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Lewis, Mark Gill

DETERMINATION OF A MECHANISM OF FELINE RETROVIRAL SUPPRESSION OF THE FELINE IMMUNE SYSTEM

The Ohio State University

Ph.D. 1983

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DETERMINATION OF A MECHANISM OF FELINE RETROVIRAL SUPPRESSION OF THE FELINE IMMUNE SYSTEM

DISSERTATION

Presented in Partial Fulfillment of the Requirements of the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Mark Gill Lewis, B.A., M.S.

The Ohio State University

1983

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1983

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My deepest appreciation is given to my wife, Cindy, for her endless support and understanding during my academic pursuits.
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Lewis, M.G., Fertel, R., and Olsen, R.G.: The Reversal of Feline Retroviral Induced Suppression of Lymphocyte Con A Receptor Mobility by Indomethacin. (In preparation)

Abstracts:


<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
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<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF PLATES</td>
<td>xii</td>
</tr>
</tbody>
</table>

CHAPTER

I. RETROVIRAL-INDUCED EOSINOPHILIC LEUKEMIA IN THE CAT ........................................ 1
   Introduction .......................................................... 1
   Methods ................................................................. 2
   Results ................................................................. 3
   Discussion .............................................................. 7
   Summary ................................................................. 10

II. THE REVERSAL OF FELINE RETROVIRAL-INDUCED SUPPRESSION OF LYMPHOCYTE CON A RECEPTOR MOBILITY BY INDOMETHACIN AND PGE₂ .................................................. 17
   Introduction .......................................................... 17
   Methods ................................................................. 19
   Results ................................................................. 21
   Discussion .............................................................. 23
   Summary ................................................................. 26

III. THE IN VITRO EFFECT OF PROSTAGLANDIN INHIBITORS AND CYCLIC AMP ENHANCERS ON THE SUPPRESSIVE ACTION OF FeLV P15E ..................................................... 32
   Introduction .......................................................... 32
   Methods ................................................................. 34
   Results ................................................................. 37
   Discussion .............................................................. 40
   Summary ................................................................. 43
TABLE OF CONTENTS (Continued)

CHAPTER

IV. THE EFFECT OF FeLV UPON PGE₂ AND CYCLIC NUCLEOTIDE PRODUCTION IN VITRO ................. 52

Introduction ............................................. 52
Methods ................................................. 54
Results .................................................. 58
Discussion .............................................. 60
Summary .................................................. 62

CONCLUSION ........................................... 67

BIBLIOGRAPHY .......................................... 70
LIST OF TABLES

CHAPTER I

Table

1.1. Viral Proteins and FOCMA Antibodies in Cats Infected with PR-8 Isolate of FeLV .......................... 11
1.2. Hematologic Data for Cats with Eosinophilia .......................... 12

CHAPTER IV

Table

4.1. The Effect of FeLV and Indomethacin on cAMP Levels of Lymphocytes ............... 63
LIST OF FIGURES

CHAPTER II

Figure

2.1. Effect of indomethacin on FeLV-induced suppression of Con A receptor mobility. Results represent a mean ± SE of 4 tests. (*p < .001) (**p < .05) ............... 25

2.2. Effect of indomethacin on FeLV p15E-induced suppression of Con A receptor mobility. Results represent a mean ± SE of 3 tests. (*p < .05) (**p < .005) .... 26

2.3. Effect of PGE₂ on normal Con A cap formation and FeLV-induced suppression. Each point represents the mean ± SE of 5 experiments ..................... 27

2.4. Effect of PGE₂ on FeLV p15-induced suppression of Con A cap formation. Each point represents the mean ± SE of 3 experiments ...................... 28

2.5. Effect of indomethacin on Con A receptor cap formation on cells from FeLV-infected cats. Each point represents the mean ± SE of 4 experiments ......................... 29

CHAPTER III

Figure

3.1. The effect of FeLV and PGE₂ upon the normal feline Con A LBT. The results are expressed as a mean ± standard error of 5 experiments ................. 42
Figure

3.2. The effect of arachidonic acid on the normal Con A LBT and a Con A with FeLV. The results are expressed as a comparison to normal control cell. Each point represents a mean ± SE of 6 experiments. (*p<.02 vs. control) ....... 43

3.3. The effect of indomethacin on FeLV (n=20) and FeLV p15E (n=4) -induced suppression of the Con A LBT. Results expressed as comparison of normal control cells. (*p<.01 vs. control) (**p<.001 vs. control) .................. 44

3.4. The effect of NDGA on FeLV-induced suppression of the Con A LBT. Each point represents the mean ± SE of 6 experiments. (*p<.05 vs. control) (**p<.001 vs. control) .................. 45

3.5. The effect of papaverine on the Con A LBT with and without FeLV. Each point represents the mean ± SE of 6 experiments. (*p<.01 vs. control) ........... 46

3.6. The effect of IBX on the Con A LBT with and without FeLV. Each point represents the mean ± SE of 6 experiments. (*p<.01 vs. control) .................. 47

3.7. The effect of forskolin on the FeLV-induced suppression of the Con A response of normal cells. The results are expressed as percentage of viral-induced inhibition. Each point represents the mean ± SE of 6 experiments ..................... 48
LIST OF FIGURES (Continued)

CHAPTER III

Figure

3.8. The effect of indomethacin upon Con A stimulation of FeLV-infected cats. Each point represents the mean ± SE of 8 experiments .......................... 51

CHAPTER IV

4.1. PGE₂ levels expressed as percentage of normal cells. Each point represents the mean ± SE of 8 experiments. (*p < .05 vs. Con A-stimulated cells) .... 64

4.2. Cyclic AMP levels of Con A-stimulated cells with FeLV and indomethacin. Each point represents the mean ± SE of 6 to 10 experiments. (*p < .001 vs. Con A-stimulated cells and Con A plus indomethacin) .......................... 65

4.3. Cyclic GMP levels of Con A-stimulated cells with FeLV and indomethacin. Each point represents the mean ± SE of 4 experiments .......................... 66
LIST OF PLATES

CHAPTER I

Plate

I. Eosinophilic precursors from the bone marrow of a cat inoculated with the PR-8 isolate of FeLV. Most of the cells have fine nuclear chromatin, prominent nucleoli, numerous azurophilic granules and faintly stained eosinophilic granules. H and E stain, X1680 ..................... 13

II. Eosinophilic progranulocytes in the bone marrow of a cat inoculated with the PR-8 isolate of FeLV. X8400.

Inset shows lamellar appearance of electron dense granules, X53,000 .......... 15
CHAPTER I
RETROVIRAL-INDUCED EOSINOPHILIC LEUKEMIA
IN THE CAT

INTRODUCTION

Infection with the Rickard strain of feline leukemia virus (FeLV-R) induced thymic lymphomas in susceptible cats (1). The onset of an experimentally induced lymphoma usually is 3 to 6 months after detectable virus appears in peripheral blood. Although the mechanism of retroviral-induced leukemia has been under investigation for a number of years, studies with recombinant murine retrovirus isolates have had limited success (2,3). Haberman and Velicer (4) have reported the isolation of a putative recombinant feline virus (PR-8) having properties of both the exogenous FeLV-R and the endogenous RD-114 virus, analogous to recombinants isolated within the murine leukemia system (3,5,6).

In order to determine if the PR-8 isolate was infectious to the cat, we infected specific-pathogen-free (SPF) cats of different ages with the in vitro-derived virus. We report here the infection of SPF cats with PR-8 and the subsequent development of eosinophilic leukemia in one cat and extreme eosinophilic hyperplasia in another after an incubation period of 1-1/2 to 2 years.
METHODS

Cats and Challenge

Cats were obtained from The Ohio State University specific-pathogen-free cat colony. These cats were determined to be free of any previous exposure to FeLV.

Fourteen cats were challenged with PR-8 by a single 1 ml intraperitoneal injection. Five newborn kittens received PR-8. Nine older cats received a 10 mg/kg dose of methylprednisolone acetate (Depo-Medrol, The Upjohn Co., Kalamazoo, MI) (10 mg/kg body weight) at the time of the infection. All of the cats were tested weekly for the first eight weeks and monthly thereafter for circulating FeLV group-specific antigen (p27) and antibody to the transformation-specific antigen (FOCMA) as has been previously described (7,8).

Euthanized cats received an overdose of ketamine hydrochloride. Representative tissues were fixed in neutral phosphate buffered formalin for histopathologic study. For electron microscopy, bone marrow aspirates or solid tissues trimmed in 1 cubic millimeter pieces were fixed in 3% glutaraldehyde with 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in 1.33% osmium with S-collidine buffer at pH 7.4, dehydrated through ascending concentrations of ethanol, transferred to propylene oxide, and embedded in Epon 812 (Shell Chemical Company, New York, NY). Thin sections were stained with uranyl acetate and lead citrate and examined with a Phillips 300 electron microscope.
RESULTS

Challenge and Monitoring

Table 1.1 details the results of infection of 14 cats with the PR-8 virus. Twelve developed detectable FeLV p27 in their peripheral blood leukocytes; 6 of these died within six weeks of the challenge. Ten of the cats developed antibodies to FOCMA. Of the five cats developing persistent viremia (>10 weeks), 53B-4 and 76 developed marked eosinophilia with mature eosinophilic leukocytes in the peripheral blood. Cat 53-B4 was losing weight and had splenomegaly at week 114 and was euthanized. Cat 76 had mild weight loss and was euthanized at week 120.

Immunofluorescence to FeLV p27 was detected in the peripheral blood PMNs of both 53-4 and 76 along with a few myeloid precursor cells. The eosinophils were negative as were the aberrant cells. A similar immunofluorescent profile was also found in the bone marrow. Cats 53-4 and 76 were found to be negative for FOCMA antibody at the time of euthanasia.

To test for viral or viral-induced antigen on the surfaces of the eosinophilic precursors, an indirect immunofluorescence assay was performed. The cells were uniformly unreactive with serum from an FeLV-inoculated cat. Also, autologous sera and serum from a FeLV-immunized goat were uniformly negative.

Hematologic Changes

The hematologic data for cats 53-4 and 76 are presented in Table 1.2. The changes noted include eosinophilia with circulating
eosinophilic metamyelocytes, eosinophilic myelocytes, and cells classified as eosinophilic progranulocytes. Lymphocytosis was present in both cats. The lymphocytes were small with normal morphology. Both cats had non-regenerative anemia. Thrombocytopenia was present in cat 53-4. The bone marrow findings were similar in both animals. Both cats had hypercellular bone marrows with increased myeloid/erythroid ratios (22.4 in cat 53-4, 31.1 in cat 76). The predominant cell was a large cell with abundant cytoplasm containing numerous azurophilic granules, variable numbers of faintly staining eosinophilic granules, a round-to-oval nucleus with finely stippled chromatin and a prominent nucleolus (Fig. 1.1). These cells, interpreted as eosinophilic progranulocytes, comprised 72.8% of the bone marrow of 53-4 and 44.2% of that of cat 76. Ultrastructurally, the cells were characterized by numerous mitochondria, prominent rough endoplasmic reticulum, numerous ribosomes, and distinctive rod-shaped, electron-dense granules with a crystalline lamellar core (Fig. 1.2). Some of the granules were degenerating with separation of the fibrils into structures resembling myelin figures. Eosinophilic myelocytes were increased in the marrow of both cats (9.6% for 53-4, 20.2% for 76) as were eosinophilic meta­myelocytes (2.0% in 53-4, 6.0% in 76). The other notable finding was a disproportionate number of progranulocytes (2.8% in 53-4, 13.4% in 76). The eosinophilia in the peripheral blood gradually subsided in cat 76 over a period of 8 months, but the eosinophilic hyperplasia persisted in the bone marrow. Progressive nonregenerative anemia
developed in this cat, but it was not accompanied by granulocytopenia or thrombocytopenia.

Necropsy and Histopathologic Findings

At necropsy, cat 53-4 had, other than pale tissues, lesions in the spleen, liver, thymus, lymph nodes, and bone marrow. The spleen was markedly enlarged (1.5 cm x 3.0 cm x 12 cm) and firm. The cut surface was red but relatively dry with numerous small tan nodules (follicles). The liver was of normal size but had an accentuated lobular pattern. A 0.5 cm x 0.5 cm x 1.5 cm firm, tan mass was found in the cranial mediastinum in the region of the thymus. The bone marrow was firm and reddish-tan throughout the length of the long bones. Histopathologic examination of cat 53-4 revealed diffuse infiltration of the bone marrow by a homogenous population of large round cells with round nuclei containing prominent nucleoli and intracytoplasmic eosinophilic granules. Numerous mitoses were present. The thymic mass was comprised of a similar uniform population of eosinophilic leukocytes. In the spleen, the general architecture was preserved but a massive infiltrate of immature eosinophilic leukocytes was present in the red pulp. Multifocal intertubular infiltrates of immature eosinophilic leukocytes were present in the kidney. In the liver, small multifocal infiltrates of similar eosinophilic cells were present in the regions of the portal triads but were not restricted to those areas. Moderate numbers of eosinophilic precursors were distributed diffusely in the medullary sinuses of the mesenteric lymph nodes along with a few distinct but
non-encapsulated foci of a homogenous eosinophilic leukocytes in the cortex. No lesions were detected in the lungs and myocardium.

Gross lesions in cat 76 included splenomegaly and red firm bone marrow. Histopathologic examination revealed marked hypercellularity of the bone marrow due to eosinophilic hyperplasia. Eosinophilic infiltrates were not detectable in other tissues. Moderate lymphoid hyperplasia and diffuse reticuloendothelial hyperplasia were present in the spleen.
DISCUSSION

Infection of SPF cats with a putative FeLV-R/RD-114 recombinant virus produced viremia in twelve of fourteen cats. Of the twelve cats that had detectable p27 in their peripheral blood, six died within eight weeks of the challenge, one cleared the virus from its system, and five became persistently infected. After a long incubation period, two of the five persistently infected cats developed extreme eosinophilia, one was diagnosed with hypereosinophilia and the other with eosinophilic leukemia. Although eosinophilic leukemia has been reported in pet cats (9), it is rare and has never been associated with a retroviral infection (10). Cats with naturally occurring eosinophilic leukemias have tested negative for FeLV infection (10). Eosinophilic leukemia has been observed in other mammals, including humans (11,12), but it has not been associated with retroviral infection. Our data indicates that a previously unaffected cell type has been transformed by possible viral recombinant of an exogenous and an endogenous retrovirus. Although PR-8 virus has been shown to have envelope properties of both FeLV-R and RD-114 (4), we have not demonstrated that is due to a genetic recombination. The manifestation of a different neoplastic disease may be due to a change in envelope glycoprotein allowing the R-FeLV genome to enter a previously resistant cell type. Further work is necessary to determine if the PR-8 isolate is a genetic recombinant or phenotypic mixing of the two parent viruses.
The distinction between eosinophilic leukemia and nonmalignant hypereosinophilic syndromes is often controversial (11,12). The diagnosis of eosinophilic leukemia in cat 53-4 was based on persistent eosinophilia with disproportionate numbers of immature eosinophilic granulocytes in the blood and bone marrow, eosinophilic infiltrates in the lung, heart and intestine, and the presence of anemia and thrombocytopenia. The marked eosinophilia with predominance of eosinophilic precursors in the bone marrow of cat 76 was interpreted as extreme eosinophilic hyperplasia. The cause of the eosinophilic hyperplasia was not determined. The lack of abnormal eosinophilic precursors in the blood and the absence of eosinophilic infiltrates in the other tissues precludes the diagnosis of leukemia. The lack of infiltrates in other tissues also does not lend support to the diagnosis of hypereosinophilia syndrome or eosinophilia secondary to allergy or parasitic infestation. The cats used in this study were derived from our specific-pathogen-free colony and were tested and found negative for external and internal parasites. The lack of an obvious cause of eosinophilia could be interpreted as evidence for preleukemia. Hypereosinophilia has been associated with lymphoblastic malignancy in human patients (13-15). It has been suggested that the eosinophilia may be caused by soluble factors released by neoplastic T-lymphocytes (15). In our cats, we found no evidence of lymphoma, but lymphocytosis was present in both animals. The lymphoma induced by FeLV-R is usually of T-cell origin.
(16) and the possibility does exist that the PR-8 infection may induce the production of an eosinopoietic factor.

The extreme eosinophilia induced in two cats by inoculation with a putative recombinant of FeLV-R and an endogenous retrovirus of cats suggests that this agent either directly transforms eosinophilic leukocytes or induces the production of eosinopoietic or chemotactic factors by other cells. This syndrome should provide viral-induced animal models for eosinophilia leukemia or hypereosinophilia associated with lymphoid malignancy.
SUMMARY

Twelve of 14 cats challenged with a putative recombinant feline retrovirus developed infection. Two cats developed eosinophilia, one was diagnosed with eosinophilic leukemia and the other with extreme eosinophilic hyperplasia. The recombinant virus has characteristics of an exogenous (FeLV-R) and an endogenous (RD-114) feline retrovirus. Changes of the viral envelope properties appear to have altered the pathogenicity of exogenous virus.
Table 1.1.

Viral Proteins and FOCMA Antibodies
in Cats Infected with PR-8 Isolate of FeLV

<table>
<thead>
<tr>
<th></th>
<th>Weeks p27 detected in blood</th>
<th>Highest FOCMA titer</th>
<th>Weeks survived after challenge</th>
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<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53B-1</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>112</td>
<td>16</td>
<td>114</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>106</td>
<td>64</td>
<td>108</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>66</td>
<td>4a</td>
<td>128</td>
<td>b</td>
</tr>
<tr>
<td>69</td>
<td>0</td>
<td>8</td>
<td>b</td>
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</tr>
<tr>
<td>72</td>
<td>0</td>
<td>64</td>
<td>b</td>
</tr>
<tr>
<td>76</td>
<td>118</td>
<td>128</td>
<td>120</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>64</td>
<td>6</td>
</tr>
</tbody>
</table>

a Transient p27 in peripheral blood.
b Alive at time of printing.

Group 1 - mean age 1 day at time of infection.
Group 2 - mean age 12 weeks at time of infection.
Table 1.2. Hematologic Data for Cats with Eosinophilia

<table>
<thead>
<tr>
<th>Cat number</th>
<th>Weeks after challenge</th>
<th>53-4 (114)</th>
<th>76 (86)</th>
<th>76 (102)</th>
<th>76 (120)</th>
<th>Reference Value*</th>
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<tr>
<td></td>
<td></td>
<td>Packed Cell Volume</td>
<td>0.18</td>
<td>0.42</td>
<td>0.31</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemoglobin g/dl</td>
<td>5.8</td>
<td>14.2</td>
<td>10.2</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erythrocytes X10^12/liter</td>
<td>3.15</td>
<td>9.43</td>
<td>6.81</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean Corpuscular Volume femtoliter</td>
<td>58</td>
<td>45</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean Corpuscular Hemoglobin g/dl X10^12/liter</td>
<td>31.8</td>
<td>34.2</td>
<td>33.4</td>
<td>33.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reticulocytes X10^9/liter</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leukocytes X10^9/liter</td>
<td>70.8</td>
<td>43.1</td>
<td>44.8</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Band neutrophils X10^9/liter</td>
<td>0</td>
<td>0.8</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Segmented neutrophils X10^9/liter</td>
<td>5.7</td>
<td>12.1</td>
<td>17.9</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophilic progranulocytes X10^9/liter</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophilic myelocytes X10^9/liter</td>
<td>19.1</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophilic metamyelocytes X10^9/liter</td>
<td>10.6</td>
<td>1.3</td>
<td>3.1</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>Band eosinophils X10^9/liter</td>
<td>2.1</td>
<td>9.1</td>
<td>12.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Segmented eosinophils X10^9/liter</td>
<td>5.0</td>
<td>8.2</td>
<td>4.5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nucleated red cells X10^9/liter</td>
<td>0.7</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphocytes X10^9/liter</td>
<td>21.9</td>
<td>9.9</td>
<td>4.9</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monocytes X10^9/liter</td>
<td>0.8</td>
<td>0.8</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platelets X10^9/liter</td>
<td>79.0</td>
<td>465.0</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>


+ Not counted separately from segmented eosinophils.
Plate I. Eosinophilic precursors from the bone marrow of a cat inoculated with the PR-8 isolate of FeLV. Most of the cells have fine nuclear chromatin, prominent nucleoli, numerous azurophilic granules and faintly stained eosinophilic granules. H and E stain, X1680.
Plate II. Eosinophilic progranulocytes in the bone marrow of a cat inoculated with the PR-8 isolate of FeLV. X8400.

Inset shows lamellar appearance of electron dense granules, X53,000.
CHAPTER II

THE REVERSAL OF FELINE RETROVIRAL INDUCED SUPPRESSION OF
Lymphocyte Con A RECEPTOR MOBILITY BY INDOMETHACIN AND PGE$_2$

INTRODUCTION

Feline retroviral infection in cats causes leucopenia, atrophy of thymic and paracortical lymph nodes and immune deficiency. The latter is characterized by retention of allografts, depressed in vitro lymphocyte blastogenesis and a loss of lymphocyte surface membrane mobility (1-5). The immunodysfunction caused by feline leukemia virus (FeLV) infection can be experimentally reproduced with inactivated virus and also with a purified virus envelope protein component FeLV p15E (6-12). It has been shown that inactivated FeLV or FeLV p15E inhibits interleukin II stimulation (13), erythropoiesis (14), mitogenesis (4,7-10), graft rejection (3) and lymphocyte capping of Con A (5,10-12).

The mechanism by which FeLV causes immunosuppression has not been established. However, Nichols et al. (11) found that colchicine, a microtubular disrupting agent, was able to reverse the FeLV inhibition of Con A receptor capping. This suggests a possible microtubular stabilizing effect by the virus, which could be mediated by the cyclic nucleotide system. Recent work demonstrates that
prostaglandins (PG) alter both the mobility and expression of cell surface receptors (15-17). Also, prostaglandins have been associated with immune modulating functions including immunosuppression (18). In order to investigate the involvement of the prostaglandin system in the suppression of Con A capping by FeLV, we have studied the effects of exogenous PGE$_2$ and indomethacin, a PG inhibitor, on the receptor capping of lymphocytes.
METHODS

Animals

Adult cats were used as blood donors in these experiments. These animals were from the specific-pathogen-free cat colony of the Department of Veterinary Pathobiology, Ohio State University. For the study of FeLV viremic animals, four cats received a 20% (w/v) cell-free tumor homogenate containing $8 \times 10^5$ focus forming units/ml of in vivo passed Richard FeLV (19). All 4 cats demonstrated chronic viremia as tested by an indirect immunofluorescence test (20).

Blood preparation

Venous blood was drawn and defibrinated with glass beads. Mononuclear cells were isolated using a Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient. Cells were removed and washed in MEM-E (GIBCO, Grand Island, NY).

Receptor mobility

Lymphocytes were tested for capping with FITC conjugated Con A or goat anti-cat globin (Miles Biochemicals, Elkhart, IN). PGE$_2$ (a gift from Dr. J. Pike, Upjohn, Kalamazoo, MI) and indomethacin (Sigma, St. Louis, MO) were dissolved in 95% ethanol at .01M and diluted to specified concentrations with MEM-E. No ethanol effect was observed in the testing. Lymphocytes ($1 \times 10^6$) were incubated for 15 min. at 37°C in 12 x 75 polypropylene tubes with the specific treatments. Following incubation, the cells were washed twice with MEM-E and then counted.

The percentage of cells capped was determined by examining 100 fluorescent lymphocytes with an epifluorescent Zeiss microscope using
a X100 oil immersion lens. Only single cells or small clumps (2 to 3 cells) were scored for capping. A lymphocyte was considered capped if 50% of the visible cell surface was fluorescent.

**Virus isolation**

The purification of Kawakami-Theilen FeLV (KT-FeLV) from tissue culture media has been described (21). Sucrose-banded virus was dialyzed 2X in Hank's Balanced Salt Solution (GIBCO) and 1X in 1640 (GIBCO) with 1.0% antibiotics (penicillin G, streptomycin sulfate). The virus was inactivated by ultraviolet light using the method of Yohn et al. (22). Protein determination was performed using a BioRad Protein Kit (BioRad, Richmond, CA).

**Purification of p15E**

The purification of FeLV p15E from KT-FeLV has been described previously (7). A few modifications were used. Briefly, the purified virus was extracted with a Triton X containing buffer (1.0% Triton X100, 0.05M Tris-HCl, 0.6M KCl, 0.01M EDTA, 0.01M Dithiothreitol) and ultracentrifuged to remove undissolved elements. The supernatant fraction was extracted with ether and dialyzed against distilled water. The precipitate was collected by centrifugation, resuspended in Triton X buffer and added to a Sephacryl 200 (Pharmacia) column. Fractions containing the protein were collected, ether extracted and dialyzed against distilled water. Purity was determined by polyacrylamide electrophoresis. The purified protein was dialyzed and UV irradiated.

**Statistical analysis**

Statistical evaluation was performed using Student's t-test.
RESULTS

Effect of Indomethacin and PGE₂ on Con A cap formation

Indomethacin, at micromolar concentration between 0.1 and 10 μM (Fig. 2.1 and 2.2), and PGE₂, 1.0 μM (Fig. 2.3 and 2.4), significantly reversed the suppressive effect of inactivated FeLV or FeLV pl15E on the Con A receptor cap formation of normal feline peripheral blood lymphocytes (PBL). Inactivated FeLV and FeLV-pl15E both decreased lymphocyte Con A receptor motility (capping) (p < .001). The indomethacin had no effect upon normal cellular cap formation at any of the concentrations tested. PGE₂, at a concentration of 1 μM, had no effect on the receptor capping of normal lymphocyte, with or without indomethacin present at 1.0 or 0.1 μM.

Effect of FeLV on immunoglobulin receptor mobility

The capping of immunoglobulin receptors of PBL of normal cats was unaffected by inactivated FeLV coincubation. In four experiments, PBL from normal cats formed caps on 33.5 ± 1.6 percent of the fluorescent cells while cells incubated with inactivated FeLV had visible cap formations on 31.3 ± 1.3 percent.

Effect of indomethacin on Con A receptor capping of FeLV-infected cats

Figure 2.5 summarizes the results of the in vitro effect of indomethacin upon Con A receptor capping of PBL from cats that were chronically infected with FeLV. Twenty-six and three tenths percent of the cells of the infected cats formed Con A caps as compared to 50.2 percent in uninfected cats. Indomethacin at a concentration of
10 or 1.0 um statistically increased the percentage of capping of PBL from infected cats (p<.01 and p<.04), while having no effect upon cap formation of normal PBL.
DISCUSSION

Feline lymphocyte cap formation of Con A receptors was significantly decreased by inactivated FeLV and its envelope protein FeLV p15E. Micromolar concentrations of indomethacin were able to reverse this observed suppression. Micromolar concentrations of prostaglandin E$_2$ also reversed the suppressive effects of the virus and p15E. The reversal of the suppressed Con A receptor capping was also observed in peripheral blood lymphocytes from cats with chronic FeLV infections. Although the virus was able to suppress Con A cap formation, which includes both B- and T-lymphocytes (23), immunoglobulin receptor capping, involving B-lymphocytes, was unaffected by FeLV.

Lymphocytes from FeLV infected cats exhibit decreased Con A receptor mobility, as do normal PBL of cats and humans when incubated with FeLV or FeLV p15E. We have found that the FeLV and FeLV p15E inhibited other hematopoietic cell functions including mitogenesis (4,7-10), interleukin II sensitivity (13), antigen recognition (3,6,24) and the mixed lymphocyte reaction (25). Others have shown similar retroviral suppression of in vivo macrophage migration, graft rejection and antibody formation in birds, mice and monkeys (26-28). Our studies of the receptor cap forming mechanism have indicated that the action of FeLV p15E may be at or near the cellular membrane surface (5,11,12). This would correspond to the probable site of FeLV p15E incorporation into the cell, since the protein is highly hydrophobic (29) and would most likely bind to a lipid layer of the cell membrane. Once inserted, it may cause malfunction of the normal
cell membrane. Work with colchicine has indicated a possible microtubular interaction (11). The close association of microtubules, cyclic nucleotide and prostaglandins to the capping event (15-17,30), prompted us to look at the effects of indomethacin and PGE\textsubscript{2} on the viral suppression of Con A receptor cap formation. Also, prostaglandins have been shown to be potent modulators of the immune system (18) and the possibility exists for a connection between FeLV infection and altered prostaglandin synthesis.

Indomethacin, at concentrations between 10 and 0.1 um, has been shown to block the synthesis of prostaglandins by the inhibition of cyclooxygenase (31). We found that prostaglandin production was not necessary for cap formation of normal feline PBL, but at high concentrations (>100 μM) we did observe a decline in the numbers of capped cells. Tests to determine if this was due directly to prostaglandin inhibition were not performed. The addition of exogenous prostaglandin, PGE\textsubscript{2}, at greater than physiologic levels had no statistically significant effect upon the normal cellular receptor capping, but at this concentration it was able to increase the FeLV-suppressed receptor capping of normal cells. Indomethacin and PGE\textsubscript{2}, in most cases, have opposite effects upon immune cells and outwardly these results may appear contradictory, but Kantor and Hiroshi (32) have shown that indomethacin is able to increase cellular cAMP levels. This is also one of the known actions of PGE\textsubscript{2}. Since neither PGE\textsubscript{2} nor indomethacin had any effect upon normal Con A receptor capping, but both reversed the FeLV or FeLV p15E-induced suppression, their effect may be similar and could be cyclic nucleotide related. As we
have seen previously, FeLV and FeLV p15E interfered with microtubular action, which again may be related to alterations in cyclic nucleotides. In order to determine if the virus is causing a disturbance of the cyclic nucleotide system, research was initiated to measure cAMP and cGMP levels in cells incubated with FeLV.

The resistance of immunoglobulin receptor capping to FeLV suppression is interesting. The tumors normally found in cats infected with FeLV are of T cell origin (33) and the inhibitory effects of FeLV in vitro can, in all cases, be related to a T cell defect. To our knowledge, there are no reports of specific inhibition of B cell related to retroviral cocultivation. The mechanism of a T cell-specific inhibitory effect is unknown, and most of the observed in vivo and in vitro inhibition could be associated with cellular regulatory products either being released in greater quantities, such as prostaglandins, or being inhibited from release or action, such as interleukens.

The ability of indomethacin to increase lymphocyte cap formation in FeLV-infected cats may be useful for a possible therapy. With the correlation of the in vivo and in vitro testing with indomethacin, it shows promise as an immune modulator that may be able to stimulate the immune system of the FeLV-infected cat.
SUMMARY

Indomethacin and PGE$_2$ were able to reverse the in vitro suppressive effects on Con A receptor capping induced by both inactivated FeLV and FeLV pl5E. Normal Con A receptor capping was not affected by indomethacin or PGE$_2$. Indomethacin enhanced the Con A receptor cap formation of lymphocyte from chronically FeLV-infected cats. Immunoglobulin receptor cap formation in normal cat lymphocytes was not affected by FeLV coincubation.
Figure 2.1. Effect of indomethacin on FeLV-induced suppression of Con A receptor mobility. Results represent a mean ± SE of 4 tests. (*p < .001) (**p < .05)
Figure 2.2. Effect of indomethacin on FeLV p15E-induced suppression of Con A receptor mobility. Results represent a mean ± SE of 3 tests. (*p < .05) (**p < .005)
Figure 2.3. Effect of PGE$_2$ on normal Con A cap formation and FeLV-induced suppression. Each point represents the mean ± SE of 5 experiments.
Figure 2.4. Effect of PGE$_2$ on FeLV pl5E-induced suppression of Con A cap formation. Each point represents the mean ± SE of 3 experiments.
Figure 2.5. Effect of indomethacin on Con A receptor cap formation on cells from FeLV-infected cats. Each point represents the mean ± SE of 4 experiments.
CHAPTER III

THE IN VITRO EFFECT OF PROSTAGLANDIN INHIBITORS AND CYCLIC AMP ENHANCERS ON THE SUPPRESSIVE ACTION OF FeLV P15E

INTRODUCTION

Retroviral infections are known to cause a dramatic immune dysfunction in the infected host (1). In particular, feline leukemia virus (FeLV) or the p15E component of its envelope, interferes with the normal mitogenic response of peripheral blood lymphocytes (2-6), the feline mixed lymphocyte reaction (7), interleukin II stimulation of human lymphocytes (8), erythropoiesis in feline bone marrow cultures (9) and Con A receptor capping of both human and feline lymphocytes (10-13).

The mechanism of the suppressive action of FeLV must be involved with both the infectious process and also the ability of the virus to remain in host immune system for extended periods of time. Work using indomethacin during Concanavalin A (Con A) receptor capping has indicated that the prostaglandin or cyclic nucleotide systems may be involved with the viral suppressive action (13). Also, the prostaglandin system has been linked closely with immune system control.
mechanisms (14). The close association between mitogenic stimulation, prostaglandins and cyclic nucleotides indicates that FeLV may be immunosuppressive via disruption of either necessary prostaglandin or cyclic nucleotide signals during the response.

The studies reported here were undertaken to determine if an alteration in cellular prostaglandins or cyclic nucleotides were related to FeLV-induced suppression of Con A stimulation. Our results indicate that the suppression by FeLV may be related to PGE$_1$ or E$_2$ levels, possibly through cyclic AMP mediation.
METHODS

Animals

Adult cats were used as blood donors in these experiments. These animals were from the specific-pathogen-free cat colony of the Department of Veterinary Pathobiology, Ohio State University. For the study of FeLV viremic animals, cats received a 20% (w/v) cell-free tumor homogenate containing $8 \times 10^5$ focus-forming units/ml of in vivo passed Rickard FeLV (15). All cats demonstrated chronic viremia as tested by an indirect immunofluorescence test (16).

Blood preparation

Venous blood was drawn with 0.4% sodium citrate. Mononuclear cells were isolated using a Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient. Cells were removed and washed in L-15 medium (GIBCO, Grand Island, NY) with 0.1% EDTA Sigma and 1.0% antibiotics (penicillin G, streptomycin sulfate).

Virus isolation

The purification of Kawakami-Theilen FeLV (KT-FeLV) from tissue culture media has been described (17). Sucrose-banded virus was dialyzed 2X in Hank's Balanced Salt Solution (GIBCO) and 1X in 1640 (GIBCO) with 1% antibiotics. The virus was ultraviolet inactivated using the method of Yohn et al. (18). Protein determination was performed using a BioRad Protein Kit (BioRad, Richmond, CA).

Purification of p15E

The purification of FeLV p15E from KT-FeLV has been described previously (3). A few modifications were used. Briefly, the purified virus was extracted with a Triton X containing buffer (1.0%
Triton X100, 0.05M Tris-HCl, 0.6M KCl, 0.01M EDTA, 0.01M Dithiothreitol) and ultracentrifuged to remove undissolved elements. The supernatant fractions were exhaustively extracted with ether and dialyzed against distilled water. The precipitate was collected by centrifugation, resuspended in TX buffer and added to a sephacryl 200 (Pharmacia) column. Fractions containing the protein were collected, ether extracted and dialyzed against distilled water. Purity was determined by polyacrylamide gel electrophoresis. The purified protein was dialyzed and UV irradiated.

**Lymphoblast transformation assay**

The lymphoblast transformation test (LBT) is a modification of one which has been previously described (2). Briefly, purified blood lymphocytes (PBL) were added at a concentration of $1 \times 10^5$ cells per well to a 96 well plate. Con A, FeLV, FeLV p15E, prostaglandins and/or prostaglandin inhibitors were added to bring the total volume per well to 200 ul. Five percent fetal calf serum (Sterile Systems, Salt Lake City, UT) was added and the suspension was incubated 4 days. Eighteen hours before harvest the cultures were pulsed with $[^3]H$ thymidine (New England Nuclear, Boston MA). The plates were harvested on glass wool using a Mash unit (Brandel, Gathersburg, MD) and assessed for thymidine uptake using a liquid scintillation counter (Packard Instruments, Downers Grove, IL).

**Reagents**

Indomethacin, Nordihydroguaniretic acid (NDGA), Imidazole, Tranylcypromine (Tranyl), 3-isobutyl-1-methyl xanthine (IBX) (Sigma),
forskolin (Calbiochem, La Jolla, CA), arachidonic acid and the prostaglandins E₁, E₂, D₂, F₂α (gifts of Dr. J. Pike, Upjohn, Kalamazoo, MI) were dissolved in 95% ethanol to a concentration of 0.01M and diluted to needed concentrations with culture media. No alcohol effect was observed in any of the culturing conditions. Papaverine (Sigma) and 6 keto PGF₁α (Dr. J. Pike) were dissolved in sterile distilled water. Two X Con A (Miles Biochemical, Elkhart, IN) or Con A (Sigma was titrated and used at its maximal stimulatory dose, .4 μg/well and 20 μg/well, respectively. Culturing media consisted of RPMI 1640 (GIBCO) with hepes, 1.0% glutamine and 1.0% antibiotics.

**Statistical analysis**

Statistical evaluation was performed using Student's t-test.
RESULTS

Effect of exogenous prostaglandins on the feline LBT

To determine if prostaglandins had an effect upon a normal mitogenic response of feline PBL, exogenous prostaglandins E₁, E₂, D₂, F₂α and 6-keto-F₁α were added to the Con A LBT in various concentrations. Only prostaglandins of the E series were found to have any significant effect: Both PGE and PGE₂ (0.1 μM) induced a 40 and 50% suppression of the ³H-thymidine uptake in PBL. In the presence of FeLV and PGE₂, the total counts decreased to 28% of the control as compared to 50% without PGE₂ (Fig. 3.1). Arachidonic acid (Fig. 3.2), at concentrations between 10 μM to 0.1 μM caused a slight suppression of the LBT at 10 μM, with viral protein, arachidonic acid caused no change in viral suppression at any of the concentrations tested.

Effect of prostaglandin inhibitor upon viral and p15E suppression

The prostaglandin inhibitors used in this study were indomethacin and NDGA, both of which are known inhibitors of cyclooxygenase (19,20). NDGA has also been shown to have lipoxygenase inhibitory activity. Also, the effects of tranyl, an inhibitor of prostacyclin synthetase (20), and imidazole, an inhibitor of thromboxane synthetase (21), were studied. Indomethacin enhanced thymidine uptake by Con A stimulated lymphocytes, at concentrations above .01 μM. For all the inhibitors tested, concentrations of greater than 10 μM significantly inhibited Con A stimulation and these concentrations were not used in further studies.
Both of the cyclooxygenase inhibitor had the ability to reverse the viral suppression of Con A stimulation. Indomethacin (Fig. 3.3) significantly reversed the suppression at concentrations of 0.1 μM (p < .001) and above. It also was able to reverse p15E suppression at concentrations of 1.0 μM (p < .001). Reversing the FeLV p15E suppressive effect by indomethacin was observed at a concentration of 0.1 μM (Fig. 3.3). It was only effective if added within the first 24 hr of culture; by 48 hr there was no effect. NDGA was less effective but did significantly (p < .001) reverse the FeLV effect at 1.0 μM.

Tranyl and imidazole, at concentrations between 10 μM and 0.1 μM had no effect on normal Con A PBL simulation. In addition, it did not reverse the FeLV immune suppression of Con A LBT.

Effect of cyclic AMP enhancers upon FeLV suppression

Papaverine and IBX, both inhibitors of phosphodiesterase activity (23, 24), and forskolin, an activator of adenylate cyclase (25), were added to the LBT as cAMP level enhancers. The phosphodiesterase inhibitors strongly decreased normal Con A stimulation at 10 μM, but, at lower concentrations, they had no effect (Figs. 3.5 and 3.6). In addition, neither papaverine or IBX had any effect on the FeLV suppression on the Con A LBT. Forskolin had no effect upon normal Con A stimulated cultures, some stimulation was seen at the higher concentrations tested but this was not significant. At a concentration of 1.0 μM, the FeLV-induced inhibition was reversed by forskolin (p < .04) (Fig. 3.7).
Effect of indomethacin on Con A LBT of viral-infected cats

Indomethacin significantly increased the Con A simulation of infected cats (Fig. 3.8). At concentrations between 1 and 0.01 μM, Con A stimulation was increased significantly. At a concentration of 10 μM, a significant depression of stimulation occurred.
DISCUSSION

Previous work with FeLV pl5E has shown that this polypeptide can suppress a number of normal cellular functions including Con A-induced mitogenesis and receptor capping (2-13). The addition of indomethacin or PGE$_2$ reversed the suppressive action of FeLV pl5E upon the receptor capping process (13). Also, colchicine, a microtubular destabilizing agent, was able to augment the suppression of capping (11). These studies indicated a possible relationship between prostaglandin, cyclic nucleotides, microtubules and FeLV suppression.

In this study, indomethacin caused slight augmentation of the Con A LBT response. Others have found that cyclooxygenase inhibitors can enhance mitogen responses (14,26-28) of both normal cells and cells from patients with other suppressive disorders (29-32). NDGA inhibited the LBT at high concentrations (>1 μM) but had no effect at lower concentrations. The inhibitory effect may be related to its lipoxygenase inhibition (20). Both indomethacin and NDGA were able to significantly reverse the FeLV-induced suppression of the LBT, indomethacin being more efficient. Indomethacin was also able to enhance the Con A stimulation of PBL of infected cats. The mechanism for the reversing effect of these drugs is probably through the inhibition of prostaglandin production, probably the PGE$_2$ series, which has been linked with suppressor cell actions (27). When prostacycline or thromboxine production was inhibited by tranyl or imidazole, no effect on the LBT or FeLV suppression was observed, so that neither of these cyclooxygenase products appear to be essential.
for the LBT in the cat or in FeLV suppression, but measurements of their levels were not taken. Arachidonic acid, which induces increased prostaglandin production (33) caused suppression of the normal LBT and had no effect upon the FeLV inhibition. These results would indicate that the prostaglandins may be involved with the FeLV-induced suppression of the Con A response. If FeLV caused an increase in PGE₂ levels, our results indicate that the LBT would be inhibited. Blocking prostaglandin production stopped FeLV suppressive effects.

The studies with the cAMP inducers were undertaken because Con A stimulation of PBL is known to cause a rise in the cellular cAMP levels (34,35). One possible mechanism of FeLV suppression, suggested by the suppression of receptor capping by FeLV, was a disruption of cAMP levels induced by either FeLV p15E insertion into the cell membrane or by alteration of cyclic nucleotide metabolism. If cAMP production were decreased or if cAMP breakdown was increased, then the Con A response could be inhibited. By inhibiting cAMP-phosphodiesterase, the enzyme important for cAMP breakdown, the cellular cAMP should be elevated. Indomethacin has been reported to have this action (22). However, when cAMP-phosphodiesterase inhibitors were added, they had no effect upon the FeLV suppressive effect and inhibited the Con A response. This does not eliminate the possibility the cAMP may be involved, since effects of these agents on cAMP levels were not measured. Forskolin causes the production of cAMP by activating the adenylate cyclase, the enzyme which synthesizes cAMP (25). When it was included in the LBT alone, there was no
effect (results not shown), at a concentration of 1.0 μM the drug reversed the FeLV effect, indicating that cAMP production may be interfered with.

In order to determine if prostaglandins and/or cyclic nucleotides are involved with the suppressive action of FeLV pl5E, further testing will be necessary, including measurements of their levels during the test period with the various drugs. Tests with other inhibitors and activators of both systems would also be informative.
SUMMARY

Indomethacin, a cyclooxygenase inhibitor (19), was able to reverse the inhibitory action of FeLV on the Con A mitogenic response of normal feline peripheral blood lymphocytes. It also caused an enhancement of the Con A response of FeLV-infected cats. Nordihydro-guariaretic acid, another inhibitor of prostaglandins and also lipoxigenase, was similarly able to reverse FeLV suppression in vitro. Inhibitors of prostacyclin and thromboxane, tranylcypromine and imidazole, respectively, had no effect on the normal LBT or on the FeLV inhibition.

To determine if the enhancing effect of the cyclooxygenase inhibitors was due to an increase in cellular cyclic AMP, three enhancers were used. Two phosphodiesterase inhibitors, papaverine and 3-isobutyl-1-methyl xanthine, were studied and found to inhibit the LBT of normal cells, while having little effect upon the FeLV suppression. Forskolin, an adenylate cyclase activator, was able to reverse the FeLV effect.
Figure 3.1. The effect of FeLV and PGE$_2$ upon the normal feline Con A LBT. The results are expressed as a mean ± standard error of 5 experiments.
Figure 3.2. The effect of arachidonic acid on the normal Con A LBT and a Con A LBT with FeLV. The results are expressed as a comparison to normal control cell. Each point represents a mean ± SE of 6 experiments. (*p < .02 vs. control).
Figure 3.3. The effect of indomethacin on FeLV (n=20) and FeLV-p15E (n=4)—induced suppression of the Con A LBT. Results expressed as comparison of normal control cells. (*p<.01 vs. control) (**p<.001 vs. control).
Figure 3.4. The effect of NDGA on FeLV-induced suppression of the Con A LBT. Each point represents the mean ± SE of 6 experiments. (*p<.05 vs. control) (**p<.001 vs. control).
Figure 3.5. The effect of papaverine on the Con A LBT with and without FeLV. Each point represents the mean ± SE of 6 experiments. (*p < .01 vs. control)
Figure 3.6. The effect of IBX on the Con A LBT with and without FeLV. Each point represents the mean ± SE of 6 experiments. (*p < .01 vs. control).
Figure 3.7. The effect of forskolin on the FeLV-induced suppression of the Con A response of normal cells. The results are expressed as percentage enhancement of thymidine uptake during incubation with FeLV and Con A inhibition. Each point represents the mean ± SE of 6 experiments.
Figure 3.8. The effect of indomethacin upon Con A stimulation of FeLV-infected cats. Each point represents the mean ± SE of 8 experiments.
CHAPTER IV

THE EFFECT OF FeLV UPON PGE$_2$ AND CYCLIC NUCLEOTIDE PRODUCTION IN VITRO

INTRODUCTION

The suppressive effect of retroviruses on the immune system, both in vivo and in vitro, is well documented (1). Administration of inactivated FeLV into young kittens induced immune dysfunctions similar to defects observed in leukemic cats (2). It was subsequently found that an inactivated FeLV vaccine predisposed the cat to oncogenic disease. The addition of inactivated virus to in vitro cell culture systems also causes an inhibitory effect (3-13).

Work with feline leukemia virus (FeLV) and its envelope protein p15E has shown that both suppress a number of immune functions both in vivo and in vitro, including antibody response in vivo (14), Con A receptor capping (4-6,16), mitogen-induced blastogenesis (3,15-17), mixed lymphocyte reactions (8), erythropoiesis (9) and interleukin II stimulation (7). Our main focus has been to determine a mechanism for the FeLV p15E suppression. Experiments with the Con A receptor mobility indicated a possible involvement of the microtubular-microfilament network in the cell membrane (5,6). We also have found that indomethacin was able to reverse the inhibitory effect of FeLV in
both the capping and mitogen activation of peripheral blood lymphocytes (10,13). These experiments have indicated a possible involvement of the prostaglandin (PG) and/or cyclic nucleotide systems, both of which are involved with normal immune system functions (18,19). To determine if FeLV does cause a disruption of normal prostaglandin and cyclic nucleotide levels we have measured the levels of PGE$_2$, cAMP and cGMP during incubation with FeLV.
METHODS

Blood preparation

Venous blood was drawn from specific-pathogen-free cats from The Ohio State University cat colony. The syringe included a .4% (v/v) solution of sodium citrate. Peripheral blood lymphocytes were isolated using the Ficoll-Hypaque density gradient. Mononuclear cells were removed and washed in L-15 medium (GIBCO, Grand Island, NY) with 1.0% EDTA and antibiotics (penicillin G and streptomycin sulfate).

Virus isolation

The purification of Kawakami-Theilen FeLV (KT-FeLV) from tissue culture media has been previously described (20). Sucrose banded virus was dialyzed 2X in Hank's Balanced Salt Solution and 1X in RPMI 1640 with 1.0% antibiotics. The dialyzed virus was inactivated with ultraviolet light using the method of Yohn et al. (21). Protein determination was performed using a BioRad Protein Kit.

Lymphoblast transformation assay

The lymphoblast transformation test (LBT) has been previously described (22); some modifications were used. Briefly, purified blood lymphocytes (PBL) were added at a concentration of 1 x 10^5 cells per well to a 96 well plate. Con A, FeLV, and/or prostaglandin inhibitors were added and the total volume per well was 200 µl. Five percent fetal calf serum (Sterile Systems, Salt Lake City, UT) was added and the suspension was incubated 4 days. Eighteen hours before harvest the cultures were pulsed with [3H] thymidine (New England Nuclear). The plates were harvested on glass wool using a Mash unit.
(Brandel, Gathersburg, MD) and assessed for thymidine uptake using a liquid scintillation counter (Packard Instruments, Downers Grove, IL).

Culture conditions for prostaglandin and cyclic nucleotide measurements

Purified blood lymphocytes (5 x 10^5/well) were plated in a 24 well plate (CoStar) with RPMI 1640 media containing 1.0% glutamine, Hepes, antibiotics and 5.0% fetal calf serum. At the specified times the cells were removed and centrifuged. The supernatant was removed and frozen for later prostaglandin quantitation. The cell pellets were solubilized with 6.0% trichloroacetic acid. Ether extracted residues were then tested for their cyclic nucleotide (cAMP or cGMP) levels.

Radioimmunoassay for cAMP and cGMP

The samples were analyzed for cyclic AMP and cyclic GMP content by a modification of the radioimmunoassay (RIA) procedure of Steiner et al. (23). To increase the sensitivity of the cyclic AMP and cyclic GMP RIA, 10 μl of a 2:5 mixture of acetic anhydride and triethylamine (v:v) was added to duplicates of the (100-300 μl) samples and standards (24). Cyclic AMP and cyclic GMP (Sigma) were radiolabeled with 125I (New England Nuclear) using the procedures of Steiner et al. (23), or Miyachi et al. (25). Labeled cyclic AMP and cyclic GMP were diluted in 0.25% bovine gamma-globulins (Sigma) to give 20,000-30,000 cpm per sample. Rabbit anti-cAMP and cGMP were developed in this laboratory and are highly specific and sensitive enough to measure 10 femtomols of either nucleotide. The antiserum
was diluted in 50 mM sodium acetate buffer, pH 6.3, with 0.2% bovine serum albumin (Sigma) to give a trace binding of 25-35%. After an 18-24 hr incubation at 4°C, the bound and free fractions were separated by a 20 min incubation with 60% ammonium sulfate at room temperature. After a 20 min centrifugation at 2000 X g, the supernatant fraction was removed and the remaining precipitate was counted in a Beckman 7000 gamma counter. The cyclic nucleotide concentrations were determined by a comparison with a 16 point standard curve.

**Assay for PGE₂**

The concentration of PGE₂ was assessed by radioimmunoassay (RIA). Antibodies were obtained from chicken egg yolks after injections of PG coupled to keyhole limped hemocyanin (26). The concentration of antibody used for each metabolite was sufficient to bind 30-40% of its specific PG ligand. Twelve concentrations of unlabeled PG standard ranging from 1 to 10,000 pg/0.1 ml were utilized. All reagents were adjusted to the appropriate concentration with 50 mM Tris buffer (0.1% bovine serum albumin, 0.05% sodium azide, pH 7.5). Each 1 x 75-mm glass tube contained 0.05 ml of antibody, 0.05 ml of ³H-PG (17 pg or 15,000 dpm) (New England Nuclear) and either 0.01 ml of PG standard or medium sample. The final volume of 0.2 ml was mixed by vortexing and incubated at 4°C for 18 hr. The unbound PG was removed by mixing with a 0.5 ml suspension of dextran-coated charcoal (250 mg Norit A, 25 mg Dextran T-70, 100 ml Tris buffer) and centrifuged at 2000 for 15 min. The supernatant containing the antibody-bound PG was decanted into a 3 ml scintillation vial containing 3 ml Thrift-Solv scintillation cocktail.
(Beckman) and counted on a Beckman 7000 LSC. The results were expressed as the percentage of labeled PG bound versus the concentration of unlabeled PG present. The sample PG content was extrapolated from the standard displacement curve which had a minimum sensitivity of 1 pg/0.1 ml.

Statistical analysis

Statistical evaluations were performed using Student's t-test.
RESULTS

Mitogen response

The mean Con A response of the normal cats was significantly (p < .001) depressed by co-incubation with inactivated FeLV (125 μg/ml). Of the 10 cats studied, the mean suppression of the Con A LBT was 66 ± 22 percent. Indomethacin (0.1 μM) significantly (p < .001) reversed (111 ± 19% of Con A control) the FeLV suppression in the cats tested.

Measurement of prostaglandin production

Figure 4.1 summarizes data for assays for PGE₂ from the supernatants of the PBL cultures that included Con A, FeLV and/or indomethacin. During the first 24 hr period, Con A and FeLV both decrease the release of PGE₂ as compared to control amounts. The combination of FeLV and Con A did not alter the amount of PGE₂ released and it remained equal to that of the control cells during the first 24 hrs of culture. This difference was significantly higher (p < .05) than the Con A stimulated cells. By 48 hr and 72 hr no differences were observed. Indomethacin (0.1 μM) caused a 10-fold decrease in PGE₂ levels at all times measured.

Measurement of cyclic AMP levels

As has been previously reported (27), Con A caused a significant increase in cellular cAMP over the 72 hr culturing period (Fig. 4.2). FeLV with Con A led to a slight initial rise in cAMP, but by 72 hr the level was lower than Con A or Con A with indomethacin and FeLV (p < .001). The rise in cAMP was seen with indomethacin and FeLV even
when Con A was not present (Table 4.1). Indomethacin or FeLV alone did not cause a significant change in cAMP levels when compared to the control cells.

**Measurement of cGMP levels**

Neither Con A nor FeLV had any effect upon the normal cellular levels of cGMP (Fig. 4.3). Indomethacin with FeLV, Con A, or both appeared to cause a rise in cGMP levels, but due to the small test sample (n=4) the rise did not prove to be significant. Indomethacin alone had no effect.
DISCUSSION

Viral infections often lead to immune system dysfunction (1) and consequently can predispose the host to secondary infections. The mechanism for the suppressive action may be multiple, including direct infection of the immune system and indirect immune interference. Research with FeLV has indicated that a viral envelope protein, FeLV pl5E, may be a cause for the suppressive action (15-17). The purpose of this study was to further characterize the mechanism for the suppression. Previous work has indicated that FeLV pl5E caused a disruption of normal receptor capping and mitogenic stimulation of lymphocytes possibly through a change in normal cyclic nucleotide and prostaglandin levels (3-6,10,13). In order to determine if the virus had direct effects upon the levels of these compounds we have measured their production by lymphocytes during co-incubation with FeLV.

FeLV did not cause an increase in PGE\textsubscript{2} production by lymphocytes. However, Con A stimulation caused a significant decrease in PGE\textsubscript{2} production over the culturing period. If FeLV was combined with Con A the PGE\textsubscript{2} levels remained equal to the control cell during the first 24 hrs. This difference may allow for the activation of a suppressor cell by the endogenous PGE\textsubscript{2} as suggested by Goodwin (18). The PGE\textsubscript{2} sensitive cells are active only during the first 24 hr of culture so that the difference in PGE\textsubscript{2} levels during the first 24 hr of culture may cause an increase in the active suppressor population. Indomethacin caused a 10-fold decrease in PGE\textsubscript{2} production over the culturing period and has been shown to block production of
prostaglandin-induced suppressor type cells (28). Thus, its ability
to reverse the viral suppression may be due to the inhibition of PGE
production.

Cyclic AMP has been shown to be a second messenger for PGE$_2$
(29). Its levels are also elevated by Con A stimulation (27).
Previously we found that elevation of cAMP by an adenylate cyclase
activator, forskolin, caused a reversal of FeLV suppression of Con A
stimulation, but had no effect upon normal stimulation. We have also
seen that PGE$_2$ blocks FeLV p15E suppression of Con A receptor cap
formation (10). Both studies indicate that FeLV p15E may interfere
with normal cyclic nucleotide production. Measurements of cAMP
levels during Con A stimulation support this hypothesis. FeLV
blocked the rise in cellular cAMP induced by Con A. Indomethacin
reversed the viral inhibition, possibly by its ability to increase
cAMP levels in the presence of FeLV.

Indomethacin has been reported to cause an inhibitory effect
upon the cAMP-phosphodiesterase, the enzyme that hydrolyzes cAMP
(30). This would lead to an accumulation of cAMP. Although in this
study, indomethacin alone did not elevate cAMP level, the levels did
rise when indomethacin was combined with FeLV. Thus, this increase
in cAMP levels may represent the mechanism of indomethacin reversal
of FeLV suppression. Further research will be necessary to determine
if the cause for FeLV suppression is due to PGE$_2$ suppressor cell
activation or inhibition of cAMP production. FeLV effects upon the
adenylate cyclase and cAMP phosphodiesterase may further delineate
the mechanism of FeLV p15E suppressive action.
SUMMARY

Measurements of the effect of inactivated FeLV upon cyclic nucleotide and PGE$_2$ levels of feline PBL were taken over a 72 hr period. There were no observed effects on cGMP levels over the period, however, cAMP levels were significantly lower in the presence of FeLV. Con A stimulation caused an increase in cAMP and a decrease in PGE$_2$ during the culturing period. Indomethacin decreased PGE$_2$ amounts 10-fold as compared to control cells and was able to block the FeLV inhibitory effect on cAMP production.
Table 4.1. The Effect of FeLV and Indomethacin on cAMP Levels of Lymphocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>FeLV</td>
<td>133 ± 8(^a)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>122 ± 17</td>
</tr>
<tr>
<td>Indomethacin + FeLV</td>
<td>146 ± 13</td>
</tr>
</tbody>
</table>

\(^a\)Each number represents the mean ± SE of 6 experiments.
Figure 4.1. PGE$_2$ levels expressed as percentage of normal cells.

Each point represents the mean ± SE of 8 experiments.

(*p < .05 vs. Con A-stimulated cells).
Figure 4.2. Cyclic AMP levels of Con A-stimulated cells with FeLV and indomethacin. Each point represents the mean ± SE of 6 to 10 experiments. (*p<.001 vs. Con A-stimulated cells and Con A plus indomethacin).
Figure 4.3. Cyclic GMP levels of Con A-stimulated cells with FeLV and indomethacin. Each point represents the mean ± SE of 4 experiments.
CONCLUSION

Since the discovery of FeLV, there has been an intense interest in the mechanism of its infection of the domestic cat population. With the isolation of the human T-cell leukemia virus (1) and its similar route of infection, and association with the acquired immune deficiency disease (2-5), the research with FeLV has gained greater importance. The main research effort from our lab has been to determine the mechanism of FeLV immune suppression both in vivo and in vitro. The affinity of FeLV towards the T-lymphocyte and how the virus, even when inactive, is able to inhibit normal immune functions, were the major questions of this work.

Using a putative recombinant virus having different envelope determinants than FeLV-R, we found that the affinity of the virus had changed, causing transformation of a cell type normally not seen associated with FeLV infection. This transformation may have occurred because of the altered envelope determinants. Further work with the PR-8 isolate will indicate if the virus is actually a genetic recombinant or if it is a FeLV-R core with an altered envelope. Study of the virus isolated from the infected cats is necessary to determine if it is PR-8 or possibly another virus. Isolation and growth of the transformed cells should allow for the re-isolation of the infectious virus, which will allow further genetic studies.
Studies of the receptor capping mechanism by Nichols et al. (6) and Dunlap et al. (7) indicated the FeLV could inhibit Con A receptor mobility both on infected cat cells and normal cat cells. This inhibitory effect was found to be colchicine-sensitive. They concluded that FeLV may be interfering with the normal microtubule action of the lymphocytes. Although colchicine's main effect is on microtubule formation, a recent report (8) indicates that it may also have effects on the site of cAMP production. This site has three subunits; first, the surface receptor site that binds the external stimulator such as hormones or prostaglandins, second, the GTP bind-site or G-site and, third, the catalytic site containing adenylate cyclase. Colchicine is reported to enhance the G-site mediating activation of adenylate cyclase, possibly by disrupting their microtubule supports. If FeLV were able to stabilize the microtubule, the G unit would lose its mobility and in so doing would lower the cAMP production. The finding that immunoglobulin capping was not altered by FeLV is also important, not only because it reaffirms the specificity of FeLV for T-cells, it also indicates a different mechanism of capping for B-cells. Rogers et al. (9) have reported the capping mechanism for B-cells is different from that for Con A. The difference is in the action of the microtubules. Con A capping is enhanced by microtubule disrupting agents, whereas Ig capping is not affected. Another difference observed in B-cells is that their adenylate cyclase activity is much higher than T-cells (10,11), which may explain why membrane fluidity is more critical to T-cells than B-cells.
The work with the Con A LBT and the modulators of the prostaglandin and cyclic nucleotide systems indicated that both PGE$_2$ and cAMP may be involved with FeLV suppression. Although the reversal of suppression by indomethacin may be PGE$_2$-related, the changes in cAMP levels by FeLV and indomethacin appear more significant. PGE$_2$ causes an increase in cellular cAMP by activation of the receptor site. If FeLV is altering the production of cAMP, then the PGE$_2$-induces rise in cAMP may also be altered. In addition, the apparent contradiction between the PGE$_2$-reversing effect of FeLV-suppressed capping and its augmenting effect on the FeLV suppression of the Con A LBT, indicate the PGE$_2$ may not be involved with the FeLV suppression. The addition of forskolin which, to date, has not been shown to have any effect on the prostaglandin system, was able to reverse the suppression of FeLV presumably by raising cAMP levels.

Further work will be needed to determine the actual mechanism of the suppressive effect of FeLV. This should include testing the influence of FeLV on the subunits of the cyclic nucleotide system. By using specific inhibitors or enhancers of these units a clearer understanding should result. Also, measurements of adenylate cyclase and cAMP phosphodiesterase from normal and infected cats' lymphocytes should indicate if FeLV is altering their function. Another system which has not been studied and may be involved is the calmodulin control of calcium, which has been shown to be closely associated with cyclic nucleotides and microtubules (12).
CHAPTER I


6. Troxler, D.H., Lowry, D., Howk, R., Young, H. and Scolnick, E.M. Friend stain of spleen focus-forming virus is a recombinant


CHAPTER II


CHAPTER III


21. Gryglewski, R.J., Bunting, S., Morcada, S., Flowers, R.J. and Vane, J.R. Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they


CHAPTER IV


CONCLUSION


89


