Inhibition of Normal Lymphocyte Mitogenic Reactivity by Serum from Feline Leukemia Virus-infected Cats

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SUMMARY

The effect of serum from 12 cats with lymphosarcoma induced by feline leukemia virus (FeLV) on the response of normal cat peripheral blood lymphocytes to phytohemagglutinin-induced blastogenesis was studied. The majority of FeLV sera, when tested at a concentration of 20% of the incubation medium, caused a 40 to 70% reduction in the mean blastogenic response to concanavalin A compared to the response obtained with a similar concentration of normal feline serum. Results with pokeweed mitogen were similar, but the depression in blastogenesis was less than with concanavalin A. Further studies showed that the blastogenic inhibitory activity of FeLV serum (a) was heat labile at 56° for 30 min, (b) could not be overcome by greater concentrations of mitogens, (c) was proportional to the concentration of FeLV serum in the incubation medium, and (d) could not be demonstrated when lymphocytes were preincubated in FeLV serum followed by washing and reincubating in normal feline serum. The results suggested that a substance present in the serum of FeLV-infected cats contributes to altered immunological reactivity during leukemogenesis in the cat.

INTRODUCTION

Cats infected with FeLV have depressed immunological reactivity in the preneoplastic period as indicated by prolonged cutaneous allograft rejection (18) and lymphocyte hyporeactivity to mitogenic stimulation (4). These defects appear as early as 5 weeks after experimental infection and are not related to changes in circulating T- or B-lymphocytes or the presence of circulating morphologically transformed lymphoblasts (4). Since the mitogen hyporeactivity correlates most closely with the onset of viremia, we suggested that the immunodepression was due to a direct viral effect upon lymphocyte function rendering lymphocytes nonresponsive to mitogen stimulation whether or not they are yet transformed (4). Recently, other investigators have demonstrated the presence of immunosuppressive factors present in the serum of tumor-bearing humans and animals that are capable of inhibiting transformation of normal homologous lymphocytes (7, 11, 20, 21, 23). To elucidate further the mechanisms of altered immune reactivity during leukemogenesis in the cat, in the present report we have studied the effect of serum from cats with lymphosarcoma induced by FeLV on the response of normal cat PBL to phytohemagglutinin-induced blastogenesis.

MATERIALS AND METHODS

NFS. Sixteen adult SPF cats (8 male, 8 female) were bled bimonthly. Sera were collected, pooled, filtered, and stored at −70° until use. The mitogen-induced response of normal feline PBL in this pooled serum sample was indistinguishable from that obtained with individual serum samples making up the pool.

FeLV Serum. Serum samples from 12 SPF cats experimentally infected with the Rickard strain of FeLV (19) were collected and stored at −70° until use. These cats were positive for FeLV group-specific antigen in circulating leukocytes (viremia) when serum samples were taken, and all cats died of lymphosarcoma. Table 1 details age at infection and postinfection week at the time of serum collection and death. The volume of serum available from individual cats was variable and not sufficient for all 12 sera to be tested on lymphocytes from each donor cat in all experiments.

Normal Feline PBL. Four adult SPF cats were maintained as a source of normal feline PBL. Lymphocytes were isolated by centrifugation of heparinized blood on a Ficoll-Hypaque gradient (3).

Lymphocyte Blast Transformation Assay. Details of this assay have been described (3). Briefly, 1 × 10^6 PBL were incubated with either 10 μg Con A (Sigma Chemical Co., St. Louis, Mo.) or 4 μg PWM (Grand Island Biological Co., Grand Island, N. Y.) for 72 hr. Stimulation was measured by incorporation of [3H]Tdr (specific activity, 6.7 Ci/m mole; New England Nuclear, Boston, Mass.), added during the final 18 hr of incubation. Unless otherwise stated, all sera were used non-heat inactivated at a final concentration of 20% of the culture medium. For a given experiment, a single PBL isolation was used to compare the effect of NFS and FeLV serum. Data were calculated as the mean of quadruplicate assays.

RESULTS

Effect of FeLV Serum on Mitogen-induced Blastogenesis.
sia. Serum samples from 12 FeLV-infected cats were incubated with normal feline PBL from 2 to 4 SPF cats and stimulated with Con A or PWM. Chart 1 compares these results to those obtained under identical conditions except that NFS was used instead of FeLV serum. The mean blastogenic response to Con A of PBL from all 4 normal cats was 30 to 60% of the mean response in NFS for the majority of FeLV sera tested. Results with PWM were similar, but the depression in blastogenesis was less than with Con A. The response of PBL from Cat 927 consistently was less inhibited, and in fact stimulated, by FeLV serum than were the responses of the 3 other normal cats. PBL from this cat also responded to the least degree of all 4 cats with NFS.

**Effect of Heat Inactivation of FeLV Serum on Mitogen-Induced Blastogenesis.** In this and all further experiments, 4 FeLV sera (187B, 188B, 613-1, 613-4) were selected to characterize the nature of the inhibitory factor. This selection was based on demonstrated inhibitory activity to at least 1 mitogen. In the previous experiment the mean response of PBL from 4 normal cats was 30 to 60% of the mean response in NFS for the majority of FeLV sera tested. Results with PWM were similar, but the depression in blastogenesis was less than with Con A. The response of PBL from Cat 927 consistently was less inhibited, and in fact stimulated, by FeLV serum than were the responses of the 3 other normal cats. PBL from this cat also responded to the least degree of all 4 cats with NFS.

Alloquots of NFS and FeLV sera were inactivated at 56° for 30 min. Chart 2 compares the results obtained when PBL from normal SPF cats were stimulated with Con A or PWM in the presence of heat-inactivated sera. Heat inactivation of NFS caused a 20 and 41% increment in Con A- and PWM-stimulated [3H]TdR uptake, respectively, compared to non-heat-inactivated NFS. Similar treatment of FeLV sera caused increases of 197, 37, 141, and 201% for Con A-, PWM-, Con A- and PWM-induced blastogenesis, respectively, in comparison to non-heat-inactivated FeLV sera. In most cases the resulting mitogen-induced blastogenesis in heat-inactivated FeLV sera was equal to or greater than the response in heat-inactivated NFS.

**Effect of FeLV Serum on Mitogen Dose Responsiveness.** Chart 3 depicts mitogen dose-response curves for normal feline PBL in the presence of either NFS or FeLV serum. Maximal [3H]TdR uptake was seen at similar mitogen concentrations, irrespective of type of serum used in the culture medium, and agreed with previous findings for normal cat PBL (3). In addition, examination of PBL after 3 days of incubation indicated no difference in percentage of viable cells (as determined by trypan blue dye exclusion) whether cultured with NFS or FeLV sera at optimal mitogen concentrations (10 μg Con A, 4 μg PWM).

**Effect of Different Concentrations of FeLV Serum.** Culture media were prepared containing 5, 10, 15, and 20% FeLV sera, supplemented with NFS to produce a final total serum concentration of 20%. Chart 4 illustrates results of mitogen-induced stimulation of normal feline PBL with differing concentrations of FeLV sera compared to the response obtained with 20% NFS. A progressive reduction in [3H]TdR incorporation was seen with increasing concentrations of FeLV sera. Lower concentrations of FeLV sera approached or exceeded the response with NFS.

**Effect of Preincubation of Normal Feline PBL in FeLV Serum.** Normal feline PBL were cultured with mitogens either in medium containing FeLV sera or preincubated in this medium for 1 hr at 37°, washed, resuspended, and cultured with mitogens in NFS-containing medium. Chart 5 illustrates the percentage of change in cpm under these conditions compared to the response obtained by using NFS with no preincubations. Preincubation in FeLV sera with subsequent culture in NFS produced only minimal...
Immunodepression during Leukemogenesis in the Cat

Chart 3. Mitogen dose response for Con A- and PWM-induced blastogenesis of normal feline PBL in the presence of serum from normal cats and FeLV-infected cats (187B, 188B, 613-1, and 613-4). All sera were non-heat inactivated and used at a final concentration of 20%. Data are calculated as the average response of PBL from 2 normal SPF cats.

Chart 4. The effect of different proportions of serum from normal cats and FeLV-infected cats (187B, 188B, 613-1, and 613-4) on Con A- and PWM-induced blastogenesis of normal feline PBL. All combinations of sera were non-heat inactivated and used at a final total concentration of 20%. In each group: 1st bar, 15% NFS, 5% FeLV serum; 2nd bar, 10% NFS, 10% FeLV serum; 3rd bar, 5% NFS, 15% FeLV serum; 4th bar, 0% NFS, 20% FeLV serum. Data are calculated as the average response of PBL from 2 normal SPF cats. NT, not tested.

Chart 5. The effect of preincubation in sera from FeLV-infected cats (188B, 613-1, and 613-4) on Con A- and PWM-induced blastogenesis of normal feline PBL. Stippled bar, response of PBL in FeLV serum; open bar, response of PBL in NFS after preincubation (1 hr, 37°C) in FeLV serum. All sera were non-heat inactivated and used at a final concentration of 20%. Data are calculated as the average response of PBL from 2 normal SPF cats.

FeLV serum had a greater inhibitory effect upon the response of normal feline PBL to Con A than to PWM. In other species, such as humans and mice, Con A is thought to stimulate T-cells, while PWM stimulates B- as well as T-cells (1, 12). Although the tropism of these mitogens for feline lymphocytes is not definitively known, the greater inhibition of Con A as compared to PWM-induced blastogenesis may indicate a selective inhibition of T-cell function with continued B-cell response. A similar effect has been shown with serum fractions from human cancer patients (13). However, we cannot exclude the possibility that this differential mitogen effect merely reflects the difference in sensitivity of normal feline PBL to Con A and PWM. Con A usually induces a 3- to 4-fold greater stimulation of feline PBL than does PWM (3). It may be easier to demonstrate the inhibitory effect of FeLV serum with mitogens that induce greater lymphocyte stimulation; PBL from cats that

DISCUSSION

The results of this study present preliminary evidence for the inhibition of Con A- and PWM-induced blast transformation of normal feline PBL by serum from cats with lymphosarcoma induced by FeLV. Initial studies to characterize the properties of the factor(s) responsible for this inhibitory activity indicate that (a) it is heat labile at 56°C for 30 min, (b) inhibition cannot be overcome by greater concentrations of mitogens, (c) the degree of inhibition is proportional to the concentration of FeLV serum in the culture media, and (d) blastogenesis is not inhibited by preincubation in FeLV serum followed by washing and reincubation in NFS.
responded minimally to Con A in NFS were less apt to show inhibition of blastogenesis with either Con A or PWM in FeLV serum.

Several mechanisms must be considered to explain the lymphocyte antiproliferative effect of FeLV serum. These include (a) nutritional insufficiency, (b) cytotoxic factors, (c) products of tumor cells, (d) virus or viral antigens, (e) humoral antibodies, and (f) immunoregulatory factors.

The heat lability of inhibitory activity suggests that the mechanism is not simply related to a deficiency of critical nutrients needed for mitogenesis.

No cytotoxic activity for PBL could be demonstrated in FeLV serum as assayed by the ability of cells to exclude trypan blue. Furthermore, preincubation in FeLV serum had no effect upon subsequent blastogenesis in NFS.

Inhibitory activity may be due to specific factors released from tumor cells. Certain malignant cells have been shown to produce substances in vitro with either immunosuppressive or immunostimulatory effects (2, 10, 14, 24). All cats used in this study died with lymphosarcoma, and serum was collected during the phase of expected rapid tumor growth. However, in other experiments, we have found that serum taken from FeLV-infected cats early in the preneoplastic period has a similar inhibitory activity. Therefore, inhibitory activity of FeLV serum is manifest whether or not tumor cells are present.

All cats from which serum was tested were viremic at the time of serum collection. Therefore, it must be considered that the inhibitory activity of FeLV serum is due to a direct effect of virus upon lymphocyte function. However, if this were the case, preincubation of cells in FeLV serum for 1 hr should have had a detrimental effect upon blastogenesis during subsequent incubation in NFS. Longer periods of time for contact between virus and PBL prior to washing must be used to provide more evidence for this point. In addition, cats are known to produce antibodies of several specificities in response to FeLV infection (6, 17). The direct effect of virus, viral antigen, antiviral, or anti-tumor cell-specific antibodies, or antigen-antibody complexes on lymphocyte transformation is unknown. Additional studies are needed to compare the inhibitory capacity of FeLV serum before and after precipitation with antiviral antibody, viral, or tumor cell antigens or by assaying the effect of NFS after addition of these reagents.

Tumor growth-"enhancing" factors have been described in the serum of tumor-bearing humans and animals that can block direct cellular cytotoxicity of immune lymphocytes against tumor cells (9, 22). Such factors, however, are specific in their abrogation of the immune response to tumor cell-specific antigens only. The inhibitory activity that we have found in FeLV serum is similar to that of a serum peptide fraction isolated from human cancer patients that is capable of inhibiting nonspecific phytomitogen-induced blastogenesis (8, 15). This factor has been likened to an immunoregulatory α-globulin found in trace amounts in pooled normal human serum (5, 16). It is possible, therefore, that the inhibitory activity described in this report is due to a normal host immunoregulatory substance acting in a feedback manner to inhibit the expanding neoplastic cell population. Paradoxically, however, such inhibition may contribute to the suppression of cell-mediated tumor defense mechanisms.

The immunosuppressive effects of FeLV infection are well documented, but the mechanisms remain to be determined. Our results suggest that a substance present in the serum of FeLV-infected cats contributes to altered immunological reactivity during leukemogenesis in the cat. This system may prove to be a valuable animal model for the study of similar effects noted in less controlled human cancers or in more artificial systems with murine leukemia viruses and inbred mice.

ACKNOWLEDGMENTS

We wish to acknowledge the capable technical assistance of Lynn Hebebrand and Steven Hunziker.

REFERENCES

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