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Polymorphonuclear Leukocyte Dysfunction Associated with Feline Leukaemia Virus Infection

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SUMMARY

The chemiluminescent characteristics of enriched (>95%) peripheral blood polymorphonuclear leukocyte populations (PMN) from normal and feline leukaemia virus (FeLV)-infected cats were investigated. FeLV-infected cats demonstrated a significantly lower ($P < 0·001$) PMN chemiluminescent response when compared to the response of normal age-matched controls. Normal PMN treated with FeLV-infected cat serum exhibited a depressed response in comparison to control cells. A titration of serum from infected cats supplemented with normal serum revealed a titratable suppression of chemiluminescence with increasing concentration of serum from the infected cats. However, PMN from FeLV-infected cats treated with normal serum displayed a slight increase in chemiluminescence over the same cells in autologous serum. The addition of inactivated FeLV to normal PMN caused a titratable decrease in chemiluminescence.

INTRODUCTION

Infection of cats with feline leukaemia virus (FeLV) has two alternative consequences (for review, see Neil & Onions, 1985). The majority of affected cats develop and recover from a mild, transient infection with high titred antiviral and antitumour antibodies. In contrast, some cats develop a chronic progressive form of the disease characterized by chronic viraemia and immunosuppression, leading to the establishment of secondary, opportunistic infections and/or leukaemia. Animals developing the progressive form usually succumb to unrelated infections as a result of a virally associated immunosuppression (Hardy, 1981). The objective of this study was to examine the polymorphonuclear leukocyte (PMN) population from FeLV-infected cats for evidence of cell-mediated immune dysfunction. We chose to determine if the chemiluminescence (CL) response of the PMN was altered during FeLV infection and if the alteration was due to PMN dysfunction or to external factors acting on the PMN.

CL is the result of the oxidative metabolic burst associated with the membranes of phagocytic cells and is measured by a light release assay (Babior *et al.*, 1981; Klebanoff, 1980). The CL response associated with particle uptake by PMN has been directly associated with their microbiocidal activity (Horan *et al.*, 1982). Previous studies have shown that viruses can alter PMN functions (Faden *et al.*, 1979, 1981; Masihi *et al.*, 1984; Niwa *et al.*, 1984, 1985; Ras *et al.*, 1984; Roth & Kaeberle, 1983). These studies suggest that the loss of neutrophil CL activity is directly associated with subsequent secondary bacterial infections.

METHODS

Cats. All animals used in this study were specific-pathogen-free (SPF) cats obtained from a hysterectomy-derived breeding colony maintained by the Department of Veterinary Pathobiology, Ohio State University. All cats used in this study were more than 6 months old at the time of testing.

Virus. The Rickard strain of FeLV (FeLV-R) (Rickard *et al.*, 1969) was used in all of the *in vivo* studies. The FeLV-R inoculum was a 20% (w/v) homogenate of a lymphoma of the thymus gland, and represented the seventh *in vivo* passage of the original R-FeLV isolate. All infected cats received an inoculum that contained 10^5 f.f.u./ml as

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assayed in clone 81 feline cell cultures (Fischinger *et al.*, 1974; Schaller & Olsen, 1975). Virus challenge of 8-week-old, SPF kittens was by oronasal inoculation of 1·0 ml on 2 consecutive days. The Kawakami–Theilen strain of FeLV (FeLV-KT) was used for *in vitro* testing. The purification of FeLV-KT from tissue culture media has been described (Mathes *et al.*, 1977). Sucrose-banded virus was dialysed twice in Hanks' balanced salt solution (Gibco) and once in RPMI 1640 medium (Gibco) with 1·0% antibiotics (penicillin, streptomycin). The virus was inactivated by using u.v. light according to the method of Yohn *et al.* (1976). Protein determination was performed using a Bio-Rad protein kit.

Indirect immunofluorescent assay for FeLV viraemia. FeLV group-specific antigen (GSA) was detected by an indirect immunofluorescence assay modified from that developed by Hardy *et al.* (1973). The primary reagent was goat antiserum against ether-disrupted FeLV; the antiserum was absorbed extensively with normal feline blood cells to remove heterologous and non-specific reactivity (Hoover *et al.*, 1978).

Collection and separation of peripheral blood leukocytes. Thirteen ml of blood was drawn from the jugular vein of each test animal. Ten ml of each sample was immediately defibrinated with glass beads while 3 ml was allowed to clot for serum separation. After defibrination, enriched leukocyte preparations were obtained by centrifugation through Ficoll–Hypaque (Pharmacia) gradients.

Isolation of PMN. PMN were isolated by centrifugation of Ficoll-separated leukocytes over a three-step (50%, 62%, 80%) Percoll gradient (Pharmacia). Gradients were constructed from a stock 90% (v/v) Percoll solution in 10 × normal saline. Final dilutions were made in normal saline. Centrifugation was performed at 400 *g* for 40 min at 4 °C. Cell bands generated at the 80% interface were harvested and washed twice in normal saline and assayed for viability by trypan blue exclusion. This fraction gave >96% PMN as defined by MacNeals Tetrachrome blood stain and new methylene blue.

CL assay. A modification of the procedure of Jacobsen *et al.* (1982) was used to detect neutrophil CL. A 10% mixture of latex beads (0·797 μm, Seragen Diagnostics, Indianapolis, Ind., U.S.A.) were opsonized for 10 min at room temperature in autologous serum and used as the particle to be phagocytosed. The separate PMN were resuspended to $8{\cdot}5 \times 10^5$ cells/ml in normal saline. The assay system consisted of 0·8 ml of the PMN suspension mixed with 50 μl Luminol (Sigma) [5×10^{-5} M in 0·1% (v/v) bovine serum albumin in normal saline], and 25 μl of the opsonized beads. Upon addition of the PMN, the sample was immediately loaded into the scintillation counter (Packard Model 3385). Counts of light emitted were measured at 0·20 min intervals for a total of 5 min. Experimental samples were run in triplicate. Light release values were recorded and a light release index (LRI) was calculated for each sample according to the equation: LRI = peak c.p.m. of cells, luminol and latex beads/background c.p.m. of cells and luminol.

Statistics. Student's *t*-test was used to determine significance.

RESULTS

CL of PMN from normal SPF cats and FeLV-infected cats

Four healthy SPF control cats were tested and their response reached its peak between 36 and 48 s, and ranged from $6{\cdot}5 \times 10^4$ to $8{\cdot}9 \times 10^4$ c.p.m. (Fig. 1*a*). Cell viability before and after CL remained unaffected, ranging between 93% and 98%.

Isolated PMN from four clinically normal cats GSA-positive for ≥20 weeks were tested to determine their CL response (Fig. 1 *b*). These cats demonstrated lower peak responses ranging from $1{\cdot}5 \times 10^4$ to $3{\cdot}6 \times 10^4$ c.p.m. than those demonstrated by the age-matched controls. Three of the four viraemic cats shown also exhibited a delay in the attainment of a peak response, with the response peaking at 72 to 84 s after the addition of the cells. Again, cell viability before and after CL remained unaffected. The PMN used in the CL assay were collected and found to contain FeLV GSA.

An overall comparison of the LRI of seven healthy control cats and seven viraemic cats is shown in Fig. 1 (*c*, *d*). CL responses of control normal cats were significantly ($P < 0{\cdot}001$) higher than those of the GSA-positive cats (2948 compared to 967).

Effect of normal serum on viraemic PMN from GSA-positive cats

In order to determine if the depressed CL response was due to PMN dysfunction or the presence or absence of a serum factor, neutrophils from five GSA-positive cats were tested with autologous and pooled normal serum from eight SPF cats. The LRI was calculated from the peak response elicited with each serum source.

The effect of normal serum on the CL of neutrophils obtained from the GSA-positive cats is given in Table 1. The LRI increased slightly when incubated with normal serum in three of the

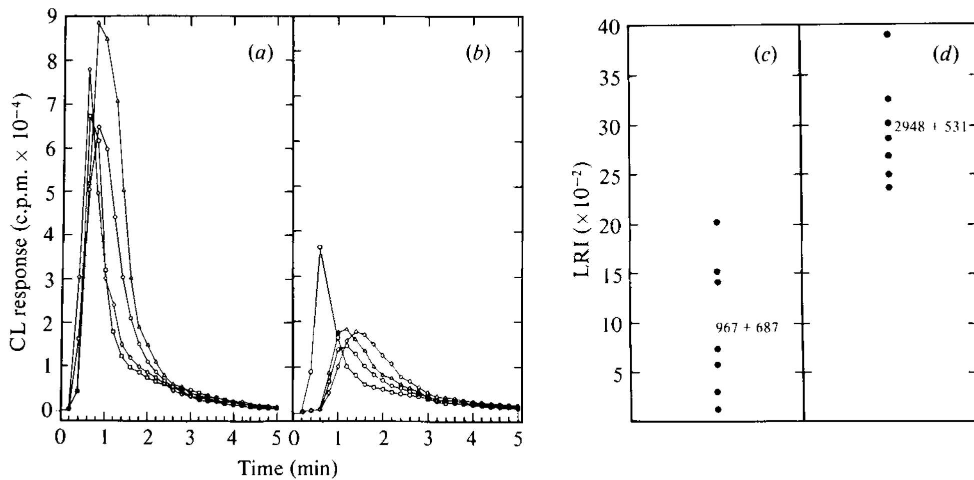

Fig. 1. (*a*) CL response (expressed in c.p.m.) of PMN from four normal SPF cats. (*b*) CL response (expressed in c.p.m.) of PMN from four FeLV-infected cats. FeLV GSA was observed in the PMN of these cats for > 20 weeks before testing. (*c, d*) Comparison of LRI of PMN from seven healthy SPF (*d*) and seven FeLV-infected (*c*) cats. All values represent triplicate runs for each sample.

Table 1. *Effect of different cat sera on the CL response of PMN isolated from normal and FeLV-infected cats*

Cat no.	GSA status	Serum source	LRI
1	+	Autologous	247·0
		SPF	548·6
2	+	Autologous	1355·8
		SPF	1222·1
3	+	Autologous	239·4
		SPF	838·6
4	+	Autologous	47·7
		SPF	112·0
5	+	Autologous	599·6
		SPF	535·9
6	−	Autologous	3278·4
		GSA-positive	1080·3
7	−	Autologous	2588·8
		GSA-positive	196·4
8	−	Autologous	2926·8
		GSA-positive	1023·4
9	−	Autologous	2675·2
		GSA-positive	1056·6
10	−	Autologous	2870·5
		GSA-positive	1838·9

cats, but was still below the LRI observed with normal cells. The two remaining cats showed no difference in the CL response.

Effects of GSA-positive cat serum on normal PMN function

The effect of GSA-positive serum on normal SPF neutrophil CL is summarized in Table 1. Normal PMN stimulated with latex beads in the presence of pooled GSA-positive serum gave a suppressed CL response ($P < 0{\cdot}001$). The response of these cells ranged from 8% to 64% of the response of the same cells incubated with autologous serum.

In a titration experiment GSA-positive serum and normal serum were mixed in varying

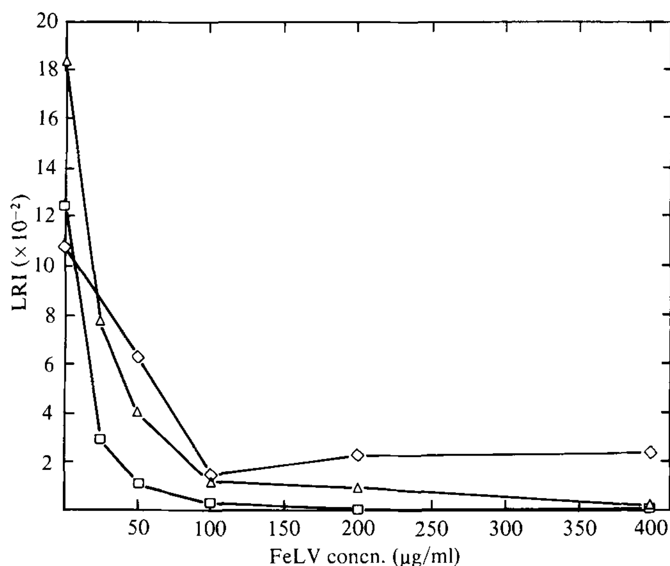

Fig. 2. Effect of increasing amounts of inactivated FeLV-KT on the CL response of normal PMN. The cells were incubated with FeLV-KT for 1 h prior to testing. Each point represents the LRI calculated as described in Methods. Each line represents an experiment on an individual cat.

Table 2. *Effect of viraemic serum concentration on the CL response of normal PMN supplemented with normal serum*

Cat no.	% Viraemic serum of total serum used	LRI	Peak response (%)
1	0	3965·9	100
	20	3116·2	78
	40	1123·2	28
	60	1811·4	28
	80	351·5	5
	100	457·0	7
2	0	6731·7	100
	20	2094·4	31
	40	1784·6	26
	60	2048·5	26
	80	1703·3	22
	100	1410·6	18
3	0	4288·1	100
	20	4078·3	95
	40	3110·4	72
	60	2113·2	51
	80	3055·6	49
	100	1875·8	45

amounts. The normal PMN from three cats gave a titratable suppression of CL with increasing levels of GSA-positive serum (Table 2). Total serum concentration (100 µl/test) was held constant with increasing amounts of GSA-positive serum added in place of an equal amount of normal serum. Increasing the amount of GSA-positive serum correlated with a decrease in LRI, with a mixture of 40% GSA-positive serum causing significant ($P < 0{\cdot}05$) loss of LRI.

Effect of u.v.-treated FeLV on normal cat PMN

In order to determine if the suppression of LRI observed with GSA-positive serum was due to the presence of FeLV, we incubated inactivated FeLV-KT with normal PMN for 1 h and then tested their CL response. The inactivated virus gave a dose-dependent decrease in PMN LRI, with a dose of 25 µg/ml causing significant suppression (Fig. 2). Media controls remained

unaffected. There was no observable loss of viability of the PMN due to the incubation with the virus.

DISCUSSION

The results of these studies demonstrate that FeLV *in vivo* and inactivated FeLV *in vitro* can suppress the function of cat PMN. The stimulation of PMN by latex beads has been directly correlated to their ability to kill microorganisms (Horan *et al.*, 1982). The observed loss of PMN responsiveness in FeLV-infected cats may explain the increase in secondary infection associated with FeLV infections, including chronic infections in the skin and respiratory tract (Hardy, 1981). This observed depression of PMN function by FeLV may occur by two possible mechanisms: first, by an infection of the granulocytic precursor cell by the virus and/or second, by the association of the PMN with FeLV virions or viral proteins, or viral immune complexes.

Retrovirus-associated immunosuppression has been well documented (Teich *et al.*, 1978). Many of the studies associating retroviruses with immunosuppression have shown involvement of lymphocytes. There are also studies showing that other immune cell types are similarly affected (Cianciolo *et al.*, 1980; Ras *et al.*, 1984). The present studies were performed to determine the effect of a retrovirus on the neutrophil. Previous work with viruses has shown that neutrophils are susceptible to impairment due to the presence of virus (Faden *et al.*, 1979, 1981; Masihi *et al.*, 1984; Niwa *et al.*, 1984, 1985; Ras *et al.*, 1984; Roth & Kaeberle, 1983). The loss of PMN function in cells isolated from FeLV-infected cats is in accord with these observations.

The site at which FeLV causes its effect may be multiple and has yet to be defined. The CL response measures the generation of oxygen radicals, which is the end reaction of a complex oxidative pathway. Its inhibition by FeLV may occur at any of a number of sites by a number of possible mechanisms. Studies with Newcastle disease virus (Faden *et al.*, 1981) have shown that the virus can inhibit NADPH-oxidase, and that the inhibition is not dependent on an active infection. Niwa *et al.* (1984, 1985) have shown that T lymphocytes from patients with infectious mononucleosis and measles are capable of inhibiting PMN function, suggesting a possible T cell defect causing a PMN dysfunction in virus-infected hosts. The possibility that the PMN dysfunction in FeLV-infected cats is secondary to the site of the defect as was shown by Niwa *et al.* (1984, 1985) for mononucleosis and measles would appear to be unlikely due to the purity of the cell preparation and to the continued dysfunction after incubation with normal serum. The measurement of different enzyme systems associated with PMN activation and the use of other PMN stimulators such as stimulatory peptides, phorbol esters and calcium ionophores will help to elucidate further the exact site of the defect associated with FeLV infection.

The observed loss of PMN function in the presence of inactivated FeLV indicates that the virus need not be infectious to induce PMN dysfunction. We have previously observed that inactivated FeLV and an envelope protein from FeLV (p15E) can inhibit a number of cellular functions including mitogenesis and membrane mobility (Copelan *et al.*, 1983; Dunlap *et al.*, 1979; Hebebrand *et al.*, 1977, 1979; Mathes *et al.*, 1978). Orosz *et al.* (1985*a*, *b*) suggested that FeLV may bind at the cell surface, blocking the passage of an external signal through the surface membrane. A similar process may occur in the presence of FeLV, causing a blocking in the binding of the bead to the PMN surface membrane interfering with the uptake of the particle or transmission of a stimulatory signal. *In vivo*, the PMN may accumulate FeLV antigens, possibly in the form of immune complexes, thus causing a depression similar to that observed with the *in vitro* addition of u.v.-treated FeLV.

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