Inhibition of Concanavalin A Stimulation of Feline Lymphocytes by Inactivated Feline Leukemia Virus

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SUMMARY

In a lymphocyte blast transformation assay, the response of feline lymphocytes to concanavalin A was suppressed 20 to 65% in the presence of inactivated feline leukemia virus. The decrease was not due to viral cytotoxicity, as determined by trypan blue viability counts, nor was the virus binding the concanavalin A and interfering with its mitogenic stimulation. The virus may be biochemically repressive in itself, interfering with cell-mediated immunity within the feline system.

INTRODUCTION

It is now well accepted that a variety of infectious viruses exert immunosuppressive effects upon their hosts (2). Viral infections may result in decreased circulating antibody titers or impaired alloreactivity rejection. In vitro tests have revealed inhibition of normal PBL2 response to phytomitogens in the presence of virus or tumor extracts (6, 9).

While previous studies have dealt primarily with the immunosuppressive properties of live virus, we have recently reported a similar phenomenon with UV-inactivated FeLV. Immunization of cats with inactivated FeLV resulted in abrogation of tumor immunity and increased tumor incidence as compared to nonimmunized controls, following challenge with infectious FeSV (4, 8). At this point the mechanisms accounting for increased tumor incidences are unknown. Inactivated virions may exert direct immunosuppressive effects on lymphocytes or, alternately, may stimulate synthesis of blocking antibody.

The objective of this study was to measure the direct inhibiting properties of UV-inactivated FeLV on feline lymphocyte function in a controlled in vitro assay.

MATERIALS AND METHODS

Animals. Cats were chosen at random from the SPF cat colony at The Ohio State University Department of Veterinary Pathobiology (7).

Production of Virus. Production and purification of the Rickard strain of FeLV has been described (3). KT-FeLV was supplied by Pfizer Inc., Maywood, N. J. through the auspices of the Virus Cancer Program of the National Cancer Institute. All virus was exhaustively dialyzed against minimum essential media for suspension cultures (KC Biological, Inc., Lenexa, Kans.) to prevent any buffer interference of Con A (Sigma Chemical Co., St. Louis, Mo.) stimulation.

Virus Inactivation. All virus was inactivated by exposure to UV with agitation to a surface dose rate of 150 ergs/sq mm/sec for an accumulated total of 35,000 ergs/sq mm (10).

Lymphocyte Isolation and LBT Test. The LBT assay is described in a previous publication (1) with 1 alteration, namely, that Con A and virus were prepared in twice-concentrated solutions and combined in equal volumes. One-tenth of a ml of this preparation was then added to 0.1 ml of cell suspension and incubated for 5 days. Final Con A concentration was 10 μg/well. PBL used in the assay were taken from young adult SPF cats.

Virus Cytotoxicity. For assessment of possible cytotoxic effect of the virus, increasing dilutions of virus were incubated with normal PBL. In triplicate wells, 0.1 ml of each viral dilution was incubated for 5 days with 0.1 ml of 1.0 × 10⁶ PBL. Following the incubation period, cultures were assessed for cell death by trypan blue exclusion.

RESULTS

LBT in Presence of Virus. Chart 1 summarizes the LBT response of 3 normal SPF cats to Con A in the presence of decreasing dilutions of KT-FeLV. Response of PBL to Con A incubated with 18.75 μg of whole virus was suppressed 20 to 65% as compared to the response of control cultures where cells were incubated with Con A only. Virus was still suppressive at 7.5 to 1.5 μg/well, after which increasing dilutions led to normal blastogenic response. Similar results were seen with Rickard strain FeLV.

Virus Cytotoxicity. Table 1 illustrates the effect of virus in culture upon lymphocyte viability. In all dilutions of virus, total cell viability was consistent with control counts where PBL were incubated with media alone. Thus, virus did not appear to be toxic for the dormant lymphocytes.

Determination of Neutralization Effect of FeLV on Con A. For determination of whether KT-FeLV was binding to Con A and therefore neutralizing its mitogenic properties, 2 ml of Con A (200 μg/ml) were incubated with 2 ml of KT-FeLV (860 μg/ml), resulting in a final concentration of Con A (100 μg/ml) + KT-FeLV (430 μg/ml). The Con A was subsequently used as the mitogenic stimulator for feline PBL (Table 2). No significant loss of Con A mitogenic activity after KT-FeLV treatment was noted.

DISCUSSION

In previous studies we have shown that kittens immunized...
with inactivated FeSV and later challenged with live FeSV demonstrated more susceptibility to live virus than non-immunized cats. This was reflected in shorter survival time, greater tumor size, more rapid tumor growth, and more extensive metastases (8). Moreover, in recent reports it is demonstrated more susceptibility to live virus than non-immunized cats. This was reflected in shorter survival time, greater tumor size, more rapid tumor growth, and more extensive metastases (8). Moreover, in recent reports it is demonstrated more susceptibility to live virus than non-immunized cats. This was reflected in shorter survival time, greater tumor size, more rapid tumor growth, and more extensive metastases (8).

In this study we present evidence that UV-inactivated FeLV directly affects feline lymphocyte function; i.e., it renders them unresponsive to Con A. This inhibitory effect appears to be a general property of FeLV and not a property of 1 particular strain of FeLV. The partial abrogation of response to Con A did not appear to be due to viral cytotoxicity, in that culture viability was comparable to control cultures where no virus was present. However, this does not exclude the possibility of a synergistic effect between Con A and virus which may impair the lymphocyte response to Con A. Moreover, FeLV did not appear to interfere with Con A binding to lymphocytes. Similar results within the murine system have been reported by Dr. A. Hellman, at NIH, Washington, D. C.

It is possible that immunization with killed tumor antigen and/or FeLV may induce production of a blocking antibody that interferes with immunocompetence. However, the fact that significant suppression of mitogenesis of normal adult feline PBL’s in vitro is observed in the presence of killed virus may indicate that the virus is biochemically repressive in itself, capable of altering lymphocyte function and thus weakening the cellular immune response, rather than acting as an inducer of blocking antibody.

REFERENCES

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