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## Effects of Retrovirus Protein on the Feline One-way Mixed Leukocyte Reaction

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### SUMMARY

The objective of this study was to evaluate the effects of feline retrovirus protein on cat lymphocyte recognition of allogeneic cells in the mixed leukocyte reaction (MLR), using u.v.-inactivated feline leukaemia virus (FeLV) and a non-disease-producing endogenous cat virus (RD-114). Twenty micrograms of u.v.-inactivated virus protein from each virus were added to mixed leukocyte cultures. FeLV induced significant suppression of the MLR ( $P < 0.005$ ) whereas no significant suppression was found using RD-114. Since feline leukaemia infection is associated with immunosuppression, the retrovirus proteins may be interfering with the response of immunocompetent cells when they are confronted with an altered host cell.

The purpose of this research was to study the effects of u.v.-inactivated retroviruses (FeLV and RD-114) on the feline mixed leukocyte reaction (MLR); an *in vitro* correlate of cell-mediated immunity (McDevitt, 1978) and immunosurveillance (Burnet, 1970).

Feline leukaemia virus-infected cats have been shown to demonstrate suppressed allograft rejection (Perryman *et al.*, 1972). Subsequently, Cockerell *et al.* (1976) found that the *in vitro* lymphocyte blastogenic responses to T-cell mitogens were suppressed in viraemic cats even though the T-cell population remained relatively constant. To determine whether this suppression in cats was due to virus protein, Hebebrand *et al.* (1977) used u.v.-inactivated FeLV in *in vitro* mitogenic studies and noted suppression of normal mitogenic responses with 20 µg/well of virus protein. Characterization of a particular immunosuppressive envelope protein (p15E) has been accomplished by Hebebrand *et al.* (1979) and Mathes *et al.* (1978, 1979). This research has investigated the possibility that feline leukaemia virus proteins may interfere with cellular immunity in cats as evaluated by the MLR.

The feline one-way MLR was performed as a modification of the human MLR (O'Leary *et al.*, 1977). Briefly, defibrinated venous blood was obtained from specific-pathogen-free cats (SPF; over 6 months old) (Rohovsky *et al.*, 1966) using aseptic technique. Diluted blood was layered over Ficollpaque (Pharmacia) and centrifuged (Cockerell *et al.*, 1975). The leukocyte-rich interface was recovered, cells were washed and diluted in RPMI 1640 medium with 25 mM-HEPES buffer (Grand Island Biological Co.). Stimulators were treated with mitomycin C (Sigma) at 0.05 mg/10<sup>7</sup> cells. One-tenth ml of each cell suspension (10<sup>5</sup> cells) was placed in plastic round-bottom microtitre plates (Linbro Division, Flow Laboratories) and incubated for 9 days at 37 °C in 2.5% CO<sub>2</sub>, moist air. During the last 18 h of incubation, 0.5 µCi [<sup>3</sup>H]thymidine (6.7 Ci/mol; New England Nuclear) was added. Cells were collected and assayed for radioactivity by liquid scintillation. Each sample was counted for 5 min and net ct/min of quadruplicate wells were recorded. All autologous controls were tested along with the allogeneic combinations. Five percent rabbit serum (heat-inactivated) was added as the serum supplement. Viruses used *in vitro* were the feline leukaemia virus (KT strain, supplied by Pfizer, Inc., Maywood, N.J., U.S.A. through the auspices of the virus cancer program of the National Cancer Institute) and RD-114 (from RD-RD114 cell line supplied by Pfizer). RD-114 virus was

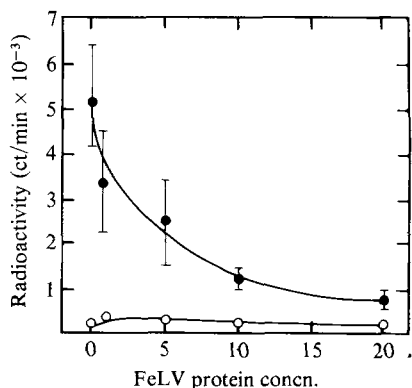


Fig. 1

Fig. 1. Effects of u.v.-inactivated feline leukaemia virus (KT strain) protein at concentrations of 0 to 20  $\mu\text{g}/\text{well}$  on the feline mixed leukocyte reaction. Cells ( $2 \times 10^5/\text{well}$ ) were incubated for 9 days in the presence of viral proteins. R, Responder; S, stimulator (mitomycin C-treated). ●, Cat G(R)  $\times$  Cat H(S); ○, Cat G(R)  $\times$  Cat G(S).

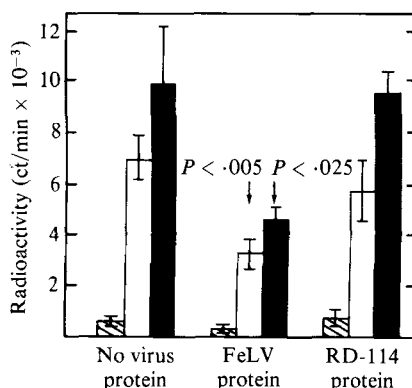


Fig. 2

Fig. 2. Effects of retrovirus u.v.-inactivated proteins (RD-114 and FeLV) at a concentration of 20  $\mu\text{g}/2 \times 10^5$  cells in an MLR. Cells were incubated in the presence of the viral proteins for 9 days. R, Responder; S, stimulator (mitomycin C-treated). The cats are designated by number. ▨, Autologous control: 1222(R)  $\times$  1222(S); □, 1222(R)  $\times$  995(S); ■, 1222(R)  $\times$  1170(S).

purified by banding twice in sucrose. FeLV was banded in sucrose once, since twice banding causes no significant differences in tissue culture studies. Whole viruses were u.v.-inactivated and dialysed (Mathes *et al.*, 1978).

MLRs were done on a number of cats to locate allogeneic combinations that result in stimulation. Initially, dose titration experiments were done using 0, 5, 10 or 20  $\mu\text{g}$  virus protein/well with these allogeneic combinations. The RD-114 virus proteins caused no significant suppression of the MLR at any of the concentrations, although slight suppression was noted at 20  $\mu\text{g}/\text{well}$ . In comparison, the FeLV proteins caused significant suppression at 5  $\mu\text{g}/\text{well}$  ( $P < 0.05$ , Student's *t*-test), 10  $\mu\text{g}/\text{well}$  ( $P < 0.005$ ) and 20  $\mu\text{g}/\text{well}$  ( $P < 0.001$ ) (Fig. 1). Viability experiments (trypan blue exclusion) indicated that this suppression was not due to virus-induced cytotoxicity.

Additional studies were done using media (control) or 20  $\mu\text{g}/\text{well}$  of each virus protein preparation in the same allogeneic combinations simultaneously. A significant suppression of the MLR with u.v.-inactivated FeLV ( $P < 0.005$ ) occurred compared to the test without virus, whereas RD-114 did not significantly suppress the response ( $P < 0.1$ ) (Fig. 2).

In these experiments RD-114, an endogenous cat virus, did cause some suppression in the MLR. It is not known whether this decrease in MLR was caused by an immunosuppressive protein which is less effective than that found with FeLV or simply the presence of extraneous protein in the reaction system. However, it is clear that FeLV virus proteins cause greater immunosuppression of the cat MLR than the endogenous cat retrovirus (RD-114) which has not been shown to cause any disease (Gardner *et al.*, 1974).

In studies of the murine leukaemia viruses, Cianciolo *et al.* (1980) found that the subviral proteins (including p15E) inhibited macrophage function in the inflammatory response. Therefore, it is possible that the feline leukaemia subvirus proteins are interfering with the monocyte's role in the MLR (Bach, 1976).

In addition to suppression of lymphocyte blastogenic transformation (Hebebrand *et al.*, 1979; Olsen *et al.*, 1980), allograft rejection (Perryman *et al.*, 1972), and lymphocyte capping (Dunlap *et al.*, 1979), we find that FeLV virus proteins also suppress the cell-mediated response of the MLR. These data therefore suggest that FeLV virus protein may inhibit the lymphocyte response to transformed or virus-infected cells.

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