Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 237–240, January 1987
Immunology

Suppressive effect on polyclonal B-cell activation of a synthetic peptide homologous to a transmembrane component of oncogenic retroviruses

(immunomodulation/feline leukemia virus/immunoglobulin synthesis/immunosuppressive peptide)

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ABSTRACT **Purified feline leukemia virus, UV light-inactivated feline leukemia virus, and a synthetic peptide (CKS-17) homologous to a well-conserved region of the transmembrane components of several human and animal retroviruses were each studied for their effects on IgG production by feline peripheral blood lymphocytes. Using a reverse hemolytic plaque assay, both the viable virus and the UV-inactivated feline leukemia virus, but not the CKS-17, activated B lymphocytes to secrete IgG. When staphylococcal protein A, a polyclonal B-cell activator, was used to stimulate IgG synthesis by feline lymphocytes, the viable virus, the UV-inactivated virus, and the CKS-17 peptide each strongly suppressed IgG secretion without compromising viability of the lymphocytes. These findings suggest that the immunosuppressive influences of feline leukemia virus on immunoglobulin synthesis may reside in a conserved portion of the envelope glycoprotein that includes the region homologous to CKS-17.**

Many oncogenic human and animal retroviruses, including feline leukemia virus (FeLV), exert profound immunosuppressive effects on their hosts (1–6), leading to enhanced susceptibility to infections by opportunistic organisms. Of special concern are the immunosuppressive influences of human T-lymphotropic virus type III/lymphadenopathy-associated virus [HTLV-III/LAV; human immunodeficiency virus (HIV)] (7–9). From *in vitro* studies, we and others have concluded that noninfectious preparations of either FeLV or HTLV-III viruses suppress several functions of feline or human lymphoid cells, respectively. For example, we showed that UV-inactivated FeLV (UV-FeLV) inhibits the production of α-¶ or γ-interferon (11) by feline lymphocytes. Pahwa *et al.* (12) showed that noninfectious band-purified preparations of HTLV-III inhibit proliferation of B lymphocytes activated by pokeweed mitogen (PWM), *Staphylococcus aureus*, or Epstein–Barr virus. Such preparations also inhibit T-cell proliferation induced by phytohemagglutinin, Con A, or allogeneic cells. Somewhat paradoxical was the finding that the same preparation of noninfectious HTLV-III, depending on experimental conditions, produced either polyclonal B-cell activation and activation of T cells to express Tac antigen or, in contrast, produced immunosuppressive effects. It would appear from such observations that virus preparations may contain protein(s) capable of multiple effects on lymphoid cell functions. Indeed, it has been established that the transmembrane envelope protein p15E present in feline, murine, or bovine leukemia viruses, suppresses a number of lymphoid cell functions (13–16). Recently, Cianciolo *et al.* (17) synthesized a 17-amino acid peptide (CKS-17), which exhibits a striking homology to the transmembrane peptides present in many animal and human retroviruses (e.g., HTLV-I, HTLV-II, feline, murine, bovine, avian, and simian retroviruses) (18–20). This peptide also suppresses *in vitro* the respiratory burst of human monocytes (21), interleukin 2-induced proliferation of an interleukin 2-dependent murine T-cell line, and proliferative reactions of human or murine lymphocytes in mixed lymphocyte cultures (17). It also inhibits both endogenous and activated human natural killer cell lytic activity (22).

In the present report, we demonstrate that viable FeLV or UV-FeLV may either activate or suppress immunoglobulin secretion by feline lymphocytes, depending on the concentration used. In contrast, the synthetic peptide CKS-17 produces only a potent inhibition of immunoglobulin secretion by activated feline lymphocytes.

MATERIALS AND METHODS

Animals. Domestic cats (five males and seven females) were obtained from the Department of Laboratory Animal Medicine (University of South Florida, Tampa, FL). They were periodically checked for the presence of FeLV by an enzyme-linked immunosorbent assay (Leukassay F; Pitman-Moore, Washington Crossing, NJ) or by immunofluorescence as described (23). Cats used in this study were nonviremic and appeared to be healthy.

Lymphocyte Preparations. Heparinized blood was obtained by venipuncture from the jugular vein of healthy cats. The peripheral blood lymphocytes (PBL) were separated from fresh blood by Ficoll-Hypaque (Pharmacia) density-gradient centrifugation. The PBL were washed three times with Hanks' balanced salt solution and resuspended in complete medium, RPMI 1640 medium supplemented with 15% heat-inactivated fetal bovine serum/2 mM L-glutamine/penicillin (100 units/ml)/streptomycin (100 μg/ml) (GIBCO).

Viruses and CKS-17. Purified FeLV was provided by R. G. Olsen and L. E. Mathes (Ohio State University, Columbus, OH). Virus was inactivated by exposure to UV irradiation at a surface dose rate of 170 ergs·mm^{-2}·sec^{-1} for an accumulated total of 1.5×10^6 ergs/mm^2 according to the modified methods described (24, 25). The CKS-17 synthetic peptide

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Abbreviations: FeLV, feline leukemia virus; HTLV, human T-lymphotropic virus; LAV, lymphadenopathy-associated virus; HIV, human immunodeficiency virus; UV-FeLV, UV-inactivated FeLV; PWM, pokeweed mitogen; PBL, peripheral blood lymphocytes; BSA, bovine serum albumin; SPA, staphylococcal protein A; RHPA, reverse hemolytic plaque assay; PFC, plaque-forming cells.

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¶Yasuda, M., Good, R. A. & Day, N. K., Sixth International Congress of Immunology, July 6–11, 1986, Toronto, Canada, p. 361 (abstr.).

was synthesized and purified as described (17). As reported earlier (17, 21), immunosuppressive activity is only seen when the peptide is coupled to the carrier protein bovine serum albumin (BSA). Previous studies using a series of partially homologous and nonhomologous retroviral peptides have demonstrated that only the CKS-17 peptide sequence has immunosuppressive activity (17, 21). BSA that has undergone the coupling process in the absence of peptide as described (17, 21) was used as a control and is referred to as BSA*.

Cell Culture. Cat PBL were adjusted to 1×10^6 cells per ml in complete RPMI 1640 medium. The cells were dispensed in 0.5-ml volumes into plastic tubes (12 × 75 mm) (Falcon) and stimulated with PWM (GIBCO) at 5 μg/ml or staphylococcal protein A (SPA; Pharmacia) at 0.1 μg/ml. To the cell cultures was added FeLV or UV-FeLV at concentrations of 5, 10, or 40 μg/ml, or CKS-17 or BSA* at final dilutions of 1:80, 1:40, or 1:20. These dilutions represent final peptide concentrations of ≈1.9, ≈3.8, and ≈7.5 μM, respectively. The cultures were incubated for 7 days at 37°C in 10% CO_2/90% air and studied for secretion of IgG by the reverse hemolytic plaque assay (RHPA) described below. The viability of cultured cells was checked by the trypan blue dye exclusion test.

RHPA for IgG-Secreting Cells. Plaque-forming cells (PFC) were enumerated by a modified RHPA method using Cunningham chambers (26, 27). Sheep erythrocytes were obtained from the same sheep and stored in sterile Alsever's solution. Sheep erythrocytes were coupled to purified SPA by using a chromium chloride technique. Cell cultures were harvested, washed, and resuspended with an adequate amount of Hanks' balanced salt solution. The mixtures of 40 μl of resuspended culture cells, 30 μl of a 45% suspension of SPA-coupled sheep erythrocytes, 15 μl of rabbit anti-cat IgG (1 mg/ml) (Cappel Laboratories, Malvern, PA), and 5 μl of sheep erythrocyte-absorbed guinea pig complement (Cordis, Miami, FL) were incubated in 30-μl Cunningham chambers in duplicates at 37°C. After 4–6 hr of incubation, PFC were counted under an inverted microscope (Invertoscope ID 02; Zeiss, Oberkochen, F.R.G.). The data are presented as the mean PFC per 10^6 original culture cells ± SD.

Statistical Analysis. The statistical significance of the data was determined by Student's *t* test. A *P* value of <0.05 was considered significant. Each experiment was repeated five times.

RESULTS

Polyclonal B-Cell Activation by SPA or PWM Using Cat PBL. Fig. 1 shows that in the feline system, both PWM and SPA at optimal doses induce PFC response in the RHPA. SPA is, however, apparently a better inducer of IgG synthesis than PWM. Indeed, in some experiments when cells from different animals were used, PWM even suppressed formation of PFC, indicating that PWM may have activated suppressor cells (data not shown). Therefore, SPA was used in all subsequent experiments as an inducer of a PFC response in the RHPA.

Effect of Viable FeLV on Induction of IgG by Cat PBL. Fig. 2 presents the results of the RHPA in the presence or absence of different concentrations of viable FeLV (5, 10, or 40 μg/ml) incubated with feline PBL in the presence or absence of SPA. As shown, the number of PFC increased significantly when FeLV at 5 μg/ml ($P < 0.0005$) or 10 μg/ml ($P < 0.005$) was added to the cat PBL. At a higher concentration (40 μg/ml), induction of IgG was reduced. In contrast, when FeLV at the same concentrations used above was cultured with PBL plus SPA, a polyclonal activator of B cells, the SPA-induced PFC responses were inhibited significantly at 10 μg/ml ($P < 0.005$) or 40 μg/ml ($P < 0.001$) of FeLV added. This suppressive effect of FeLV was dose dependent. At a

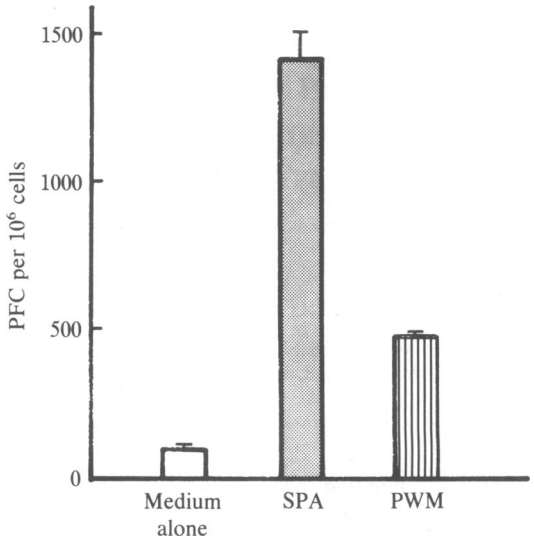

FIG. 1. Cat PBL were cultured with SPA (0.1 μg/ml) or PWM (5 μg/ml) for 7 days. Polyclonal B-cell activation was estimated by RHPA as described.

FeLV concentration of 40 μg/ml, suppression of SPA-induced PFC was virtually complete. These findings indicate clearly that immunoglobulin production by SPA-activated B-cells is dramatically suppressed by viable FeLV.

Effect of UV-FeLV on Polyclonal B-Cell Activation. As in the experiments described above, UV-FeLV also activated IgG production at low concentrations ($P < 0.0005$ at UV-FeLV of 5 μg/ml and 10 μg/ml) and inhibited B-cell activation to immunoglobulin production by SPA at a higher concentration ($P < 0.01$ at UV-FeLV of 40 μg/ml; Fig. 3). It can be concluded from these two sets of experiments that whole virus, depending on the concentration, has a dual effect on B-cell activation. Furthermore, noninfective UV-inactivated viral particles are also effective, even though their ability to suppress polyclonal B-cell activation by SPA is less dramatic than when live virus is used.

Effect of Synthetic Peptide CKS-17 on Activation of B Cells. Retroviral-encoded CKS-17 gave the following results (Fig. 4; Table 1). First, CKS-17 or BSA* alone did not influence the viability of feline PBL after 7 days of culture (data not

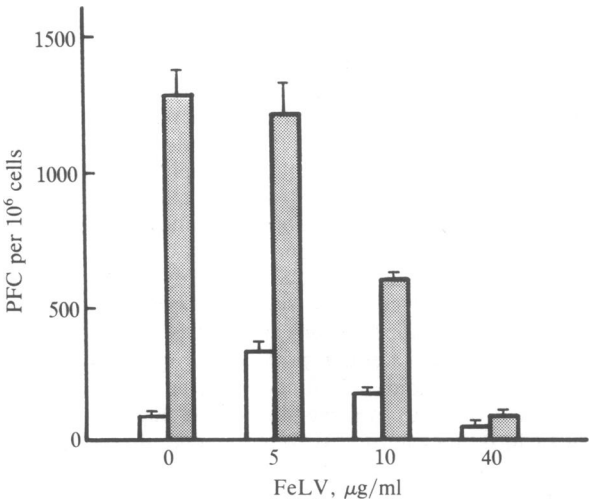

FIG. 2. Effect of FeLV on B-cell activation. FeLV was added at a final concentration of 5, 10, or 40 μg/ml to the cell cultures unstimulated (□) or stimulated with 0.1 μg of SPA per ml (▨). The cultures were incubated for 7 days and RHPA was done.

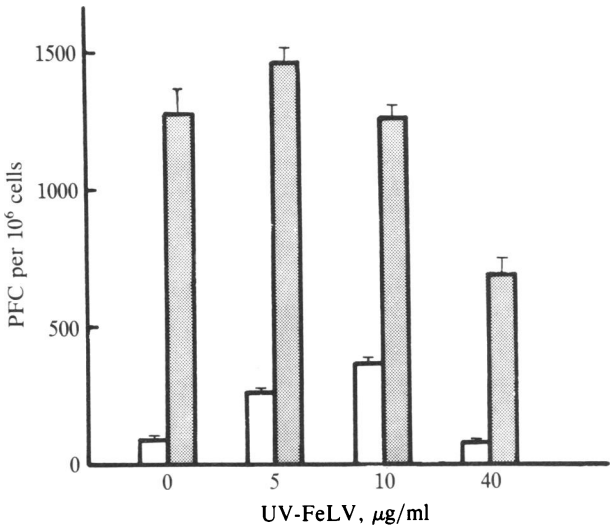

FIG. 3. Effect of UV-FeLV on B-cell activation. UV-FeLV was added at a final concentration of 5, 10, or 40 μg/ml to the PBL cultures unstimulated (□) or stimulated with 0.1 μg of SPA per ml (▨). The cells were harvested after 7 days of incubation and applied to RHPA.

shown). Second, CKS-17 at various dilutions under the conditions used did not activate feline PBL to produce IgG in the RHPA. Third, when the same concentrations of CKS-17 or BSA* alone were added to mixtures of feline PBL and SPA, a dramatic suppression of IgG secretion was observed only when CKS-17 was present ($P < 0.01$ at 1:40, $P < 0.001$ at 1:20 dilution of CKS-17) but not when BSA* was used alone. Representative experiments using PBL from two different cats and SPA plus CKS-17 or BSA* alone are shown in Table 1. Up to 99.3% or 99.9% inhibition of the PFC response was observed with the 1:40 or 1:20 dilutions of CKS-17, respectively, with PBL of cat 1 (Exp. 1). With PBL from a second cat, an inhibition of 77.4% was observed with the 1:40 dilution of CKS-17. No suppression of PFC occurred with the BSA* controls (Exp. 2). These data show that the suppressive effect of CKS-17, although regularly dramatic, may vary in degree with PBL of different cats. From this experiment, no evidence that CKS-17 stimulates polyclonal B-cell activation was obtained. By contrast, CKS-17 inhibited SPA-induced immunoglobulin secretion in a dose-dependent fashion.

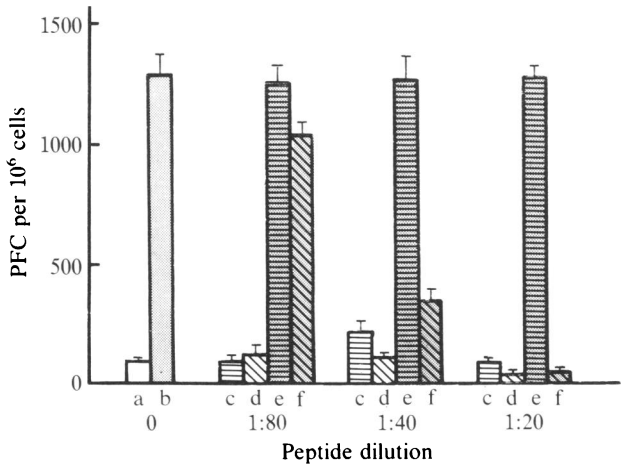

FIG. 4. Effect of CKS-17 on B-cell activation. Cat PBL cultures were left untreated (a); stimulated with BSA (c) or a synthetic peptide CKS-17 (d) at final dilutions of 1:80, 1:40, or 1:20; stimulated with SPA alone (b); stimulated with SPA plus BSA (e) or with SPA plus CKS-17 (f) at dilutions of 1:80, 1:40, or 1:20. PFC were enumerated by RHPA after 7 days of incubation.

Table 1. Suppressive effect of CKS-17 on the development of PFC

Peptide dilution	PFC per 10^6 cells cultured with BSA*	PFC per 10^6 cells cultured with CKS-17	% suppression	*P* value
		Exp. 1		
1:80	5730 ± 120	1406 ± 139	75.5	<0.005
1:40	6255 ± 285	41 ± 19	99.3	<0.005
1:20	5750 ± 696	4 ± 4	99.9	<0.005
		Exp. 2		
1:80	2790 ± 510	1744 ± 229	37.5	NS
1:40	3090 ± 30	698 ± 23	77.4	<0.0005

Cat PBL were cultured with SPA (0.1 μg/ml) added with the indicated dilutions of BSA* or CKS-17 for 7 days. % suppression was calculated by the formula (1 − PFC cultured with CKS-17/PFC cultured with BSA*) × 100. *P* values compared to BSA* control. NS, not significant.

DISCUSSION

Our results are of interest from several points of view. Prior studies with human cells have shown that the reverse hemolytic plaque assay represents a useful method for estimating and evaluating polyclonal activation of B lymphocytes (28–30). We showed that this system can also be adapted to cat cells using PWM as the stimulation (26). SPA was found to induce even more consistent plaque formation with cells from numerous cats and was therefore used as a method of choice in polyclonal B-cell activation.

In the present studies, the biphasic effects of band-purified viable or UV-FeLV were illustrated by induction of differentiation of B lymphocytes at low concentrations, whereas higher concentrations cause inhibition of polyclonal B-cell activation by other activators such as SPA. These findings are remindful of the findings of Pahwa *et al.* (12). These investigators showed that purified noninfectious preparations of HTLV-III/LAV (HIV), under conditions similar to those described here, could either induce proliferation of B cells at low dilutions of the virus preparations or inhibit polyclonal B-cell activation by several B cell activators—e.g., *Staphylococcus aureus*, Epstein–Barr virus, or PWM. These observations are also consonant with the observation that depressed humoral responses to T-cell-dependent or T-cell-independent antigens are frequently present in patients with acquired immunodeficiency syndrome (AIDS) despite the hypergammaglobulinemia that regularly accompanies this clinical condition (31, 32).

The *in vivo* and *in vitro* immunosuppressive effects of retroviruses have been recognized for many years (1–9). Disrupted virus preparations or inactivated viruses produced by freeze–thawing (33), Triton X-100 treatment (12, 34), or UV irradiation (11, 35, 36) have been used to inhibit immune responses. More recently, the immunosuppressive properties of retroviruses have been attributed to a transmembrane component, p15E, of the envelope glycoprotein of the retrovirus (13–16). Whether this retroviral protein exerts a biphasic influence such as that described above for the FeLV or HTLV-III/LAV preparations has not yet been fully addressed and needs to be further investigated.

In the present studies, CKS-17, a synthetic peptide comprised of 17 amino acids with homology to conserved regions of the transmembrane proteins of the envelopes of both animal and human retroviruses (17–20), produced dramatic inhibition of immunoglobulin secretion by SPA-activated peripheral blood feline B lymphocytes. No activation of B lymphocytes by CKS-17 was observed during the present

experiments, even though a range of concentrations was used. Throughout the experiments, PBL retained their viability in the trypan blue dye exclusion test. This finding, of course, raises the question of how the peptide exerts its influence but indicates clearly that it does not operate by killing or irrevocably damaging the cells.

The results obtained in the present study suggest that different components of the virus may be responsible for the different abilities to suppress or activate lymphoid cells. They also suggest that CKS-17 represents an amino acid sequence that is crucial to some or all of the immunosuppressive actions attributable to the viruses or their p15E transmembrane components. It is of interest that cancer cell lines but not normal lymphoid cells have been shown to contain material immunologically reactive with epitopes present in p15E (10, 13, 37). If an immunosuppressive peptide homologous to CKS-17 is contained in and released by cancer cells, vital immunosuppressive effects of these cells might then be attributed to this peptide or a closely related cellular component (13). The question of the origin, functions in the normal body economy, and perturbations in disease states of such a peptide might then become a most fundamental issue.

This work was supported by National Institutes of Health Grants CA-34103, R23-CA34671, and PO1-CA29589, and American Cancer Society Grant IM-298.

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