after administration of ethanol compared to controls. These observations suggest alterations in sensitivity of the central nervous system to ethanol, perhaps as a result of residual brain dysfunction from past thiamine deficiency.

Characteristic neuropathological findings of Wernicke-Korsakoff syndrome were found in 1 to 2 percent of all human brain specimens at autopsy in recent general hospital surveys; most cases occurred in alcoholics, only a small proportion of whom had been diagnosed during life (*5, 16*). Randomly selected alcoholics showed microscopic and biochemical evidence of demyelinization in the mammillary bodies, a brain region known to be damaged in Wernicke-Korsakoff syndrome (*17*). Certain memory abnormalities and other neuropsychologic deficits of chronic alcoholics are qualitatively similar to those of Korsakoff patients (*6, 18*). Behavioral manifestations and brain neuropathologic changes similar to those in Wernicke-Korsakoff syndrome have been reported in normally nourished rats exposed to ethanol over long periods as well as in thiamine-deficient animals not exposed to ethanol (*4*). Therefore, in the animal model, long-term consumption of ethanol, even with an adequate diet, may result in functional thiamine deficiency sufficient to cause damage to the central nervous system. These findings should only be extrapolated to humans with caution. However, it is possible that reported abnormalities in neurotransmitter function of the central nervous system in alcoholism and related psychopathological syndromes (*19*) as well as protracted withdrawal signs in abstinent alcoholics (*20*) may be related to enduring brain dysfunction sustained during cumulative subclinical episodes of thiamine deficiency throughout a course of extended alcohol abuse (*16*).

PETER R. MARTIN*
Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland 20205
EDWARD MAJCHROWICZ
EWA TAMBORSKA
CHERYL MARIETTA
Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism
ANIL B. MUKHERJEE
Human Genetics Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20205
MICHAEL J. ECKARDT
Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism

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* To whom requests for reprints should be addressed at the National Institutes of Health, Building 10, Room 3B-19, 9000 Rockville Pike, Bethesda, Md. 20205.

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Suppression of Gamma Interferon Production by Inactivated Feline Leukemia Virus

Abstract. *Supernatants from cultures of normal feline lymphocytes stimulated with* Staphylococcus *enterotoxin A showed antiviral activity, characterized as a gamma-like interferon. With the addition of inactivated feline leukemia virus, markedly less interferon was produced. The reduction in interferon production was not attributable to lowered lymphocyte viability or reduced mitogenic properties of* Staphylococcus *enterotoxin A and appears to be a direct retroviral effect. This finding may reflect clinically relevant events that may contribute to the development of the feline or human states of acquired immunodeficiency.*

Feline leukemia virus (FeLV), a contagious retrovirus transmitted primarily through salivary secretions, causes neoplastic or nonneoplastic diseases in the infected cat, including a state of immunodeficiency characterized by diminution of cellular and humoral immunity. Retrovirus-induced immunosuppression, the most frequent sequela of persistent FeLV viremia, predisposes the animal to secondary illness of infectious or autoimmune origin and accounts for most FeLV-related deaths (*1*). Viremic cats have suppressed blastogenic responses to T-cell mitogens, reduced mobility of lymphocyte membrane concanavalin A (Con A) receptors, suppressed antibody responses to synthetic polypeptides, prolonged allograft rejection times, and various degrees of hypocomplementemia, thymic atrophy, and depletion of the paracortical zones of lymph nodes (*2*). In addition, peripheral blood and splenic lymphocytes from FeLV-infected cats, when stimulated with T-cell mitogens, cannot be induced to produce interferon, or when induced produce only low titers thereof (*3*). Although little

Table 1. Titer of interferon-generated (units per milliliter) and viability of peripheral blood lymphocytes after 72 hours of culture. N.D., not done.

Cat	Number of experiments	No mitogen	SEA (1.0 μ/ml)						
				UV-inactivated FeLV (μg/ml)					
				625	400	300	200	100	50	10
301	2	<10	214	N.D.	19	52	61	134	150	196
249	3	<10	168	N.D.	39	67	124	150	141	378
1225	2	<10	47	<10	<10	<10	17	N.D.	N.D.	25
1264	2	<10	200	N.D.	39	43	99	112	83	N.D.
302	2	<10	289	N.D.	35	125	44	119	150	130
248	4	367	768	N.D.	409	89	734	980	875	815
000	2	<10	287	<10	N.D.	N.D.	160	367	N.D.	N.D.
Viability after culture										
Samples tested		15	28	N.D.	14	11	13	11	6	8
Viability (percent*)		90 ± 10	93 ± 4	N.D.	86 ± 11	90 ± 6	89 ± 6	92 ± 7	95 ± 2	96 ± 2

*Mean ± standard error.

is known about how FeLV initiates immunosuppression, the virus may mediate immunomodulating events independent of cellular infection, since FeLV inactivated by ultraviolet light and certain FeLV structural proteins have been shown to impair lymphocyte proliferative responses and membrane receptor capping to Con A in vitro (*4*). This inability of lymphocytes to respond to mitogenic stimuli during incubation with inactivated virus may be due to decreased elaboration of T-cell growth factor (possibly interleukin-2) activity (*5*). We report here that the synthesis in vitro of a gamma-like interferon by normal feline lymphocytes that have been stimulated with *Staphylococcus* enterotoxin A (SEA) is reduced when ultraviolet-inactivated FeLV is also present in culture during the period of stimulation.

Seven healthy, FeLV-free domestic cats maintained in our laboratory served as donors of peripheral blood lymphocytes. The cells were isolated from defibrinated whole blood by Ficoll-Hypaque density centrifugation. Cell viabilities were determined by vital dye exclusion. Lymphocytes (5×10^6 per milliliter) in RMPI 1640 medium supplemented with 10 percent fetal calf serum, antibiotics, antimycotics (Gibco), and $5 \times 10^{-5} M$ 2-mercaptoethanol (Eastman Kodak) were placed in flat-bottom microplate wells (16 mm in diameter). The cells were cultured in the absence of mitogen, in the presence of SEA (1.0 μg/ml; Microbial Biochemistry Branch, Division of Microbiology, Food and Drug Administration, Cincinnati), or with SEA (1.0 μg/ml) plus ultraviolet-inactivated KT-FeLV. The KT-FeLV was purified and ultraviolet-inactivated (*4*). Final culture volume in all cases was 1.0 ml. Microplates were placed in culture boxes, incubated at 37°C in a humidified atmosphere containing 10 percent CO_2, and rocked for 72 hours at eight cycles per minute. Cultures were then harvested, cells were pelleted by slow centrifugation, and supernatants were collected and stored at 4°C. The antiviral activity of supernatant fluids from these cultures were titrated for ability to inhibit the cytopathic effects of approximately 40 plaque-forming units of vesicular stomatitis virus (VSV) on a monolayer of feline lung (FL) cells (American Type Culture Collection, Rockville, Maryland) by means of a plaque reduction method (*6*). All titrations were carried out in duplicate. Interferon (units per milliliter) was measured as the reciprocal of the dilution that reduced the number of plaque-forming units by 50 percent. A reference sample of feline interferon (162 U/ml) was used to monitor the assays.

To determine whether the inactivated FeLV suppressed mitogen-induced proliferation of lymphocytes, four separate lymphocyte blastogenesis assays were performed by a method previously reported (*4*), except that Con A (2.5 to 10.0 μg per well) or SEA (0.05 to 0.5 μg per well) served as mitogens and 18.75 μg of inactivated FeLV was used in the test wells. Under these conditions the amount of inactivated virus per lymphocyte was 18×10^{-5} μg per cell and the residual proliferative response, as determined by measuring the incorporation of tritiated thymidine, ranged from 22.4 to 78.5 percent, which is comparable to reported values.

Two, three, or four separate culture series were established with lymphocytes from each of the seven cats. Table 1 shows induced antiviral activity of the supernatant as an average of two to four determinations. A value of <10 U/ml denotes an undetectable interferon level. Supernatant from wells containing only lymphocytes and complete medium had no antiviral activity (except for two of the four cultures of cells from cat 248). Cultures containing SEA-stimulated lymphocytes produced interferon at between 47 and 768 U/ml. When 625 μg of inactivated FeLV was incubated with SEA in lymphocyte cultures, the supernatant showed no antiviral effect. With the addition of 400 μg of virus to SEA-stimulated lymphocyte cultures, the induced antiviral activity of the supernatant was markedly reduced compared to the positive control. Under these conditions there was 8×10^{-5} μg of virus per lymphocyte and the residual antiviral activity ranged from 8.8 to 53.2 percent.

Table 2. Characterization of the antiviral activity of culture supernatants as a gamma-like interferon (units per milliliter).

Treatment	Cat					
	248	249	1264	1276	301	302
pH						
*p*H 7.2	767	339	246			
*p*H 2	<10	<10	<10			
Heat						
Control			246	44		
56°C (30 minutes)			<10	<10		
Activity against other viruses						
VSV					150	
FCV					267	
FHV-1					<10	
Heterologous cells						
FL					150	200
MDBK					<10	<10

With further dilution of the virus the induced antiviral activity of the supernatant increased. In some cases, when small amounts of inactivated FeLV were incorporated in cultures the resultant antiviral effect exceeded that in the positive control (cats 249 and 248).

To characterize the antiviral activity of the supernatant, we conducted separate tests in which they were dialyzed at *p*H 2, heat-treated at 56°C for 30 minutes, or subjected to ultracentrifugation (110,000*g* for 90 minutes). No antiviral activity remained in samples dialyzed at *p*H 2 or subjected to heat, while ultracentrifugation did not reduce the antiviral effect (Table 2). Supernatants that effected plaque reduction when VSV was used as the challenge virus also demonstrated this protective antiviral effect when feline calicivirus (FCV, clone F-9) was substituted as the challenge virus, showing that the antiviral substance effectively inhibited the replication of other viruses. Feline herpesvirus type 1 (FHV-1, clone C27) was insensitive to any antiviral activity in any of the tested supernatants. Variation in the sensitivity of different viruses to interferon has been described (*7*). Samples with antiviral activity in FL cell monolayers showed no activity in a heterologous system of interferon assay with Madin-Darby bovine kidney (MDBK) cell monolayers (American Type Culture Collection) and VSV as a challenge virus. These findings indicate that the antiviral effect of treating FL cell monolayers with supernatants from cultures containing SEA-stimulated lymphocytes was attributable to an interferon. This interferon was a gamma-like (immune) interferon on the basis of its susceptibility to *p*H 2.

The reduction in interferon production in lymphocyte cultures where cells were incubated with virus and SEA was not solely attributable to a reduction in the number of viable lymphocytes. By resuspending individual cell pellets in 1.0 ml of complete medium after harvesting the supernatants, we determined mean viability after 72 hours of incubation in 106 cultures from three experiments involving four cats (Table 1). Lymphocyte viabilities after culture differed only slightly, regardless of the culture conditions.

To show that the addition of virus did not neutralize the mitogenic properties of SEA, we incubated inactivated FeLV (1250 μg) and SEA (50 μg) together at 37°C for 18 hours in 4.0 ml of complete medium. The virus was then removed by ultracentrifugation (100,000*g* for 90 minutes). The remaining SEA-containing supernatant, used in place of stock SEA for the stimulation of lymphocytes, induced comparable interferon titers when compared to stock SEA (Table 3). To determine whether interferon was produced but subsequently neutralized by the presence of inactivated virus, 400 μg of FeLV was added to SEA-stimulated lymphocyte cultures for the final 2 hours of incubation. Subsequent testing of these supernatants did not show any reduction in antiviral activity (Table 3), suggesting that the FeLV-induced impairment of antiviral activity was a result of reduced interferon synthesis and not reduced effectiveness caused by the presence of FeLV.

Table 3. Failure of FeLV to alter the mitogenic properties of SEA or the antiviral activity of SEA-stimulated lymphocyte supernatants. Values are interferon titers (units per milliliter).

Treatment	Cat		
	1276	249	301
Treatment of SEA			
Stock SEA	550	150	
SEA after incubation with FeLV	727	139	
Treatment of culture supernatant			
None	123, 150, 138		
FeLV (400 μg)	80, 150, 143		

The reduction in interferon synthesis mediated by FeLV in vitro may reflect clinically relevant impairment of lymphocyte function in cats persistently infected with FeLV. Gamma interferon appears to occupy a pivotal regulatory position in cellular immune responses, enhances antimicrobial activity of human macrophages, and augments cytotoxic and natural killer cell activities and the expression of interleukin-2 receptors on T cells (*8*). Deficient synthesis of this lymphokine may play a role in the pathogenesis of immunodeficiency in FeLV-infected cats. This finding is especially provocative in light of the proposed retroviral [human T-cell leukemia (lymphotropic) virus type 3] etiology of the acquired immune deficiency syndrome (AIDS) in humans (*9*). A recent study of 16 patients with AIDS who had opportunistic infections showed that mononuclear cells from 11 produced subnormal amounts of gamma interferon in response to a mitogen (*10*). Although this subnormal synthesis of interferon may reflect a quantitative deficiency in the T cells responsible for interferon synthesis, impaired interferon synthesis in the 11 patients and substantial synthesis in the other five cannot be attributed with assurance to alterations in lymphocyte numbers. In addition to or independent of a reduction in the number of interferon-synthesizing cells, the presence of immunosuppressive quantities of retrovirus at the cellular level may markedly impair synthesis of interferon and perhaps other lymphokines in vivo. The immunologic amplification attributable to these mediators would then be significantly reduced, resulting in a diminution of functional immunity and increased susceptibility to serious secondary illness.

R. W. ENGELMAN
Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City 73104

R. W. FULTON
Department of Microbiology, College of Veterinary Medicine, Oklahoma State University, Stillwater 74078

R. A. GOOD
N. K. DAY
Cancer Research Program, Oklahoma Medical Research Foundation

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