PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **188**, 353–363 (1988)

Altered Interleukin Production during Friend Leukemia Virus Infection (42746)

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Abstract. Spleen cells from BALB/c mice, infected 14 to 28 days earlier with Friend leukemia virus (FLV), were shown to be inhibited in their ability to produce interleukin 2 (IL-2) when stimulated with mitogen. Likewise, these spleen cell populations failed to respond following mitogenic stimulation or exogenous addition of recombinant IL-2. By contrast, the FLV-infected spleen cell populations produced normal levels of interleukin 1 (IL-1) and thymocytes from FLV-infected mice responded normally to addition of exogenous IL-1. This suggests that FLV infection selectively affects the ability of spleen cells to produce cytokines. Spleen cell populations enriched for T lymphocytes and depleted of tumor cells by density gradient centrifugation in Ficoll were unable to produce IL-2. This indicates that the failure to detect IL-2 in cells from FLV-infected mice was not due to a dilution of T lymphocytes by tumor cells but was a functional inability to produce IL-2. Furthermore, enriched T lymphocytes from FLV-infected mice failed to respond blastogenically to exogenous IL-2. Additional studies indicate that tumor cells, but not macrophages or T lymphocytes from FLV-infected spleens, suppressed the blastogenic response to mitogens and IL-2 production by normal splenic T lymphocytes.

Infection of susceptible mice with Friend leukemia virus (FLV) results in a dysfunction of immune responsiveness which resembles the human acquired immune deficiency syndrome (AIDS). Although FLV infection is the most extensively studied model of retrovirus-induced immunosuppression, the underlying mechanisms for suppression remain to be elucidated (1). Susceptible mice infected with FLV rapidly develop severe immune deficiency affecting both humoral and cellular responses (2). *In vivo* and *in vitro* antibody plaque forming cell responses to sheep red blood cells (SRBC) become suppressed shortly after FLV infection (3–5). It has been shown also that FLV infection of susceptible strains of mice may suppress cell-mediated functions as assessed *in vivo* and *in vitro* (6, 7). Studies in this and other laboratories showed that natural killer (NK) cell activity was also suppressed in FLV-infected mice (8, 9).

Earlier studies have also indicated that macrophages are involved in FLV-induced immune suppression. When macrophages from noninfected mice were transferred to FLV-infected animals the immune response was partially restored (10, 11). Furthermore, culture supernatants from normal macrophages stimulated *in vitro* with endotoxin partially reversed FLV-associated immune suppression. Macrophages derived from retrovirus-infected animals have been shown to suppress immune functions as diverse as antibody production, T cell cytotoxicity, NK activity, lectin, or antigen-driven proliferation as well as lymphokine production (8, 12–18).

The functional relationship between interleukin 1 (IL-1) and interleukin 2 (IL-2) as an essential element in T cell activation is well known. This relationship involves the conversion of a macrophage-derived maturational signal (IL-1) into a secondary lymphocyte-derived proliferation signal (IL-2) resulting in the amplification of specific immune responses (19, 20).

The present investigation studied the role of IL-1 and IL-2 in regard to their possible contribution to the mechanism(s) of suppression induced by FLV infection. Interleukin levels were assessed in culture supernatant fluids of spleen cells from FLV-infected mice. These studies revealed that infection

0037-9727/88 $1.50

did not suppress the ability of splenocytes from leukemic mice to produce IL-1 or respond to exogenous IL-1. However, IL-2 production was severely inhibited in cells derived from FLV-infected mice. Inhibition was moderate at 14 days post infection (p.i.) but became very severe by 28 days p.i. The ability to respond to IL-2 was also compromised in these animals. Therefore, the deficiency in the production of and response to IL-2 but not IL-1 appeared to be related to the inability of these virus-infected mice to mount normal immune responses.

Methods. *Mice.* BALB/c and C_3H/HeJ female mice purchased from Jackson Laboratories (Bar Harbor, ME) at 4 to 6 weeks of age were used for these studies. Mice were housed in plastic cages and allowed food and water *ad libitum.*

Friend leukemia virus infection. FLV was maintained at −70°C as a stock solution of 10% (w/v) spleen homogenate in phosphate-buffered saline (PBS) (8). Mice were infected by intraperitoneal injection with 0.2 ml of a 10-fold dilution (PBS) of the virus stock. Virus stock solution had a virus titer of 5.5×10^4 infections dose 50 (ID_{50}).

Spleen cell isolation. The spleen of individual FLV-infected BALB/c mice was aseptically removed at 14, 21, or 28 days p.i. Uninfected mice of the same age were used as a source of control spleens. The FLV-infected spleens were weighed as an indication of viral infection and those weighing more than 500 mg (indicative of advanced infection) were included in the study. Individual spleens were teased apart through a sterile wire mesh and the dispersed cells were washed three times by centrifugation at 300*g* at 4°C for 10 min with RPMI 1640 medium supplemented with antibiotics (1% penicillin (10,000 U/ml)/streptomycin (10,000 μg/ml) (GIBCO, Grand Island, NY). Red blood cells were lysed with ammonium chloride potassium solution.

Macrophage cultures. For generation of IL-1 supernatant fluids spleen cells were cultured in aliquots of 1 ml containing 10×10^6 cells in 24-well plates (Costar, Cambridge, MA) for 24 hr at 37°C in 5% CO_2, 95% air. After the incubation period, nonadherent cells were removed by thorough washing and the adherent cells were further incubated (24 hr, 37°C, 5% CO_2) in 1 ml RPMI 1640 medium supplemented with 10% fetal calf serum (FCS, GIBCO) with or without *Escherichia coli* lipopolysaccharide (LPS, Sigma Co., St. Louis, MO) at a concentration of 20 μg/ml. The LPS was used to induce IL-1 production in the macrophage cultures (21). The supernatant fluids were recovered and tested for IL-1 activity (22).

Cell fractionation. T cells were isolated from spleens by discontinuous Percoll (Pharmacia, Uppsala, Sweden) density gradients. Briefly, 100×10^6 spleen cells in 1 ml RPMI medium supplemented with 10% FCS were placed on top of the gradients. Percoll gradients ranged from 30 to 70.1% in steps of 4.5%. The gradients were centrifuged at 300*g* for 30 min at room temperature. The fractions were removed and washed thoroughly in RPMI medium. Purification of T cells was confirmed using flow cytometry, which showed that the high density Percoll fractions were at least 90% T cells. Macrophage populations were identified by nonspecific esterase staining (23).

IL-1 assay. Thymus glands from young healthy C_3H/HeJ and uninfected or FLV-infected BALB/c mice were aseptically removed and teased apart through a sterile wire mesh. Cells were vigorously washed (300*g* for 10 min) and suspended in RPMI 1640 medium supplemented with antibiotics, 5×10^{-5} *M* 2-mercaptoethanol and 10% FCS. Thymocyte density was adjusted to 1.5×10^6 cells/ml, and submitogenic concentrations of concanavalin A (Con A, 0.2 μg/ml) (Sigma Co.) were added per culture. These thymocyte suspensions were placed in individual wells of a Linbro 96-well round-bottom microtiter plate (Flow Labs, McLean, VA) and 100 μl of macrophage culture supernatant fluids was added to obtain a final volume of 200 μl per well. The cultures were incubated for 54 hr at 37°C and the plates pulsed with 0.5 μCi [^{3}H]thymidine (ICN Radiochemicals, Irvine, CA) per well for an additional 18 hr of culture. The plates were harvested with a semiautomated harvester (Model M-12, Brandel, Rockville, MD) and [^{3}H]thymidine incorporation was measured by liquid scintillation.

The presence of IL-1 was detected by proliferation of the thymocytes which is shown as an increase in the thymidine uptake (24, 25).

IL-2 supernatants. Spleen cells and purified T cells from uninfected and FLV-infected BALB/c mice obtained at various intervals p.i. were cultured in aliquots of 1 ml containing 1 × 10^6 cells in 24-well plates (Costar). Phytohemagglutinin (PHA-P Sigma Co.) at a concentration of 2.5 μg/ml was added to the cultures to stimulate the production of IL-2. Spleen cell cultures were incubated for 24 hr at 37°C in 5% CO_2. Supernatant fluids were recovered, centrifuged, filter sterilized, and tested for IL-2 activity (26).

IL-2 assay. This assay, derived from the work of Gillis *et al.* (26), utilizes IL-2-induced proliferation of an IL-2-dependent cell line, CTLL-2. CTLL-2 cells (1 × 10^4 cells) were suspended in 100 μl RPMI 1640 medium supplemented with 10% FCS and 5 × 10^{-5} *M* 2-mercaptoethanol and mixed with 100 μl test supernatant fluid. The cells were cultured for 24 hr at 37°C in 5% CO_2 in triplicate in 96-well round-bottom microtiter plates. The cultures were then pulsed with 1 μCi [^{3}H]thymidine for 6 hr and harvested and radioactivity was counted by liquid scintillation. The presence of IL-2 in the supernatant fluids was detected by proliferation of the CTLL-2 cells which is shown as an increase in the thymidine uptake. One unit of IL-2 induces 50% of the maximal proliferative response of CTLL-2 cells.

Lymphocyte blastogenic transformation assay. Spleen cells and T lymphocytes from uninfected and FLV-infected animals were resuspended at a concentration of 10^6/ml in RPMI medium supplemented with 10% FCS. PHA-P (Sigma Co.) was added at a concentration of 2.5 μg/ml. Supernatant fluids containing crude IL-2 (8 U/ml) or recombinant IL-2 (rIL-2), kindly supplied by W. Benjamin, Hoffmann–LaRoche (Nutley, NJ), were added to the cultures as indicated. A total volume of 250 μl/well was used in 96-well round-bottom microtiter plates (Flow). Tritiated thymidine (0.5 μCi/well) was added to the wells for the final 18 hr of a total 72-hr incubation period at 37°C, 5% CO_2, after which the cells were harvested and thymidine incorporation was measured by liquid scintillation (27). Each test was performed with three replicate samples.

Statistical analysis. Statistical analysis of data was performed using the Student's *t* test.

Results. *IL-1 activity in adherent spleen cell culture supernatant fluids after FLV infection.* The ability to produce IL-1 by FLV-infected mice was examined 14 days post infection. Supernatant fluids were prepared from cultures of adherent spleen cells derived from control and infected mice after *in vitro* stimulation with *E. coli* LPS (20 μg/ml) for 24 hr. Measurement of IL-1 activity in the supernatant fluids, as shown in Table I, indicated that cells from 14-, 21-, and 28-day FLV-infected animals had an IL-1 response that was comparable to cells from control animals. Supernatant fluids from adherent spleen cell cultures from noninfected or 14-, 21-, and 28-day FLV-infected animals not stimulated with LPS showed no detectable IL-1 activity.

These results indicate that the ability to produce IL-1 is not affected in macrophages of FLV-infected mice. The data also show that IL-1 activity was undetectable in supernatant fluids of spleen cells from leukemic mice not stimulated with LPS suggesting that FLV infection did not have the ability to induce spontaneous production and/or release of IL-1 in these cultures.

In vitro FLV infection of normal spleen cell cultures was performed to determine the possible effect of FLV on the production and release of IL-1. Whole spleen cell cultures and spleen macrophage cultures were prepared from normal mice as described under Materials and Methods. These cultures from noninfected BALB/c mice were infected with 0.1 ml of a 1:10 dilution of the virus stock preparation so that each culture received approximately 100 ID_{50}. Whole spleen cell cultures from noninfected BALB/c mice were inoculated with the virus at 0 hr and incubated for an additional 20 hr and LPS (20 μg/ml) was added to the cultures to induce production of IL-1. Spleen macrophage cultures received virus after nonadherent cells were removed (20 hr) and at the same time LPS was added to the cultures. Subsequently the cultures were then incubated for an addi-

TABLE I. IL-1 Activity in Supernatant Fluids from *in Vivo* or *in Vitro* FLV-Infected Whole Spleen Cells and Splenic Macrophage Cultures

BALB/c supernant fluids[a]	LPS[b]	Virus added[c]	C_3H/HeJ thymocyte proliferation (cpm ± SD)[d]	SI[e]
Uninfected splenic	–	–	215 ± 71	
macrophage cultures	+	–	3531 ± 2884	16.42
FLV 14 day p.i.	–	–	399 ± 149	
splenic macrophage cultures	+	–	4643 ± 2882	11.64
FLV 21 day p.i.	–	–	183 ± 63	
splenic macrophage cultures	+	–	2562 ± 245	14.0
FLV 28 day p.i.	–	–	161 ± 42	
splenic macrophage cultures	+	–	2484 ± 173	15.43
Whole spleen cells	–	+	175 ± 22	
	+	+	3580 ± 653	20.45
Splenic macrophage cultures	–	+	286 ± 66	
	+	+	2721 ± 1894	9.51

[a] Supernatant fluids from adherent spleen cells stimulated with *E. coli* LPS (20 μg/ml) for 24 hr.

[b] Two mice/group.

[c] Whole spleen cell and splenic macrophage cultures were infected with 550 ID_{50} of virus.

[d] Means of six experiments.

[e] Stimulation index (SI) = ratio of cpm with LPS/cpm without LPS.

tional 24 hr at 37°C, 5% CO_2. Supernatant fluids were recovered, centrifuged, filter sterilized, and tested for IL-1 activity using thymocytes from healthy C_3H/HeJ mice. As shown in Table I, IL-1 production by whole spleen cell or splenic macrophage cultures was not suppressed by *in vitro* addition of FLV. These results showed that the FLV infection did not alter the ability to produce and release IL-1. Therefore, the efferent arm of the IL-1 system did not appear to be altered. The afferent arm of the IL-1 response in spleen cells from FLV-infected animals was similarly studied. For this purpose IL-1 was induced in whole spleen or splenic macrophage cultures from animals infected 14, 21, and 28 days prior to assay. The ability of these cells to respond to IL-1 was tested by using thymocytes from FLV-infected and uninfected BALB-c mice as responder cells.

Table II shows the results obtained when the supernatant fluids were tested using thymocytes from uninfected BALB/c mice. The ability of cells from 14-, 21-, and 28-day FLV-infected animals to produce IL-1 was comparable to control values. Table III shows the IL-1 activity in culture supernatant fluids of cells obtained from FLV-infected animals tested using their own thymocytes. The ability of cells from 14-, 21-, and 28-day FLV infected animals to respond to IL-1 was normal as compared to control values. These data showed that the ability of lymphoid cells from FLV-infected animals

TABLE II. IL-1 Activity in Splenic Macrophage Culture Supernatant Fluids at Different Times post-FLV Infection

BALB/c supernatant fluids	LPS[a]	Noninfected BALB/c thymocytes proliferation (cpm ± SD)[b]	SI[c]
Control	–	221 ± 88	
	+	1757 ± 142	7.95
FLV 14 day	–	594 ± 200	
	+	2707 ± 578	4.55
FLV 21 day	–	786 ± 323	
	+	2516 ± 531	3.20
FLV 28 day	–	460 ± 163	
	+	1934 ± 346	4.20

[a] IL-1 containing supernatant fluids were obtained from control and FLV-infected macrophage cultures stimulated with *E. coli* LPS (20 μg/ml) for 24 hr.

[b] Means of four experiments. Each experiment was performed using three replicate cultures per group.

[c] Stimulation index (SI) = ratio of cpm with LPS/cpm without LPS.

TABLE III. RESPONSIVENESS OF CONTROL AND FLV-INFECTED THYMOCYTES TO IL-1

BALB/c supernatant fluids	LPS[a]	Thymocyte proliferation (cpm ± SD)[b]	SI[c]
		Control thymocytes	
Control	–	221 ± 88	
	+	1757 ± 142	7.95
		FLV 14 day thymocytes	
FLV 14 day	–	158 ± 67	
	+	3215 ± 404	20.35
		FLV 21 day thymocytes	
FLV 21 day	–	175 ± 50	
	+	2414 ± 612	13.79
		FLV 28 day thymocytes	
FLV 28 day	–	91 ± 58	
	+	841 ± 89	9.24

[a] IL-1 containing supernatant fluids were obtained from control and FLV-infected macrophage cultures stimulated with *E. coli* LPS (20 μg/ml) for 24 hr.

[b] Means of four experiments. Each experiment was performed using three replicate cultures per group.

[c] Stimulation index (SI) = ratio of cpm with LPS/cpm without LPS.

to produce and respond to IL-1 was essentially not altered by infection.

IL-2 activity in spleen culture supernatant fluids after FLV infection. Supernatant fluids were prepared from spleen cells of control and FLV infected animals at different times p.i. Table IV shows the IL-2 activity of such supernatant fluids. IL-2 production was rapidly decreased after FLV infection. This suppression increased as the time between infection and assay was lengthened. These animals, as a result of the FLV infection, showed splenomegaly which directly increased with time p.i. Due to the large amount of tumor cells present in the spleen cell cultures, the suppression of IL-2 activity in the supernatant fluids could be due to a dilution effect and not necessarily to lack of activity of the IL-2 producing cells. To examine this possibility, tumor cells were separated from lymphocytes by Percoll gradients. Due to their larger size, the tumor cells were concentrated in the low density layers in the gradient (38.6–52% Percoll). Lymphocytes were concentrated in the high density layers. For this reason lymphocytes isolated from these layers were referred to as high density lymphocytes (HDL). To confirm the "purity," i.e., homogeneity of the HDL, antibody against a T cell marker (Lyt 1.2) was used for flow cytometry. The results from these experiments showed that at least 98% of HDL were T cells (98.2% FLV infected; 99.5% uninfected).

Supernatant fluids were prepared from control and FLV-infected HDL cultures at different times p.i. Table V shows that the IL-2 activity of HDL supernatant fluids was comparable to that of whole spleen cell cultures. IL-2 activity in these supernatant fluids was decreased after FLV infection. Even though on occasion IL-2 activity was slightly larger in FLV-infected HDL cultures as compared to FLV-infected whole spleen cell cultures, it was still suppressed as compared to control values. Therefore, suppression of IL-2 activity in the supernatant fluids

TABLE IV. IL-2 ACTIVITY IN SPLEEN CULTURE SUPERNATANTS AFTER FLV INFECTION

BALB/c supernatant fluids[a,b]	CTLL-2 proliferation (cpm ± SD)[c]	% Control	Significance *P*[d]
Control	13,922 ± 774	100	
FLV 14 day	3,433 ± 540	25	<0.05
FLV 28 day	1,540 ± 838	11	<0.05
Control media	712 ± 98	—	

[a] Supernatant fluids from whole spleen cell cultures stimulated with PHA (2.5 μg/ml) for 24 hr.

[b] Two mice/group.

[c] CTLL-2 cells proliferation (measured as ^{3}H uptake) in the presence of control and/or FLV supernatant fluids. Numbers represent the means of five experiments. Each experiment was performed using three replicate cultures per group.

[d] Significance calculated using Student's *t* test comparing control cpm to experimental cpm.

TABLE V. EFFECT OF FLV INFECTION ON IL-2 PRODUCTION BY BALB/c WHOLE SPLEEN CELLS AND PURIFIED T CELLS

Cultures	CTLL-2 proliferation (cpm ± SD)[c]	% Control	Significance P^d
Control[a]	10,278 ± 1008	100	
Control HDL[b]	11,395 ± 1034	100	
FLV 14 day[a]	1,809 ± 786	18	<0.05
FLV 14 day HDL[b]	2,778 ± 401	24	<0.05
FLV 21 day[a]	1,162 ± 392	11	<0.05
FLV 21 day HDL[b]	510 ± 258	4	<0.05
FLV 28 day[a]	339 ± 252	3	<0.05
FLV 28 day HDL[b]	599 ± 202	5	<0.05
Control media	207 ± 42		

[a] Whole spleen cells.

[b] T cell-enriched population.

[c] Means of five or more experiments.

[d] Significance calculated using Student's *t* test comparing control cpm with experimental cpm.

of FLV-infected spleen cells could not have been due to a dilution effect by tumor cells. The lymphoproliferative response to PHA was examined in whole spleen cell cultures and HDL cultures from control and FLV-infected animals. The results (Table VI) showed that the lymphoproliferative response was markedly suppressed by FLV infection. This suppression correlated with suppression of IL-2 activity. Depressed proliferation as well as suppression of IL-2 activity increased as the interval between infection and assay was lengthened. As in the IL-2 assays, PHA induced slightly higher proliferation in the HDL cultures as compared to whole spleen cell cultures, although the response was still suppressed as compared to control values.

Previous reports (28–30) have indicated that microbial inhibition of lymphocyte mitogenesis is caused by interference with synthesis of functionally active IL-2. Those studies also reveal that reversal of inhibition is possible by addition of IL-2. Therefore, IL-2 responsiveness of control and FLV-infected spleen cells was studied. Table VII shows the proliferative activity of control and FLV whole spleen cells and HDL cultures when exogenous IL-2 (rIL-2 100 U) was added. Addition of exogenous IL-2 to the cultures

TABLE VI. EFFECT OF FLV INFECTION ON PHA-DRIVEN PROLIFERATION OF WHOLE SPLEEN AND/OR T CELL-ENRICHED CULTURES

Cultures	Lymphocyte proliferation (cpm ± SEM)[c]	% Control	Significance P^d
Control[a]	35,399 ± 3294	100	
Control HDL[b]	27,609 ± 2434	100	
FLV 14 day[a]	4,449 ± 312	13	<0.05
FLV 14 day HDL[b]	6,135 ± 439	22	<0.05
FLV 21 day[a]	2,186 ± 371	6	<0.05
FLV 21 day HDL[b]	3,909 ± 854	14	<0.05
FLV 28 day[a]	953 ± 92	3	<0.05
FLV 28 day HDL[b]	1,325 ± 92	5	<0.05
Control without PHA	105 ± 6		
Control HDL without PHA	223 ± 45		

[a] Whole spleen.

[b] T cell-enriched population.

[c] Each experiment was performed using three replicate cultures per group. Means of five experiments.

[d] Significance calculated using Student's *t* test comparing control cpm to experimental cpm.

TABLE VII. IL-2 RESPONSIVENESS OF CONTROL AND FLV-INFECTED SPLEEN CELLS

Cultures[c]	Exogenous IL-2[d]	Lymphocyte proliferation (cpm ± SEM)[e]	SI[f]	% Control	Significance P[g]
Control[a]	−	3,047 ± 790		—	
Control[a]	+	155,225 ± 12,322	50.94	100	
Control HDL[b]	−	5,576 ± 1,475		—	
Control HDL[b]	+	258,451 ± 11,553	46.35	100	
FLV 14 day[a]	−	1,057 ± 389		—	
FLV 14 day[a]	+	10,801 ± 1,075	10.22	7	<0.05
FLV 14 day HDL[b]	−	1,474 ± 133		—	
FLV14 day HDL[b]	+	112,939 ± 16,900	76.62	43	<0.05
FLV 21 day[a]	−	1,959 ± 360		—	
FLV 21 day[a]	+	4,222 ± 520	2.15	3	<0.05
FLV 21 day HDL[b]	−	2,085 ± 507		—	
FLV 21 day HDL[b]	+	16,803 ± 4,422	8.06	7	<0.05

[a] Whole spleen cells.

[b] T cell-enriched population.

[c] Cultures were stimulated with 2.5 μg/ml PHA.

[d] Recombinant IL-2 (rIL-2) at a concentration of 100 U.

[e] Means of five experiments. Each experiment was performed using three replicate cultures per group.

[f] Stimulation index (SI) = ratio of cpm with rIL-2/cpm without rIL-2.

[g] Significance calculated using Student's t test comparing control cpm versus experimental cpm.

did not overcome the suppression caused by FLV infection. Even though there was a slight increase in response, it was still highly suppressed as compared to control values. Therefore, it could be concluded that the ability of lymphoid cells to respond to exogenous IL-2 was compromised in these animals.

The observation of low IL-2 activity in supernatant fluids from FLV-infected spleen cell cultures could reflect a factor in these supernatant fluids which interferes with IL-2-mediated proliferation of the assayed cells. To evaluate this possibility CTLL-2 cells were cocultured with FLV spleen cell supernatant fluids plus IL-2. These studies indicated that supernatant fluids from FLV-infected splenocytes had no effect on IL-2-mediated proliferation of CTLL-2 cells (Table VIII).

Discussion. These studies examined IL-1 and IL-2 in regard to their possible role in the mechanism of immune suppression during FLV infection of susceptible BALB/c mice. The ability of adherent spleen cells to produce IL-1 *in vitro* was not altered during infection. Thymic cells from FLV-infected animals showed a normal responsiveness to the IL-1 produced. *In vitro* FLV infection of normal spleen cells and enriched macrophage populations showed no alteration in the production and release of IL-1. Therefore, it is suggested that afferent as well as

TABLE VIII. EFFECT OF FLV SPLEEN CELL SUPERNATANT FLUIDS ON THE PROLIFERATIVE RESPONSE OF CTLL-2 CELLS TO EXOGENOUS IL-2

Culture supernatant fluids	Exogenous IL-2[c]	CTLL-2 proliferation (cpm ± SD)[e]
Control[a]	+	30,067 ± 153
Control HDL[b]	+	31,213 ± 933
FLV 21 day[a]	+	35,126 ± 1188
FLV 21 day HDL[b]	+	32,385 ± 3553
FLV 21 tumor cells[d]	+	33,112 ± 4059
FLV 28 day[a]	+	29,803 ± 3455
FLV 28 day HDL[b]	+	31,217 ± 1484
FLV 28 tumor cells[d]	+	35,842 ± 1420
None (media control)	−	2,116 ± 968
None (media control)	+	27,841 ± 2018

[a] Whole spleen cells.

[b] T cell-enriched population.

[c] Crude IL-2 (8 U/ml) from Con A-stimulated rat splenocytes.

[d] Enriched leukemic cell population (erythroblastoid derived) from splenocytes of FLV-infected animals.

[e] Means of seven experiments. Each experiment was performed using three replicate cultures per group.

efferent responses involving IL-1 were not altered by FLV infection, and that the presence of virus in the cultures and assay systems did not suppress the IL-1 response.

It is interesting to note in Table III and that IL-1 activity in supernatant fluids taken from spleen cell cultures obtained from FLV infected mice and tested on thymocytes obtained from FLV-infected mice was higher earlier after infection (14 days) and decreased to normal levels later (Day 28). These data imply that IL-1 activity may be increased early after infection and then return to normal. Studies suggesting that increased IL-1 reactivity could be observed in cells from FLV-infected mice 7 days post infection but returned to normal by Day 14 have been communicated to us (M. Bendinelli, personal communication). This further supports our evidence that FLV infection does not suppress IL-1 production or reactivity.

However, lymphocyte cultures from infected mice showed a progressive loss of IL-2 activity production and responsiveness. Interestingly, the kinetics of loss paralleled the depression of humoral and cell-mediated immune responses (4), NK cell activity (8), and the decreases previously reported for cytotoxic and suppressor T lymphocytes (6, 7). Deficient IL-2 production has been well documented in a number of experimental infections (31) as well as in clinical situations. For example, suppressed IL-2 production and/or responsiveness has been reported in AIDS (32), lepromatous leprosy (33), recurrent herpes simplex virus infections (34), and pulmonary tuberculosis (35).

Decreased IL-2 activity in FLV-infected mice needs to be examined further to determine what is causing the suppression. Possible explanations include: (a) a dilution of producer and responsive cells by irrelevant cells; (b) adsorption of IL-2 to tumor cells; (c) unresponsiveness to IL-2 due to a lack of expression or function of IL-2 (TAC) receptors; (d) immunomodulation by suppressor cells, including T cells, macrophages, or tumor cells, or their soluble suppressor factors.

Due to the large proportion of abnormal cells present in the spleen of FLV-infected animals from the second week of infection, a dilution effect is a clear possibility. Removal of most tumor cells by Percoll gradients showed that IL-2 activity production was slightly enhanced in T cell cultures from FLV-infected mice as compared to whole spleen cell cultures, but was still suppressed as compared to control values. These results indicate that suppression of IL-2 production was not simply due to a dilution effect caused by the presence of tumor cells. Thus, Percoll fractionation of spleen cells from FLV-infected mice clearly identified the T lymphocyte (Lyt 1.2 positive cell population) as the affected target of the inhibition of IL-2 production.

It has been reported that the addition of uv-irradiated feline leukemia virus suppresses IL-2 production by human and murine lymphocytes *in vitro* (36, 37). The addition *in vitro* of cell-free FLV did not affect IL-2 production by normal spleen cells. Nevertheless, it is not possible at present to rule out residual tumor cells (38) or soluble suppressor factors from tumor or lymphoid cells as the mediators of suppression of IL-2 production. However, the possibility that low IL-2 activity in supernatant fluids from FLV-infected spleen cell cultures could reflect a factor that is interfering with IL-2-mediated proliferation (response to IL-2) was excluded using CTLL-2 cells cocultured with FLV spleen cell supernatant fluids plus exogenous IL-2.

Viral interference by other retroviruses in the synthesis of functionally active IL-2 has been shown to cause inhibition of lymphocyte mitogenesis (39–41). The previous studies revealed that reversal of inhibition is possible by the addition of exogenous IL-2. In the present studies addition of exogenous IL-2 to spleen or T cell cultures did not overcome the suppression caused by FLV infection. These studies showed that the ability of lymphoid cells from FLV-infected animals to respond to exogenous IL-2 is compromised. Similarly, exogenous IL-2 failed to overcome the depressed lymphocyte proliferation. This is like the suppression of cytotoxic lymphocyte generation by murine splenocytes exposed *in vitro* to FeLV (42). Studies by Toossi and co-workers (35) showed that purified IL-2 failed to correct unresponsiveness of purified protein derivative-activated peripheral mononuclear cells in tuberculosis patients. This unresponsiveness to IL-2 was associated with deficient expression of IL-2 receptors as identified using anti-TAC monoclonal antibody. Due to the importance of IL-2 receptor expression for immune regulation (43) we are presently investigating this aspect in spleen cells of FLV-infected mice.

Immune suppression in humans and animals infected with retroviruses has been extensively documented (44). *In vitro* immune suppression also has been demonstrated with various retrovirus preparations, including disrupted viruses, uv-inactivated viruses and semipurified virus structural protein preparations (37, 45, 46).

Although various mechanisms for the immune suppression that occurs during retrovirus infection have been proposed, much of the research in this area has centered on the role of retroviral envelope proteins as a major mediator of immune suppression. The envelope (env) gene of type C retroviruses (e.g., FLV) codes for an 80,000- to 90,000-Da precursor protein. The precursor protein is cleaved during viral maturation to yield the major envelope glycoprotein gp 70 and the nonglycosylated, 19,000-Da protein p15E (47). The transmembrane component, p15E is capable of suppressing the *in vitro* proliferation of murine, feline, and human lymphocytes exposed to mitogenic and allogeneic stimulation (47). Likewise, p15E is well conserved among type C and type D retroviruses and recently a 26 amino acid region, which is highly conserved, has been identified in the murine leukemia viruses, including FLV, as well as feline, avian, bovine, and human retroviruses such as HTLV-I and -II (47). This region is also contained in the type D simian retroviruses such as the Mason-Pfizer virus (48). Studies by Blalock and co-workers (49) demonstrate a region of homology between the env protein of the AIDS virus and a portion of the IL-2 molecule that binds to the IL-2 receptor. The same homologous sequence is present in the env proteins of other human (HTLV-I and -II), murine, and feline retroviruses associated with immune suppression. Therefore, this suggests a possible mechanism for the retrovirus-induced immune suppression in which env protein interferes with the IL-2 activity either directly or indirectly. This relationship between retrovirus env protein and IL-2 activity has become a focus for additional investigation.

In conclusion, IL-1 production and response to this interleukin are not compromised in FLV-infected mice; however, IL-2 production and responsiveness were strongly suppressed in these mice. Such suppression correlated well with the suppression of many parameters of humoral and cell-mediated immunity which occurs in such mice.

The portion of this work performed in Italy was supported by grants from the Italian National Research Council, Special Project "Oncology," and the Italian Ministry of Public Education. Special thanks to Mrs. Sally Baker for her secretarial assistance.

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Received September 18, 1987. P.S.E.B.M. 1988, Vol. 188.
Accepted March 31, 1988.