Suppression of in vitro Neutrophil Function by Feline Leukaemia Virus (FeLV) and Purified FeLV-p15E

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SUMMARY

Feline neutrophils (PMN) were isolated and exposed to ultraviolet light-inactivated feline leukaemia virus (UV-FeLV) and purified envelope component p15E (FeLV-p15E). Functional capacity of exposed PMN was measured in vitro utilizing the chemiluminescence (CL) response. PMN exposed to UV-FeLV demonstrated depressed CL responses to Ca2+-ionophore A23187 and latex particles. However, FeLV-p15E produced significant suppression in the CL response to A23187 but failed to produce significant alterations in response to latex particles. The data indicate that FeLV-p15E may, in part, be responsible for increased morbidity and mortality among FeLV-infected cats through suppression of the PMN population.

INTRODUCTION

Suppression of cell-mediated immunity has been demonstrated in cats challenged with feline leukaemia virus (FeLV) (Rickard et al., 1969; for review, see Neil & Onions, 1985). This suppression has been associated with direct viral infection of lymphoid tissue resulting in impairment of lymphoid function (Perryman et al., 1972). Immunization of cats with u.v.-inactivated FeLV (UV-FeLV) suppresses tumour immunity (Mathes et al., 1979) while allogeneic mixed leukocyte reactions in vitro are inhibited by UV-FeLV (Stiff & Olsen, 1983). Blastogenic responses of feline lymphocytes to concanavalin A (Con A) have been shown to be suppressed by as much as 65% (Hebebrand et al., 1979).

Although the exact mechanism of suppression is unclear, strong evidence implicates the retroviral envelope protein, p15E, as an inducer of immune suppression. Retroviral p15E (mol. wt. 19000) is a hydrophobic transmembrane envelope protein cleaved from a precursor protein (mol. wt. 80000 to 90000) with the cleavage yielding p15E and gp70 (Bolognesi et al., 1978; Snyderman & Cianciolo, 1984). Mathes et al. (1979) have reported suppression of blastogenesis of feline lymphocytes in vitro to Con A using p15E. Cianciolo et al. (1980) have demonstrated inhibition of accumulation of macrophages to inflammatory stimuli using low molecular weight ultrafiltrates of Friend, Rauscher and Moloney murine leukaemia viruses.

Polymorphonuclear leukocytes (PMN) play a central role in host defence against bacterial and fungal diseases. Removal of this population would necessarily leave the affected individual open to infection by opportunistic pathogens. Upon infection with FeLV, a minority of cats become persistently viraemic with low titre humoral responses to FeLV-associated antigens (Hardy et al., 1973; Grant et al., 1980). These cats are highly susceptible to opportunistic infections from the time of diagnosis to the onset of leukaemia with a majority succumbing to opportunistic infections (Hardy, 1980).

Recent reports have shown that infection with the human retrovirus, human immunodeficiency virus, results in suppression of mononuclear phagocytic cells (Prince et al., 1985) and polymorphonuclear phagocytic cells (Ras et al., 1984). Lewis et al. (1986) have shown that, in addition to lymphoid cell suppression, FeLV-viraemic cats express severely depressed PMN
populations as compared to controls in an assay for functional capacity in vitro. In addition, we have shown (Lafrado & Olsen, 1986) that non-viraemic FeLV-challenged cats express a similar depression in functional activity in vitro. The mechanism of this PMN suppression is unclear.

The purpose of this research was to determine the mechanism of retroviral suppression on PMN function in vitro. Neutrophil function was measured using the luminol-enhanced chemiluminescence (CL) response as the parameter of function (Allred et al., 1980; Babior et al., 1981). The data presented here show that PMN function is suppressed in vitro when exposed to purified FeLV-p15E and UV-FeLV.

METHODS

Cats. All cats used in this study were from a specific pathogen-free (SPF) colony maintained at the Department of Veterinary Pathobiology, The Ohio State University. All animals tested were more than 6 months of age.

Virus. The Kawakami-Theilen strain of FeLV was used for testing in vitro. Purification of FeLV from tissue culture medium has been described (Mathes et al., 1976). Sucrose-banded virus was dialysed twice in Hanks' balanced salt solution (Gibco) and once in RPMI 1640 (Gibco) with 1%0 antibiotics (penicillin, streptomycin). The virus was inactivated by using ultraviolet light according to the method of Yohn et al. (1976). Protein determination was performed using a Bio-Rad Protein Assay Kit.

Purification of p15E. Purified virus was extracted with 1% Triton X-100 (Sigma). Following ultracentrifugation, the supernatant fraction was extracted 10 times with 10 to 15 vol. ether. The sample was dialysed against distilled water and the precipitate was collected and resuspended in a formate buffer pH 3.5 containing 1% Triton X-100. The sample was then added to a Mono S column (Pharmacia). Bound proteins were eluted with a 1 M-NaCl eluant from the formate buffer. The FeLV-p15E-containing fraction was collected, tested for purity by gel electrophoresis and dialysed against 0.9% NaCl.

PMN isolation. Heparinized venous blood was centrifuged at 150 g for 10 min at 8°C and the resulting plasma and buffy coat layers were discarded. The erythrocyte pellet containing the PMN population was mixed with an equal volume of 6% dextran and incubated for 45 min at 37°C. The upper fraction of PMN-rich cells was drawn off and resuspended in 0.9% NaCl and centrifuged at 400 g for 10 min at 8°C. Remaining erythrocytes were lysed by resuspension in 2.0 ml of 0.9% NaCl followed by the addition of 6.0 ml of cold distilled water. After 2 min, 2.0 ml of cold 3.5% NaCl was added to re-establish isomolarity. Following centrifugation at 400 g for 10 min at 8°C, resulting erythrocyte-free pellets were resuspended in 2.0 ml of 0.9% NaCl. Viability was determined by trypan blue exclusion and purity determined by new methylene blue staining. This procedure yielded a consistent viability of 98% and purity of 93 to 96%.

Exposure to UV-FeLV and FeLV-p15E. Aliquots of 10^6 PMN/ml in 0.9% NaCl were mixed with UV-FeLV (final concentration of 10 µg/ml). FeLV-p15E was added to separate incubation vials to final concentrations of 6 µg/ml and 60 µg/ml. All samples were then incubated at 37°C for 45 min with gentle agitation every 10 min. Control samples were adjusted for volume changes with 0.9% NaCl and treated as the experimental samples were handled. After 45 min, control and experimental samples were centrifuged at 250 g for 10 min at 4°C, after which the pellets were resuspended to 10^6 cells/ml in 0.9% NaCl.

CL assay. PMN were assayed for the capacity to generate a respiratory burst accompanied by the release of photons by the CL assay. Isolated PMNs were resuspended to 10^6 cells/ml in 0.9% NaCl and used in aliquots of 0.8 ml. Fifty µl 10 mM-luminol (Sigma) was added to each aliquot as a secondary light emitter, after the calcium ion concentration was adjusted to 1.0 mM with 0.1 M-CaCl_2. 2H_2O. The sample was immediately loaded into an LKB Wallac Luminometer (Model 1251) and counted for 4 min at 12 s intervals to allow the sample temperature to equilibrate to 37°C and to determine unstimulated light release levels. After 4 min, a CL stimulant, calcium ionophore A23187 (Sigma) to a final molarity of 10^{-7} M or 25 µl latex beads in a 10% solution (0.800 µm; Seragen Diagnostics, Indianapolis, Ind., U.S.A.) was added and light release in millivolts (mV) was measured over 5 min at 3 s intervals.

RESULTS

Effect of UV-FeLV on ionophore- and latex bead-induced CL

The effect of UV-FeLV on the CL response of feline PMN was determined by exposure of isolated PMN to titrated concentrations of UV-FeLV. Fig. 1 demonstrates the effect of 45 min incubation of PMN with 1, 10 and 100 µg/ml UV-FeLV on latex bead-stimulated PMN CL. At a concentration of 10 µg/ml, UV-FeLV suppressed CL by approximately 50% (P < 0.01) in five cats. Insignificant depression was noted with 1 µg/ml UV-FeLV, whereas 100 µg/ml UV-FeLV suppressed PMN CL by more than 75% (P < 0.01). Viability before and after incubation with UV-FeLV was shown to be greater than 98%.
PMN suppression by FeLV and FeLV-p15E

Fig. 1. Effects of UV-FeLV on the CL response of feline PMN. Neutrophils were isolated and exposed to 1 μg/ml UV-FeLV (■), 10 μg/ml UV-FeLV (□) or 100 μg/ml UV-FeLV (■■) and compared to unexposed PMN (□□).

Table 1. CL response (mV) of feline PMN to ionophore A23187 and latex beads after exposure to UV-FeLV (10 μg/ml)

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Control</th>
<th>+ UV-FeLV</th>
<th>Cat no.</th>
<th>Control</th>
<th>+ UV-FeLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93-3</td>
<td>20-9</td>
<td>1</td>
<td>159-1</td>
<td>82-0</td>
</tr>
<tr>
<td>2</td>
<td>84-5</td>
<td>40-2</td>
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<td>210-8</td>
<td>10-6</td>
</tr>
<tr>
<td>3</td>
<td>83-7</td>
<td>18-3</td>
<td>6</td>
<td>249-0</td>
<td>72-6</td>
</tr>
<tr>
<td>4</td>
<td>44-4</td>
<td>12-0</td>
<td>7</td>
<td>131-3</td>
<td>44-9</td>
</tr>
<tr>
<td>5</td>
<td>50-3</td>
<td>7-3</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 1 demonstrates the effect of 10 μg/ml UV-FeLV on latex bead- and calcium ionophore A23187-induced PMN CL. The latter CL response in exposed PMN was suppressed by greater than 50% in the five cats tested (P < 0.01). The data indicate a failure in the ability of exposed PMN to respond to the Ca2+ flux normally induced by the ionophore A23187. Latex bead stimulation of PMN CL was also decreased by greater than 50%.

Titration of FeLV-p15E against feline PMN

Table 2 shows that, at a concentration of 6 μg/ml, FeLV-p15E significantly depressed the ionophore-induced PMN CL (P < 0.05), whereas at 60 μg/ml FeLV-p15E completely suppressed (P < 0.01) the ability of exposed feline PMN to respond to the Ca2+ flux induced by the ionophore. In contrast, 6 μg/ml FeLV-p15E failed to induce significant suppression of latex bead-stimulation PMN CL in three of five cats tested.

Time course analysis of effect of UV-FeLV and FeLV-p15E on feline PMN CL

Fig. 2 demonstrates the effect of UV-FeLV and FeLV-p15E on PMN CL in a single cat when analysed with time. The latex bead-induced CL was suppressed by 6 μg/ml FeLV-p15E to a level similar to that induced by 10 μg/ml UV-FeLV (Fig. 2a). Suppression of ionophore-induced CL
Fig. 2. Effects of 6 μg/ml FeLV-p15E (△), 60 μg/ml FeLV-p15E (○) and 10 μg/ml UV-FeLV (▲) on the CL response of PMN from cat no. 10 to (a) latex beads and (b) Ca²⁺ ionophore A23187. Results are compared to control values (■).

Table 2. Calcium ionophore- and latex bead-induced CL response in feline PMN exposed to purified FeLV-p15E (mV)

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Calcium ionophore A23187</th>
<th>Latex beads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p15E</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6 μg/ml</td>
</tr>
<tr>
<td>8</td>
<td>23.2</td>
<td>1.0</td>
</tr>
<tr>
<td>9</td>
<td>20.3</td>
<td>2.6</td>
</tr>
<tr>
<td>10</td>
<td>34.3</td>
<td>16.6</td>
</tr>
<tr>
<td>11</td>
<td>5.1</td>
<td>1.2</td>
</tr>
<tr>
<td>12</td>
<td>13.2</td>
<td>1.0</td>
</tr>
<tr>
<td>13</td>
<td>14.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*NT, Not tested.

was greatest 30 s earlier in UV-FeLV-treated PMN and 90 s earlier in PMN exposed to 6 μg/ml FeLV-p15E when compared to untreated control PMN.

DISCUSSION

The immunosuppressive sequelae of viraemic FeLV infections have been shown to be responsible for the increased morbidity and mortality among FeLV-infected cats (Hardy et al., 1973; Grant et al., 1980). The majority of overtly FeLV-infected cats succumb to unrelated opportunistic infections associated with an immunosuppressed state (Hardy, 1980). The precise mechanism of suppression is unclear. Data presented here suggest that FeLV and its envelope component p15E may be active in suppressing the PMN population critical to host defence...
against secondary pathogens. Harrell et al. (1986) have recently reported that a synthetic analogue of p15E suppresses the generation of oxygen radicals in human monocytes.

FeLV-p15E at low (6 µg/ml) and high (60 µg/ml) doses significantly reduces the capacity of PMN to produce a CL response after stimulation with the ionophore A23187. Activation of cells by ionophore induces the mobilization of intracellular Ca^{2+} ions and flux of extracellular Ca^{2+} ions into the cytoplasm across the cellular membranes which are essential to the function and maintenance of the cell (Allan et al., 1976; Raval & Allan, 1985; Truneh et al., 1985). Recent evidence suggests that Ca^{2+} ion mobilization and protein kinase C (PKC) (Fujita et al., 1984; Nishizuka, 1984) are activators of the superoxide anion (O_2) critical to the microbiocidal action of PMN (Brestel, 1985). The production of O_2 and subsequent CL is the result of the activation of the membrane-bound NADPH/NADH oxidase (for review, see Nishizuka, 1984) by PKC and Ca^{2+} mobilization. Faden et al. (1981) have shown that Newcastle disease virus inhibits the NADPH/NADH oxidase system with reduction of the CL response in exposed PMN. FeLV-p15E may induce a suppression in Ca^{2+} ion-dependent CL suggesting a mechanism of action similar to that seen by Faden et al. (1981). Inhibition of the mobilization or flux of Ca^{2+} would necessarily lead to diminution of microbiocidal O_2 generation and CL. Depression of oxygen radical formation has been observed in measles infections (Kaul et al., 1981). In addition, influenza virus has been shown to suppress the functional capacity of PMN in vitro (Abramson et al., 1984) and to depress the CL response of murine spleen cells (Mashihi et al., 1984).

Although FeLV-p15E produced significant decreases in CL response to ionophore A23187, it was ineffective in depressing latex bead-stimulated CL. Orosz et al. (1985) have reported that UV-FeLV and p15E inhibited development of cytolytic T lymphocytes in mixed leukocyte cultures but failed to inhibit the cytolytic activity of preformed cytolytic T lymphocytes. This lack of effect on the cytotoxic capacity of T cell subpopulations suggests that FeLV does not inhibit recognition of targets by cytotoxic T cells and the same may be true in the PMN cell population. The cytolytic activity of human natural killer cells has been shown to rely on a Ca^{2+}-independent mechanism by Brahml et al. (1985). Hallett & Campbell (1983) have reported that unopsonized latex beads stimulate the neutrophil respiratory oxidative burst independent of extracellular Ca^{2+} or mobilization of intracellular Ca^{2+} pools. It has been reported also that activation of PKC and subsequent induction of CL in rat neutrophils may occur independently of Ca^{2+}-dependent activation processes (Cooke & Hallett, 1985). Our data show that FeLV-p15E at high doses induces a slight depression in latex bead-stimulated CL. These results suggest that FeLV-p15E may preferentially suppress extracellular Ca^{2+}-dependent stimulation of CL and fails to suppress latex bead stimulation. The data reported in Table 2 suggest that FeLV-p15E fails to inhibit mobilization of intracellular Ca^{2+} in response to latex beads, resulting in a near normal CL response at low exposures.

In conclusion, these data show that PMN functional capacity in vitro is suppressed when exposed to UV-FeLV and purified FeLV-p15E. It is possible that increased morbidity and mortality among FeLV-infected cats may be directly related to a suppressed PMN population, allowing establishment of opportunistic pathogens. Horan et al. (1982) have reported the correlation of neutrophil CL with microbiocidal activity suggesting that the suppression in CL response by UV-FeLV and FeLV-p15E is a measurement of phagocytic function. Finally, the data implicate a Ca^{2+}-dependent target site for p15E action.

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