCHINGER PJ, BLEVINS CS, NOMURA S. Simple quantitative assay for both xenotropic murine and ecotropic feline leukemia viruses. J Virol 1974; 14:177-179.
(20) CRANDELL RA, FABRICANT CG, NELSON-REES WA. Development, characterization, and viral susceptibility of a feline *(Felis catus)* renal cell line (CRFK). In Vitro 1973; 9:176-185.
(21) ROJKO JL, HOOVER EA, FINN BL, OLSEN RG. Determinants of susceptibility and resistance to feline leukemia virus infection. II. Susceptibility of feline lymphocytes to productive feline leukemia virus infection. JNCI 1981; 67:899-910.
(22) SCHALLER JP, OLSEN RG. Determination of subgroup-specific feline oncornavirus neutralizing antibody. Infect Immun 1975; 12:1405-1410.
(23) HARDY WD JR, HIRSHAUT Y, HESS PW, et al. Detection of the feline leukemia virus and other mammalian oncornaviruses by immunofluorescence. In: Dutcher RM, Chieco-Bianchi L, eds. Unifying concepts of leukemia. Basel and White Plains, N.Y.: Karger, 1973:778-779.
(24) ESSEX M, KLEIN G, SNYDER SP, HARROLD JB. Antibody to feline oncornavirus-associated cell membrane antigen in neonatal cats. Int J Cancer 1971; 8:384-390.
(25) HOOVER EA, MATHES LE, ROJKO JL, SCHALLER JP, OLSEN RG. Modifications of the immunofluorescence assay for feline leukemia virus group-specific antigens. Am J Vet Res 1978; 39:1877-1880.
(26) ALLISON AC, HARINGTON JS, BIRBECK M. An examination of the cytotoxic effects of silica on macrophages. J Exp Med 1966; 124:141-153.
(27) PEARSALL NN, WEISER RS. The macrophage in allograft immunity. I. Effects of silica as a specific macrophage toxin. J Reticuloendothel Soc 1968; 5:107-120.
(28) ZISMAN B, HIRSCH MS, ALLISON AC. Selective effects of anti-macrophage serum, silica, and anti-lymphocyte serum on pathogenesis of herpes virus infection of young adult mice. J Immunol 1970; 104:1155-1159.
(29) DUBUY H. Effect of silica on virus infections in mice and mouse tissue culture. Infect Immun 1975; 11:996-1002.
(30) MOGESEN SC, ANDERSEN HK. Effect of silica on the pathogenic distinction between herpes simplex viruses type 1 and 2 hepatitis in mice. Infect Immun 1977; 17:274-277.
(31) SCHLABACH AJ, MARTINEZ D, FIELD AK, TYTELL AA. Resistance of C58 mice to primary systemic herpes simplex virus infection: Macrophage dependence and T-cell independence. Infect Immun 1979; 26:615-620.
(32) HIRSCH MS, ZISMAN B, ALLISON AC. Macrophages and age-dependent resistance to herpes simplex virus in mice. J Immunol 1970; 104:1160-1165.
(33) MOGESEN SC: Macrophages and age-dependent resistance to hepatitis induced by herpes simplex virus type 2 in mice. Infect Immun 1978; 19:46-50.
(34) PERRYMAN LE, HOOVER EA, YOHN DS. Immunologic reactivity of the cat: Immunosuppression in experimental feline leukemia. J Natl Cancer Inst 1972; 49:1357-1365.
(35) TARR MJ, OLSEN RG, HOOVER EA, KOABA GJ, SCHALLER JP. The effects of methylnitrosourea on the immune system and hematopoietic system of adult specific pathogen free cats. Chem Biol Interact 1979; 28:181-199.
(36) TONIOLO A, MATTEUCCI D, PISTILLO MP, GORI Z, BENDINELLI M. Early replication of Friend leukaemia viruses in spleen macrophages. J Gen Virol 1980; 49:203.
(37) LARSON CL, USHIJIMA RN, BAKER RE, BAKER MB, GILLIESPIE CA. Effect of normal serum and antithymocyte serum on Friend disease in mice. J Natl Cancer Inst 1972; 48:1403-1407.
(38) WIRTH JJ, LEVY MH, WHEELOCK EF. Use of silica to identify host mechanisms involved in suppression of established Friend virus leukemia. J Immunol 1976; 117:2124-2130.
(39) KNYSZYNSKI A, DANON D. The role of macrophages in defense against the development of Rauscher virus leukemia. J Reticuloendothel Soc 1977; 22:341-348.
(40) LEVY MH, WHEELOCK EF. Impaired macrophage function in Friend virus leukemia: Restoration by statolon. J Immunol 1975; 114:962-965.
(41) ———. The role of macrophages in suppression of established Friend virus leukemia. J Reticuloendothel Soc 1976; 20:243.
(42) MARCELLETTI J, FURMANSKI P. Spontaneous regression of Friend virus-induced erythroleukemia. III. The role of macrophages in regression. J Immunol 1978; 120:1-8.
(43) ———. Infection of macrophages with Friend virus: Relationship to the spontaneous regression of viral erythroleukemia. Cell 1979; 16:649-659.
(44) GAZZOLO L, MOSCOVICI MG, MOSCOVICI C, VOGT PK. Susceptibility and resistance of chicken macrophages to avian RNA tumor viruses. Virology 1975; 67:553-565.
(45) CERNY J, HENSGEN PA, EISTEL SH, DEMLER LM. Interactions of murine leukemia virus (MuLV) with isolated lymphocytes. II. Infection of B and T cells with Friend virus complex in diffusion chambers and in vitro: Effect of polyclonal mitogens. Int J Cancer 1976; 18:189-196.
(46) CERNY J, ISAAK DD. Interactions of murine leukemia virus (MuLV) with isolated lymphocytes. IV. The role of mitogen-induced cellular DNA synthesis in virus infection and replication. Int J Cancer 1979; 23:260-268.
(47) HOOVER EA, ROJKO JL, OLSEN RG. Experimental modulation of the natural resistance of cats to feline leukemia virus. In: Yohn DS, Lapin BF, Blakeslee J, eds. Advances in comparative leukemia research 1979. New York: Elsevier, 1980:283-284.
(48) WERB Z, FOLEY R, MUNCK A. Interaction of glucocorticoids with macrophages. Identification of glucocorticoid receptors in monocytes and macrophages. J Exp Med 1978; 147:1684-1694.
(49) SCHULTZ RM, CHIRIGOS MA, STOYCHKOV JN, PAVLIDIUS NA. Factors affecting macrophage cytotoxic activity with particular emphasis on corticosteroids and acute stress. J Reticuloendothel Soc 1979; 26:83-92.
(50) PARAN M, GALLO RC, RICHARDSON LS. Adrenal corticosteroids enhance production of type-C virus induced by 5-iodo-2'-deoxyuridine from cultured mouse fibroblasts. Proc Natl Acad Sci USA 1973; 70:2391-2395.
(51) IHLE JN, LANE SE, KENNEY FT, FARRELLY JG. Effect of glucocorticoids on activation of leukemia virus in AKR mouse embryo cells. Cancer Res 1975; 35:442-446.
(52) VARNIER OE, LEVY JA. Differential effect of dexamethasone on replication of ecotropic and xenotropic mouse type C viruses. Virology 1979; 96:604-614.
(53) WU AM, REITZ MS, PARAN M, GALLO RC. Mechanism of stimulation of murine type-C RNA tumor virus production by glucocorticoids: Post-transcriptional effects. J Virol 1974; 14: 802-812.
(54) DUNN CD, AARONSON SA, STEPHENSON JR. Interactions of chemical inducers and steroid enhancers on endogenous mouse type-C RNA viruses. Virology 1975; 66:579-588.
(55) BLAKESLEE J, ELLIOT A, TURNER D. Induction of retrovirus non-producer human cells to producer cells by dexamethasone. In: Yohn DS, Lapin B, Blakeslee JR, eds. Advances in comparative leukemia research 1979. New York: Elsevier, 1980:87-88.
(56) LOWY DR, SCOLNICK EM. Glucocorticoids induce focus formation and increase sarcoma viral expression in a mink cell line that contains a murine sarcoma viral genome. J Virol 1978; 25:157-163.
(57) SCHALLER JP, MILO GE, BLAKESLEE JR JR, OLSEN RG, YOHN DS. Influence of glucocorticoid, estrogen, and androgen hormones on transformation of human cells in vitro by feline sarcoma virus. Cancer Res 1976; 36:1980-1987.
(58) MORTON JM, MUNCK A. In vitro actions of glucocorticoids on murine macrophages: Effects on glucose transport and metabolism, growth in culture, and protein synthesis. J Immunol 1980; 125:259-266.
(59) VAN ZWET TL, THOMPSON J, VAN FURTH R. Effect of glucocorticoids on the phagocytosis and intracellular killing by peritoneal macrophages. Infect Immun 1975; 12:699-705.
(60) WIENER E, MARMARY Y, CURELARW Z. The in vitro effect of

hydrocortisone on the uptake and intracellular digestion of particulate matter by macrophages in culture. Lab Invest 1972; 26:220-226.

(61) GREENBERGER JS. Sensitivity of corticosteroid-dependent insulin-resistant lipogenesis in marrow preadipocytes of obese-diabetic (*db/db*) mice. Nature 1978; 275:752-754.

(62) ELLIASON JF, TESTA NG, DEXTER TM. Erythropoietin-stimulated erythropoiesis in long-term bone marrow culture. Nature 1979; 281:382-384.

(63) CEGLOWSKI WS, FRIEDMAN H. Failure of peritoneal exudate macrophages to reverse immunologic impairment by Friend leukemia virus. Proc Soc Exp Biol Med 1975; 148:808.

(64) ESSEX M, COTTER SM, HARDY WD JR, et al. Feline oncornavirus-associated cell membrane antigen. IV. Antibody titers in cats with naturally occurring leukemia, lymphoma, and other diseases. J Natl Cancer Inst 1975; 55:463-467.

(65) OLSEN RG, HOOVER EA, MATHES LE, HEDING L, SCHALLER JP. Immunization against feline oncornavirus disease using a killed tumor cell vaccine. Cancer Res 1976; 36:3642-3646.

(66) PERNIS B, PARONETTO F. Adjuvant effect of silica (tridymite) on antibody production. Proc Soc Exp Biol Med 1962; 110:390-392.

(67) WILKINSON PC, WHITE RG. The role of Mycobacteria and silica in the immunological response of the guinea pig. Immunology 1966; 11:229-241.

(68) LEVY MH, WHEELOCK EF. Effects of intravenous silica on immune and non-immune functions of the murine host. J Immunol 1975; 115:41-48.

(69) KESSEL RW, MONACO L, MARCHISIO MA. The specificity of the cytotoxic action of silica: A study in vitro. Br J Exp Pathol 1962; 44:351-364.

(70) AHO S, PELTONEN J, JALKANEN M, KULONEN E. Effect of silica on culture of rat peritoneal macrophages. Ann Occup Hyg 1979; 22:285-296.

(71) KIESSLING R, KLEIN G, WIGZELL H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cell. Specificity and distribution according to genotype. Eur J Immunol 1975; 5:112.

(72) KUMAR V, BENNETT M, ECKNER RJ. Mechanism of genetic resistance to Friend leukemia virus in mice. I. Role of ^{89}Sr sensitive effector cells responsible for rejection of bone marrow allografts. J Exp Med 1974; 139:1093.