BIOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF LYMPHOCYTE REGULATORY MOLECULES

V. Identification of an Interleukin 2-producing Human Leukemia T Cell Line

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Recent studies from our laboratories (1, 2) and others (3, 4) have confirmed that interleukin 2 (IL-2;[1] formerly referred to as T cell growth factor, or TCGF) purified from either mouse, rat, or human lymphocyte conditioned medium retains biologic activity in several in vitro murine lymphocyte response assays. For example, isoelectrically pure IL-2: (*a*) stimulates the long-term in vitro proliferation of antigen-specific effector T cells; (*b*) enhances thymocyte mitogenesis; and (*c*) induces cytotoxic T cell (CTL) reactivity and plaque-forming cell responses in cultures of nude mouse spleen cells (1, 2). Because current preparative purification protocols require fractionation of several liters of conventionally prepared conditioned medium to generate submicrogram quantities of IL-2, we have initiated several studies aimed at identifying more potent sources of IL-2. After screening >40 malignant murine T cell lines, we recently identified, adapted to in vitro culture, and cloned a murine T cell lymphoma that, upon T cell mitogen stimulation, produces 1,000–10,000 times the amount of IL-2 generated by similarly activated mouse or rat spleen cells.[2] Murine lymphoma-generated IL-2 was found to be biochemically indistinguishable from IL-2 produced by ligand-stimulated normal mouse splenocytes.[3]

Based on these results we undertook a similar screening of several established human leukemic T and B cell lines for constitutive and lectin-induced IL-2 production. In this communication we report that one such leukemic T cell line (designated

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; CTLL, cytotoxic T lymphocyte line; FCS, fetal calf serum; [^{3}H]Tdr, tritiated thymidine; IL-1, Interleukin 1; IL-2, Interleukin 2; NaGO, neuraminidase galactose oxidase; PBL, peripheral blood leukocytes; PHA, phytohemagglutinin; PKW, pokeweed mitogen; PMA, phorbol myristate acetate; TCGF, T cell growth factor; TDT, cytosol terminal deoxynucleotidyl transferase.

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Jurkat-FHCRC) can be stimulated by phytohemagglutinin (PHA) or concanavalin A (Con A) to produce between 100 and 300 times the amount of human IL-2/ml normally generated by lectin-stimulated human peripheral blood lymphocytes (PBL) or spleen cells. Because Jurkat-FHCRC-derived IL-2 retained the capacity to promote the in vitro proliferation of antigen-activated human and mouse effector T cells, we feel that such a line will prove to be a valuable reagent for investigators interested in growing clonal human T cells with various antigen and effector specificities. Furthermore, Jurkat-FHCRC cells will provide a much needed source of concentrated human IL-2 conditioned medium to foster further molecular characterization of this lymphocyte regulatory molecule.

Materials and Methods

Screening of Leukemic T and B Cell Lines for IL-2 Production. Several (see Table I) in vitro cultured leukemic T and B cell lines (RPMI-1640 medium, supplemented with 10% heat-inactivated [56°C for 30 min] fetal calf serum [FCS], 50 U/ml penicillin, 50 μg/ml streptomycin, and 300 μg/ml fresh L-glutamine) were tested for both constitutive and lectin-induced IL-2 production. Cell lines designated SKI were obtained either from Dr. Roland Mertelsmann, Laboratory of Developmental Hematopoiesis, or Dr. Peter Ralph, both from the Memorial Sloan-Kettering Cancer Center, New York. Cell lines designated FHCRC were obtained from Dr. John Hansen, Histocompatibility Laboratory, Puget Sound Blood Center, Fred Hutchinson Cancer Research Center, Seattle, Wash. (5). After harvest from exponential proliferative culture, cell line samples (2×10^6 cells/ml) were resuspended in Click's medium (supplemented with 10% FCS, 25 mM Hepes Buffer, 16 mM $NaHCO_3$, 50 U/ml penicillin, 50 μg/ml streptomycin, and 300 μg/ml fresh L-glutamine) and seeded in 100-μl aliquots in replicate flat-bottomed microplate wells (3596; Costar, Data Packaging, Cambridge, Mass.). Triplicate microwell cultures were then stimulated by a 100-μl addition of either (*a*) tissue culture medium; (*b*) Con A (20 μg/ml; Miles Biochemicals Inc., Elkhart, Ind.; (*c*) PHA (PHA-M; 2% by volume; Grand Island Biological Co., Grand Island, N. Y.); or (*d*) pokeweed mitogen (PKW; 1/50 dilution; Grand Island Biological Co.). After the time periods indicated (Results), supernate samples from triplicate cultures were pooled and assayed for IL-2 activity in a standard microassay as detailed below. Optimal mitogen dose, harvest time and cell concentration for Jurkat-FHCRC-mediated IL-2 production was determined in separate experiments conducted either in 200-μl microwell cultures as detailed above or in 5-ml vol in tissue culture flasks (3013; Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.). All IL-2 production cultures were conducted at 37°C in a humidified atmosphere of 5% CO_2 and air.

IL-2 Microassay. IL-2 activity was determined using a standard TCGF microassay (6) monitoring the IL-2-dependent cellular proliferation of a mouse cytotoxic T cell line (CTLL) (7). Briefly, 3,000 CTLL cells were seeded in replicate 200-μl vol (in 96-well flat-bottomed microtiter plates) in the presence of a $\log_2$ dilution series of a given IL-2-containing sample. After 24 h of culture (at 37°C in a humidified atmosphere at 5% and air) microwell cultures were pulsed for 4 h with 0.5 μCi of tritiated thymidine ([^{3}H]Tdr; 20 mCi/mM sp act, New England Nuclear, Boston, Mass.) after which time the cultures were harvested onto glass fiber filter strips with the aid a multiple automated sample harvester (Microbiological Associates). [^{3}H]Tdr incorporation was determined by liquid scintillation counting. Using these methods, only CTLL cells cultured in the presence of IL-2 incorporated [^{3}H]Tdr in a dose-dependent manner. CTLL cells cultured in the absence of IL-2 incorporated only scintillant control levels of [^{3}H]Tdr and were >95% trypan-blue positive after 24 h of IL-2 deprivation. Units of IL-2 activity were determined by probit analysis of [^{3}H]Tdr incorporation data (6). A 1 U/ml standard was defined as the amount of IL-2 activity present in 48 tissue culture medium conditioned by the Con A (5 μg/ml) stimulation of normal rat spleen cells (10^6 cells/ml). Assay of 1 U/ml standard routinely stimulated 10,000 cpm of CTLL [^{3}H]Tdr incorporation at a dilution of 1:2.

To confirm that the IL-2 activity produced by PHA-stimulated Jurkat-FHCRC cells would support the sustained in vitro proliferation of human activated T cells, identical TCGF

microassays were conducted using an IL-2-dependent human cytotoxic T cell line as the indicator cell (10^5 cells/ml). This cell line, designated MLR-FHCRC-1 was developed from effector cells harvested from a 10-d mixed lymphocyte reaction and had been in IL-2-dependent culture for 4–6 weeks before use in TCGF microassays.

The amount of IL-2 activity generated by Jurkat-FHCRC cells was compared to that present in several other sources of conditioned medium. Conditioned media were prepared by the T cell mitogen stimulation of normal murine or rat spleen cells. IL-2-containing supernates were also generated from single donor human PBL stimulated with (*a*) PHA; (*b*) PHA and allogeneic cells from an in vitro cultured B lymphoblastoid cell line (Ar-77, obtained from Dr. R. Mertelsmann); or (*c*) neuraminidase and galactose-oxidase (NaGO). Additional IL-2-containing supernates were produced by the 1% PHA stimulation of either normal human spleen cells (obtained from Dr. Michael Bean, Virginia Mason Research Center, Seattle, Wash.) or the mouse lymphoma LBRM-33. Methods for production of all of the above IL-2-containing tissue culture media have been detailed elsewhere (6–9).[2, 3]

Results

Screening of Human T and B Leukemia Cell Lines for IL-2 Production. Table I details constitutive and lectin-induced IL-2 production by 48-h cultures of 16 cell lines. Where possible, identical cell lines were obtained from two different sources in the event that upon subsequent long-term culture, one derivative might have expressed a capacity to produce IL-2. Of the lines tested, only one, the T cell leukemia Jurkat-

TABLE I

Screening of Cultured Human T and B Cell Leukemias for IL-2 Production

	IL-2 activity present in 48-h supernate after activation with			
	Medium*	Con-A‡	PHA§	PKW‖
		U/ml		
T cell leukemias				
CEM-SKI	0.0	0.0	0.0	0.0
CEM-FHCRC	0.0	0.0	0.0	0.0
8402-FHCRC	0.0	0.0	0.0	0.0
HSB2-SKI	0.0	0.0	0.0	0.0
HSB2-FHCRC	0.0	0.0	0.0	0.0
Molt-4-SKI	0.0	0.0	0.0	0.0
Molt-4-FHCRC	0.0	0.0	0.0	0.0
Ke37-FHCRC	0.0	0.0	0.0	0.0
T-45-SKI	0.0	0.0	0.0	0.0
R-2-SKI	0.0	0.0	0.0	0.0
Peer-SKI	0.0	0.0	0.0	0.0
HPB-ALL-SKI	0.0	0.0	0.0	0.0
Jurkat-FHCRC	0.0	93.7	225.0	52.1
B cell leukemias				
Daudi-SKI	0.0	0.0	0.0	0.0
Nalm-1-SKI	0.0	0.0	0.0	0.0
8866-FHCRC	0.0	0.0	0.0	0.0
AR-77-SKI	0.0	0.0	0.0	0.0

* RPMI-1640, 10% FCS.
‡ 10 μg/ml.
§ 1.0% by volume.
‖ 1/100 dilution.

FHCRC, produced significant quantities of IL-2 after mitogen sensitization. In fact, 1% PHA stimulation of Jurkat-FHCRC cells resulted in the elaboration of conditioned media that contained 225 times the amount of IL-2 present in a standard rat spleen cell preparation of Con A conditioned medium (Materials and Methods). None of the cells tested demonstrated a capacity for constitutive IL-2 production. It should be stressed that the IL-2 production data detailed in Table I were not significantly different when supernates were harvested from 24- or 72-h medium and lectin-stimulated cultures (data not shown). Therefore, it is probable that the cell lines identified as negative for IL-2 production (using the concentrations of lectin indicated) do not yield negative values because of an alteration in production kinetics.

The amount of IL-2 activity produced by PHA-stimulated Jurkat-FHCRC cells was quite significant especially in comparison to more conventional sources of human conditioned medium. As demonstrated in Table II, only PHA-stimulated LBRM-33 cells (a radiation-induced splenic lymphoma from the B10.BR mouse) produced more IL-2 activity than Jurkat-FHCRC leukemia cells.[2, 3] Clearly, the Jurkat-FHCRC cells were the reagent of choice for production of highest titer IL-2 from human sources. The supernates generated by 1% PHA-stimulated Jurkat-FHCRC cells contained 10 times the amount of IL-2 produced by the next most successful method (dual stimulation of single donor peripheral blood leukocytes by PHA and a B lymphoblastoid cell line). It is of interest to note that NaGO stimulation of human PBL lead to the generation of IL-2-containing conditioned medium. This treatment was originally described by Novogrodsky et al. (11) as a method for initiating human T cell

TABLE II

Relative Capacity of Rat, Mouse, and Human Lymphocyte Populations to Produce IL-2

Lymphocyte source	Mitogen	Cell concentration*	IL-2 (TCGF) activity in filtered medium	
			24-h supernate	48-h supernate
			U/ml	
Mouse spleen	Con-A (1.25 μg/ml)	10^6/ml	0.10	0.35
Mouse spleen	Con-A (2.5 μg/ml)	10^7/ml	1.60	0.75
Rat spleen	Con-A (5 μg/ml)	10^6/ml	0.68	1.0 (standard)
Rat spleen	Con-A (5 μg/ml)	10^7/ml	13.60	7.20
Human PBL	PHA 1%	10^6/ml	2.30	4..30
Human PBL	B-LCL‡ (Daudi or AR-77 2×10^5–5×10^5/ml live or irradiated)	10^6/ml	0.63	1.20
Human PBL	B-LCL + 1.0% PHA	10^6/ml	8.60	10.70
Human PBL	NaGO§	10^6/ml	3.60	0.73
Human spleen	1% PHA	10^7/ml	25.00	12.60
Mouse lymphoma LBRM-3	1% PHA	10^6/ml	535.00	520.00
Human T cell leukemia Jurkat-FHCRC	1% PHA	10^6/ml	206.00	185.00

* 50-ml vol in 250-mm^2 flasks lying flat in a humidified atmosphere of 5% CO_2.

‡ B lymphoblastoid cell line.

§ NaGO treatment (8).

proliferation, and provided an excellent means of producing low titer, but mitogen-free, human IL-2.

Because the experiments described in both Tables I and II monitored IL-2 activity on the basis of its capacity to sustain the in vitro proliferation of murine activated T cells, it was not necessarily clear that PHA-stimulated Jurkat-FHCRC cells produced significant amounts of human IL-2 activity. To approach this problem, both Jurkat-FHCRC and LBRM-33 conditioned medium were tested for their capacity to induce proliferation of both murine CTLL and human MLR reactive T cell lines harvested from IL-2 dependent cultures. Results of these experiments are displayed in Fig. 1 in terms of [^{3}H]Tdr incorporation observed in cultures that contained various concentrations of conditioned medium. Jurkat-FHCRC-derived IL-2 activity was capable of sustaining the in vitro proliferation of both murine and human CTLL. LBRM-33-generated conditioned medium proved effective only when tested on murine CTLL. Tissue culture medium that contained 1% PHA was incapable of inducing proliferation of either mouse or human activated T cells. These data were consistent with our previous studies detailing the species specificity of IL-2 (6) and confirmed that Jurkat-FHCRC conditioned medium was an excellent source of human IL-2 activity.

Jurkat-FHCRC IL-2 Production Kinetics and Mitogen Dose Responses. To determine the production kinetics of Jurkat-FHCRC-derived IL-2, Jurkat-FHCRC cells were har-

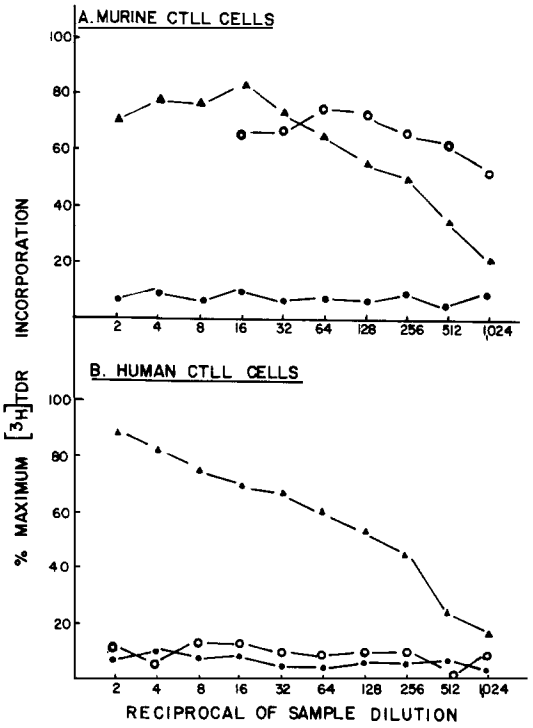

FIG. 1. [^{3}H]Tdr incorporation (4-hour pulse) by murine (A) and human (B) CTLL cells after a 24-h culture in a $\log_2$ dilution series of medium conditioned by 1% PHA-stimulated Jurkat-FHCRC (▲) or LBRM-3 cells (○). Response is also indicated after culture in 1% PHA (●). Maximal[^{3}H]-Tdr incorporation witnessed by murine and human CTLL cells was 10,984 and 6,530 cpm, respectively. LBRM-3-derived supernate contained 936 U/ml IL-2, whereas Jurkat-FHCRC-generated conditioned medium contained 225 U/ml IL-2 activity.

vested from in vitro culture and seeded in replicate flat-bottomed microplate wells (10^6 cells/ml) in the presence of 1% PHA. After 2, 4, 6, 8, 10, 12, 24, 48 h of culture, supernates were harvested and assayed for IL-2 activity on murine CTLL cells. As shown in Fig. 2, detectable IL-2 activity was first observed between 5 and 7 h after PHA stimulation. Peak levels of IL-2 were present in 16–24 h cultured supernates (100–250 U/ml) and these titers diminished only slightly during a subsequent 24-h culture. As such, Jurkat-FHCRC IL-2 production kinetics were similar to those previously detailed for cultures of lectin-stimulated rat, mouse and human mononuclear leukocytes where measurable IL-2 activity was detected within 6 h after mitogen stimulation (6). However, in addition to the magnitude of the factor produced, the kinetics of IL-2 production from Jurkat-FHCRC cells were somewhat different from that observed from normal cells in that the amount of IL-2 present in post-peak cultures did not significantly decline over time. This is routinely observed in normal spleen and/or PBL IL-2 production as activated T cells absorb and use the IL-2 to foster their own proliferation (10). In fact, Jurkat-FHCRC cells in IL-2 production cultures were >95% trypan-blue positive after a 48-h stimulation with 1% PHA (data not shown). At present, it is difficult to distinguish whether Jurkat-FHCRC cells die as a result of IL-2 production or whether IL-2 is released as a result of mitogen toxicity. Attempts to isolate active IL-2 from supernates of non-stimulated Jurkat-FHCRC cells (via sonication or heat treatment) have been unsuccessful. Therefore, it is questionable whether IL-2 is preformed in the cytoplasm of Jurkat-FHCRC cells and is released into the medium after mitogen sensitization. The observation that increasing concentrations of IL-2 are found over a 16-h time period in supernates of stimulated Jurkat-FHCRC cells (Fig. 2) also argues against the hypothesis that

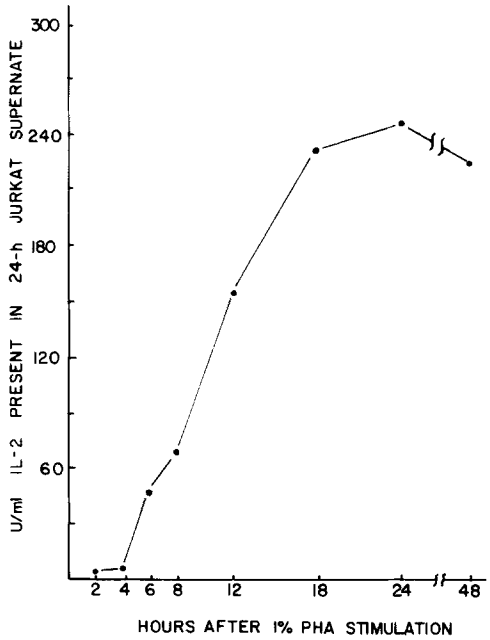

FIG. 2. Jurkat-FHCRC IL-2 production kinetics. Supernate IL-2 activity (●) monitored over time after stimulation of Jurkat-FHCRC cells (10^6/ml) with 1% PHA.

mitogen activation simply stimulates cell death and concommitant release of preformed IL-2.

Experiments similar to those displayed in Fig. 2 were conducted to determine optimal Jurkat-FHCRC cell concentration for IL-2 production. Based on the production kinetics, supernates were harvested from cultures that contained various concentrations of Jurkat-FHCRC cells (ranging from 10^6 to 10^7 cells/ml) 24 h after stimulation with 1% PHA and assayed for IL-2 activity on murine CTLL cells. As shown in Table III, optimal cell concentration for peak Jurkat-FHCRC IL-2 production was $\sim 10^6$ cells/ml. Identical experiments were also conducted to determine optimal mitogen concentration for Jurkat-FHCRC IL-2 production. Cells were plated in replicate microwell cultures (10^6 cells/ml) and stimulated with a range of concentrations of PHA (0.1–10%) and Con A (1.25–100 μg/ml). As detailed in Table IV, peak IL-2 production (>350 U/ml) was observed in either 1% PHA- or 20 μg/ml Con A-stimulated cultures.

Effect of Phorbol Myristate Acetate (PMA) on Jurkat-FHCRC IL-2 Production. Several groups have investigated the effects of macrophages on IL-2 production by normal

TABLE III

Jurkat-FHCRC-derived IL-2 Production: Optimal Cell Concentration

Jurkat-FHCRC cell concentration	IL-2 activity present in 24-h supernate after 1% PHA stimulation
	U/ml
10^7/ml	103
5×10^6/ml	151
2×10^6/ml	193
10^6/ml	225
5×10^5/ml	178
2×10^5/ml	53
10^5/ml	26
5×10^4/ml	10

TABLE IV

Jurkat-FHCRC-derived IL-2 Production: Mitogen Dose Responses

Mitogen	Concentration	IL-2 activity*
		U/ml
PHA	10%	101
PHA	5%	135
PHA	2%	171
PHA	1%	252
PHA	0.5%	234
PHA	0.2%	117
PHA	0.1%	39
Con A	100 μg/ml	124
Con A	50 μg/ml	171
Con A	20 μg/ml	207
Con A	10 μg/ml	121
Con A	5 μg/ml	14
Con A	2 μg/ml	11
Con A	1 μg/ml	8

* 24-h supernate assayed on murine CTLL cells.

murine spleen cells. These studies indicated that either supernates harvested from macrophage tumor cells (11) or, more recently, purified interleukin 1 (IL-1; previously referred to as lymphocyte-activating factor [or LAF]) could restore the capacity of adherent-cell-depleted murine T cells to produce IL-2 after mitogen stimulation (12, 3). Based on the results of Farrar and Fuller-Bonar (14) who found that the unsaturated fatty acid derivative PMA provided a similar macrophage replacement for T cell IL-2 production, we also investigated the effects of PMA on LBRM-33 (an IL-2-producing murine T cell lymphoma). We found that addition of PMA to suboptimally (0.1% PHA) stimulated LBRM-3 cells restored IL-2 production in such cultures to a level equal to that generated by cells stimulated with optimal (1% PHA) amounts of mitogen.[2]

Based on these results, we were curious to determine if PMA stimulation would affect Jurkat-FHCRC-derived IL-2 production. We therefore stimulated Jurkat-FHCRC cells with PMA (10 ng/ml), as well as with 1% and 0.1% PHA either alone or in concert with PMA. After 24 h of culture, supernates were harvested and tested for IL-2 activity. The results of three replicate experiments are shown in Table V. In contrast to our previous observations with LBRM 33 cells, PMA stimulation of Jurkat-FHCRC cells in the absence of mitogen lead to considerable IL-2 production (65 U/ml). Addition of PMA to optimally stimulated (1% PHA) Jurkat-FHCRC cells served to boost IL-2 production titer even higher (>400 U/ml) than that observed when Jurkat-FHCRC were optimally stimulated with PHA alone. Finally, as we had observed with murine IL-2 producer lymphoma cells,[2] addition of PMA to suboptimally stimulated Jurkat-FHCRC cells restored IL-2 production to levels observed when peak concentrations of mitogen were used (≃200 U/ml).

Discussion

The experiments detailed in this communication demonstrate the capacity of Jurkat-FHCRC leukemic T cells to produce human IL-2. Jurkat-FHCRC-derived IL-2 has T cell proliferation-inducing activity as tested using both murine and human cultured T cell lines. Jurkat-FHCRC cells were observed to produce IL-2 upon stimulation with appropriate concentrations of T cell mitogens; however, the quantities of factor generated far exceeded those routinely obtained from more conventional

TABLE V

Effect of PMA on Jurkat-FHCRC Cell Line IL-2 Production

Jurkat-FHCRC cells stimulated with*			IL-2 activity present in 24-h conditioned medium‡
1% PHA	0.1% PHA	PMA (10 ng/ml)	
−§	−	−	0.0 ± 0.0
−	−	+	65.0 ± 17.0
−	+	−	39.0 ± 3.6
+	−	−	210.0 ± 16
−	+	+	178 ± 42
+	−	+	462.0 ± 76

* 10^6 cells/ml in RPMI-1640 10% FCS.

‡ Mean ± 1 SD IL-2 (U/ml) activity present in supernates harvested from three separate experiments.

§ −, not present in culture; +, denotes present in culture.

modes of human IL-2 production. Peak titers of IL-2 (150–350 U/ml) were present in 16- to 24-h cultured supernates of either 1% PHA- or 20-μg/ml Con A stimulated Jurkat-FHCRC cells (10^6 cells/ml). This compares with titers of ~10–15 U/ml of IL-2 activity produced either by (*a*) PHA-stimulated spleen or (*b*) PHA and allogeneic B lymphoblastoid cell line stimulation of normal human PBL. Still higher titer IL-2-containing supernates (400 U/ml) were produced by addition of PMA (10 ng/ml) to 1% PHA-stimulated Jurkat-FHCRC cultures.

In addition to providing a new source for the isolation and characterization of human IL-2, the capacity of a continuously cultured T cell leukemia line to produce IL-2 may provide additional insight into normal IL-2 production and the role that abberrant IL-2 regulation might play in the etiology of human leukemia. For example, the observation that Jurkat-FHCRC lymphoma cells die after release of IL-2, may have some relationship to the normal condition. No evidence has been presented to date either in the mouse or human system to suggest that after ligand activation, the IL-2-producing T cell responds to IL-2 or even survives. If IL-2 producer cells were capable of responding to IL-2, one would expect that after mitogen or antigen sensitization, these cells would continue to proliferate by their own accord; if not indefinitely, at least to the point where they would represent a substantial proportion of the cells surviving ligand stimulation. Clearly, this is not the case. Activated T cells have been grown in culture only by the continual addition of IL-2. In all cases, such cell lines have been shown to mediate effector T cell function and have not been found to demonstrate capacity for IL-2 production (7, 9, 15). The notion that Il-2-producing cells die upon factor release is also supported from our previous studies conducted on an IL-2-producing murine T cell lymphoma. In these experiments, we found that the high-titer IL-2-producing LBRM 33 cell line dies after lectin stimulation and IL-2 release.[2]

The hypothesis that T cell leukemia may represent a population of IL-2 producer cells that responds to its own factor is an intriguing one. One might, therefore, envision a leukemic T cell as a cell with both IL-2 production and response (surface receptor) capacities. Such autostimulatory roles for factor production and use have recently been invoked in the relationship of sarcoma growth factor to the sustained in vitro proliferation of sarcoma virus-transformed cells (16). Similar autostimulatory viral antigen presentation has been suggested as a possible explanation for the proliferation observed in virus-induced murine thymomas (17). Given the knowledge that IL-2 is responsible for antigen-initiated T cell replication (2), the potential involvement of IL-2 in the uncontrolled replication of leukemic T cells may be considerable. For example, in screening 49 samples of adult human leukemic blasts, we observed that >95% of those patients whose malignant cells tested positive for cytosol terminal deoxynucleotidyl transferase (TDT) produced significant amounts of IL-2 (>20 U/ml) upon mitogen stimulation (18). Furthermore, IL-2 production by TDT-positive leukemic blasts was accompanied by extremely poor mitogen-induced T cell proliferation (18). One possible (among several) hypothesis for such a finding might be that leukemic T cell IL-2 receptors were previously saturated by cellular factor production. Therefore, upon increased synthesis of IL-2 one would expect little, if any, effect on already active replicatory processes. Alternatively, poor T cell proliferation in the face of high IL-2 production might suggest a lack of IL-2 receptors on the surface of leukemic blasts, or perhaps an IL-2-induced de-differentiation effect

(cessation of proliferation) on human leukemia cell replication. This possibility was further suggested by the observation that addition of exogenous purified human IL-2 to TDT-positive leukemic cells resulted in a significant diminution of leukemic cell proliferation as monitored by [^{3}H]Tdr incorporation in 24- to 72-h cultures. Finally, it is possible to interpret the above findings simply by cell death upon IL-2 release as we have noted for both Jurkat-FHCRC and LBRM-33 T cell malignancies.

In addition to serving as a useful model system for studying the involvement of IL-2 production and use in human leukemia, Jurkat-FHCRC cells will be a valuable reagent in the generation of large amounts of human IL-2 for further purification and molecular characterization. In this regard, it is fortunate that Jurkat-FHCRC cells produce large quantities of IL-2 upon mitogen stimulation. Additionally, the observation that PMA (a small molecular-weight and removable stimulant) triggers Jurkat-FHCRC IL-2 production, suggests that PMA stimulation may prove even more useful in allowing for production of large quantities of mitogen-free human IL-2-containing supernates. Eventually such stimulation protocols might prove useful for large scale production of biosynthetically radiolabeled human IL-2. Radioactive human IL-2 would be most useful not only for detailed analysis of factor function but, in addition, as a screening reagent for generation of monoclonal anti-IL-2 antibodies.

Summary

To isolate a stable tumor cell line capable of producing human interleukin 2 (IL-2; formerly referred to as T cell growth factor), 16 human T and B leukemia cell lines were screened for constitutive and mitogen-stimulated IL-2 production. We found that the T cell leukemia line designated Jurkat-FHCRC produced >200 U/ml of IL-2 activity after a 24-h stimulation with T cell mitogens. Peak mitogen-induced IL-2 activity was found in supernates harvested from 24-h Jurkat-FHCRC cell cultures stimulated with either 1% phytohemagglutinin or 20 μg/ml concanavalin A. Addition of the fatty acid derivative phorbol myristate acetate to mitogen-stimulated cultures increased Jurkat-FHCRC IL-2 production to concentrations >400 U/ml. IL-2 activity observed in such cases represented between 100–300 times that produced in conventional cultures of mitogen- or alloantigen-stimulated normal human peripheral blood or splenic lymphocytes. Jurkat-FHCRC-derived conditioned medium demonstrated equal capacity to promote the sustained in vitro proliferation of either murine or human activated T cell lines confirming the ability of Jurkat-FHCRC cells to produce human IL-2. These studies identify a new source of human IL-2 and establish a valuable reagent for the isolation and further molecular characterization of this immunoregulatory molecule.

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