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T-Cell lymphoma model for the analysis of interleukin 1-mediated T-cell activation

(T-cell growth factor/lymphocyte-activating factor/interleukins 1 and 2/T-cell differentiation/lymphoma)

STEVEN GILLIS* AND STEVEN B. MIZEL†‡

*Program in Basic Immunology, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104; and †Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

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ABSTRACT **Several laboratories have recently demonstrated that the requirement for macrophages in mitogen-induced production of murine T-cell interleukin 2 (IL-2; formerly referred to as "T-cell growth factor") could be circumvented by using the macrophage-derived peptide interleukin 1 (IL-1; formerly referred to as "lymphocyte-activating factor"). Using two cloned T-cell lymphomas, we investigated the mechanism through which IL-1 exerted its effect on IL-2 production. One of the cell lines used (LBRM-33 5A4) produces large concentrations of IL-2 upon mitogen stimulation, whereas the second (LBRM-33 1A5) is incapable of producing IL-2 in response to mitogen. It was observed that addition of purified IL-1 to nonproducer 1A5 cells converted them to a state in which subsequent mitogen stimulation triggered production of IL-2. The concentration of IL-2 produced by IL-1 treated 1A5 cells was equivalent in magnitude to that generated by mitogen-stimulated 5A4 cells (500–1000 units/ml, or approximately 1000 times the concentration of IL-2 contained in conventional preparations of murine mitogen-conditioned medium). The observations that (*i*) brief exposure to IL-1 was sufficient for 1A5 cell conversion to IL-2 production and (*ii*) IL-1 could actively be absorbed from culture medium by live or fixed 1A5 cells led us to propose the existence of IL-1 receptors on responsive 1A5 cells. On the basis of these experiments, we have postulated that IL-1 mediates its effect on immune reactivity (enhancement of thymocyte mitogenesis and induction of antibody and cytotoxic T cell responses) by maturation of a subset of immature T cells to the point where they are capable of IL-2 production. Subsequent release of IL-2 after ligand activation allows for clonal expansion of activated T cells which mediate particular effector functions.**

Recent experiments conducted in several laboratories have confirmed the pivotal roles that interleukin 1 (IL-1; formerly referred to as "lymphocyte-activating factor" or LAF) and interleukin 2 (IL-2; formerly referred to as "T-cell growth factor" or TCGF) play in the generation of T and B cell immune reactivities (1–8). Both proteins have been shown (*i*) to enhance thymocyte mitogenesis, (*ii*) to support the induction of alloantigen-primed cytotoxic T-cell reactivity, and (*iii*) to aid in the generation of helper T cells for antibody responses after stimulation with heterologous erythrocytes (1, 2, 4–7). In marked contrast, IL-2 is the sole interleukin capable of sustaining the *in vitro* exponential proliferation of effector T-cell lines (1, 2, 8, 9). Similarly, only IL-2 has been shown to be capable of allowing for *in vitro* and *in vivo* generation of cytotoxic T cells from *nude* mouse spleens (1, 2, 10,11).

Due to the well-documented macrophage requirement for mitogen-stimulated IL-2 production (12), and the observation that IL-1 is a macrophage product (13), it has been hypothesized that IL-1 may be an essential signal required by IL-2 producer T cells. Such a hypothesis was first suggested by the observations of Smith *et al.* (12) who found that IL-1 producer tumor cell line supernates could restore adherent cell-depleted, mitogen-stimulated, murine spleen cell cultures to normal levels of IL-2 production. IL-1 involvement in IL-2 production was also suggested by the studies of Farrar *et al.* (14) who showed that the tumor promotor phorbol myristate acetate [PMA; previously shown to act as a replacement for IL-1 in several immune response assays (15, 16)] could substitute for the macrophage requirement for IL-2 production by murine T cells. Finally, addition of IL-1 to mitogen-activated, adherent, cell-depleted, spleen cell cultures has been shown to reconstitute IL-2 production to normal levels (17, 18). Although the involvement of IL-1 in the production of IL-2 by ligand-stimulated T cells cannot be questioned, a precise mechanism by which IL-1 functions in this capacity has not been presented. For example, it is not known if IL-1 and mitogen affect the same cell or different cells that then must interact to initiate IL-2 production.

Experiments in our laboratories have recently documented the existence of an extremely potent IL-2 producer lymphoma cell, LBRM-33 (19). Mitogen-stimulated LBRM-33 cells produce 1000–10,000 times the amount of IL-2 generated by conventional cultures of mitogen-stimulated mouse spleen cells. Limiting-dilution cloning of LBRM-33 resulted in the isolation of both extremely high titer IL-2 producer clones and one nonproducer lymphoma cell line variant (19). On the basis of these observations, we initiated studies to evaluate the utility of IL-2 producer and nonproducer LBRM-33 clones in the dissection of the IL-1 requirement for IL-2 production.

In this communication we report that IL-1 has the capacity to convert IL-2 nonproducer tumor clones to high-titer IL-2 production. Conversion to IL-2 production occurs after brief exposure to IL-1 at concentrations that do not produce detectable thymocyte mitogenesis (13), thereby leading to the development of a more sensitive bioassay for IL-1 activity. Furthermore, the observation that LBRM-33 cells could absorb IL-1 from cultures suggests that these cells possess surface receptors for IL-1 and identifies a valuable cell population for further study of the molecular aspects of IL-1-induced immune reactivity.

MATERIALS AND METHODS

Cell Lines. LBRM-33, a radiation-induced splenic lymphoma from the B10.BR mouse (originally isolated by G. Cudcowicz, Roswell Park Memorial Institute, Buffalo, NY), has been shown (19) to produce high-titer murine IL-2 upon 24-hr stimulation with T-cell mitogens. Limiting-dilution cloning of

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Abbreviations: IL-1, interleukin 1; IL-2, interleukin 2; PHA, phytohemagglutinin; PMA, phorbol myristate acetate.

‡ Present address: Department of Microbiology, Cell Biology, Biochemistry and Biophysics, The Pennsylvania State University, University Park, PA 16802.

LBRM-33 resulted in identification of both extremely high titer IL-2 producer (clone 5A4, 1000 units of IL-2 per ml) and nonproducer cell line (LBRM-33 1A5) clones. LBRM cells were further found to be positive for cytosol terminal deoxynucleotidyl transferase and expressed TL, Thy 1, Lyt 1, Lyt 2, and Lyt 3 markers at the cell surface. 5A4 and 1A5 cells used in these studies were maintained *in vitro* in RPMI-1640 medium supplemented with 5% heat-inactivated (56°C, 30 min) fetal calf serum, 50 μM 2-mercaptoethanol, penicillin (50 units/ml), steptomycin (50 μg/ml), and fresh L-glutamine (300 μg/ml). IL-2 production cultures were conducted in this medium in either 200 μl (no. 3596, flat-bottom microplate, Costar, Cambridge, MA) or 5 ml (no. 3013 tissue culture flasks, Falcon Plastics, Oxnard, CA). IL-2 production was initiated by addition of phytohemagglutinin M (PHA; 0.1–1% by volume, GIBCO) to LBRM cell line cultures (10^6 cells per ml). In several experiments, 1A5 and 5A4 cells were also stimulated with either PMA (10 ng/ml, Sigma) or IL-1 (for concentrations see *Results*) prepared as detailed below. Supernatants harvested from 24-hr cultures were tested for IL-2 activity as detailed below. Maintenance of LBRM-33 cell lines and IL-2 production experiments were at 37°C in a humidified atmosphere of 5% CO_2 in air.

IL-2 Assay. The 24-hr culture supernatants from stimulated LBRM-33 5A4 and 1A5 cells (either in the presence or in the absence of IL-1 at various concentrations) were tested for IL-2 activity by using a standard microassay (20) based on the IL-2-dependent exponential proliferation of a murine cytotoxic T-cell line (CTLL) (8). Briefly, 3000 CTLL cells were cultured in replicate 200-μl volumes in flat-bottomed microplate wells in the presence of a $\log_2$ dilution series of putative IL-2-containing samples. After 24 hr, the cells were exposed to 0.5 μCi (1 Ci = 3.7×10^{10} becquerels) [^{3}H]dThd (20 mCi/mmol; New England Nuclear) for an additional 4 hr after which the cultures were harvested onto glass fiber filter strips with the aid of a multiple automated sample harvester (MASH 11, Microbiological Associates, Bethesda, MD). [^{3}H]dThd incorporation was then determined by liquid scintillation counting. Only CTLL cells cultured in the presence of IL-2 incorporated [^{3}H]dThd in a dose-dependent manner. Consistent with the observation that CTLL cells cultured in the absence of IL-2 are >95% trypan blue-positive, cultures lacking IL-2 incorporated <100 cpm of [^{3}H]dThd. IL-2 activity was quantified by probit analysis of [^{3}H]dThd incorporation data as described (20). A standard preparation of IL-2-conditioned medium [1 unit/ml, 48-hr supernate of concanavolin A-stimulated (5 μg/ml) rat spleen cells (10^6 cells per ml)] routinely generated 10,000–15,000 cpm of [^{3}H]dThd incorporation at a dilution of 1:2. Similarly, a supernatant containing 1000 units of IL-2 activity per ml stimulated identical levels of [^{3}H]dThd incorporation at a dilution of 1:2000. CTLL cells were routinely maintained *in vitro* in exponential proliferation in RPMI- 1640 medium supplemented with 50% rat spleen cell-conditioned medium (produced as detailed above) and 2% fetal calf serum (37°C, 5% CO_2 in air).

Preparation of IL-1 and Assay for Activity. The IL-1 used for induction of LBRM-33 IL-2 production was prepared from the culture supernatant of PMA-stimulated P388D_1 macrophage tumor cells as detailed (13). IL-1 was partially purified by a sequence of differential ammonium sulfate precipitations, DEAE-cellulose ion exchange chromatography, and Sephacryl S200 gel exclusion chromatography (21). After this, all of the biologically active IL-1 was associated with a protein of M_r ≈15,000 (21). The IL-1 used in absorption experiments was further purified by phenyl-Sepharose hydrophobic affinity chromatography (22). After Sephacryl S200 gel filtration, the specific activity of the partially purified IL-1 was approximately 10,000 units/mg of protein. Phenyl-Sepharose chromatography increased the specific activity ≈30 fold.

In some experiments, IL-1 was inactivated by the arginine-modifying agent phenylglyoxal (22). IL-1 was dialyzed into 200 mM imidazole buffer (pH 8) and incubated in 1% phenylglyoxal for 6 hr at 25°C. After this treatment, IL-1 was passed over a column of Sephadex G-25 (equilibrated in phosphate-buffered saline, pH 7.2) to remove the phenylglyoxal. Control IL-1 was mixed with 1% phenylglyoxal immediately prior to gel filtration chromatography. Phenylglyoxal treatment had no effect on mobility of IL-1 on Sephadex G-25 chromatography columns.

IL-1 activity was determined by its capacity to enhance thymocyte proliferation in response to *in vitro* stimulation with PHA at 1 μg/ml as detailed (13). Briefly, thymocytes from C3H/HeJ mice (4–6 weeks old, Jackson Laboratory, Bar Harbor, ME) were cultured in 200 μl in flat-bottom microplate wells (1.5 × 10^6 cells per well) in the presence of a $\log_2$ dilution series of putative IL-1-containing samples. Under these conditions, a standard preparation of IL-1 (100 units/ml) induced approximately 10,000–15,000 cpm of thymocyte [^{3}H]dThd incorporation at a dilution of 1:4. Half-maximal thymocyte proliferation was routinely observed after culture stimulation with 5–10 units of IL-1 per ml. Units of IL-1 activity were quantified by probit analysis as detailed elsewhere (20).

IL-1 Absorption. To test the capacity of LBRM-33 cell line derivatives to absorb IL-1, both 5A4 and 1A5 cells (10^8 cells) were washed and resuspended in RPMI-1640/2% fetal calf serum containing either 100 or 0.5 unit of IL-1 per ml. After a 4-hr incubation at 47 or 37°C, the cells were pelleted by a 10-min centrifugation at 300 × g and the supernatant was tested for residual IL-1 activity by its capacity to enhance thymocyte mitogenesis or to induce LBRM-33 1A5 IL-2 production. In some experiments, LBRM-33 5A4 and 1A5 cells were fixed with 2% glutaraldehyde (15 min, 4°C) and washed five times with 50 ml of culture medium prior to use in absorption tests.

RESULTS

Tumor Cell Line IL-2 Production. The results presented in Table 1 review the relative capacities of LBRM-33 5A4 and 1A5 clones to produce IL-2 after incubation with 1% PHA. As previously observed (19), 5A4 cells cultured for 24 hr (10^6 cells per ml) in the presence of 1% PHA produced approximately 1000 units of IL-2 activity per ml whereas LBRM-33 1A5 cells produced no detectable IL-2. It should be stressed that the amount of IL-2 generated by mitogen-stimulated 5A4 cultures was be-

Table 1. IL-2 production by LBRM-33 5A4 and 1A5 cell lines

Stimulated with*			IL-2 present in 24-hr supernate, units/ml
1% PHA	0.1% PHA	PMA (10 ng/ml)	
	With LBRM-33 5A4†		
+	−	−	813
−	+	−	26
−	−	+	0
+	−	+	869
−	+	+	762
	With LBRM-33 1A5†		
+	−	−	0
−	+	−	0
−	−	+	0
+	−	+	32
−	+	+	12

* +, Present in culture; −, absent from culture.
† 10^6 cells per ml in RPMI-1640/2% fetal calf serum.

tween 1000 and 10,000 times the amount of IL-2 produced by identical concentrations of normal murine spleen cells. We routinely found 1% PHA to be an optimal mitogen dose for eliciting production of IL-2 by the LBRM-33 5A4 cell line. Reduction of this mitogen concentration by a factor of 10 (0.1% PHA) significantly diminished the amount of IL-2 produced by clone 5A4; stimulation of nonproducer 1A5 cells with 0.1% PHA did not lead to IL-2 production.

It was interesting that incubation of 5A4 cells with 0.1% PHA and PMA at 10 ng/ml resulted in maximal IL-2 production. Of potential importance was the observation that PMA in the presence of PHA stimulated comparatively weak but significant, IL-2 production by 1A5 cells. Therefore, it was conceivable that 1A5 cells possessed the capacity to secrete IL-2 but apparently required additional signals to activate the IL-2 production process. Several laboratories have demonstrated an adherent cell requirement for murine IL-2 production (12, 14, 17, 18). In the absence of macrophages, concanavalin A-stimulated, purified T-cell populations could be restored to normal levels of IL-2 production by addition of either macrophage tumor cell line supernatants (12) or the monokine IL-1 (17, 18). Farrar *et al.* (14) found that addition of PMA to mitogen-stimulated, macrophage-depleted, spleen cell cultures also reconstituted normal IL-2 production. These results coupled with those detailed in Table 1 led us to examine the effect of IL-1 on both the PHA-responsive LBRM-33 5A4 (IL-2 producer) and the PHA-unresponsive LBRM-33 1A5 (IL-2 nonproducer) cloned lymphoma cell lines.

Effect of IL-1 on Production of IL-2 by Tumor Cell Line. Addition of $P388D_1$ macrophage-derived IL-1 to cultures of PHA-stimulated LBRM-33 5A4 and 1A5 cells had profound effects on IL-2 production. As with PMA, addition of IL-1 at 10 units/ml to suboptimally mitogen-stimulated 5A4 cells resulted in restoration of peak levels of IL-2 production (Table 2). Furthermore, IL-1 at 10 units/ml also induced optimal IL-2 production by PHA-stimulated 1A5 cultures. IL-1 induced high-titer IL-2 production by 1A5 cells when the cells were stimulated with optimal (1%) or suboptimal (0.1%) concentrations of PHA. Addition of IL-1 to cultures of non-mitogen-stimulated 1A5 cells was not a sufficient stimulus for conversion of the 1A5 cell line to IL-2 production.

Perhaps the most striking aspect of the capacity of IL-1 to foster 1A5 cell IL-2 production was the extremely low concentrations of IL-1 at which such a conversion was observed. Dose–response curves for both IL-1-induced murine thymocyte proliferation and IL-1-dependent conversion of 1% PHA-stimulated 1A5 cells to IL-2 production are shown in Fig. 1.

Table 2. Effect of IL-1 on IL-2 production by LBRM-33 5A4 and 1A5 cell lines

Culture*	IL-2 in 24-hr supernate, units/ml
5A4 + 1% PHA	565
5A4 + 0.1% PHA	35
5A4 + IL-1	0
5A4 + IL-1 + 1.0% PHA	604
5A4 + IL-1 + 0.1% PHA	525
1A5 + 1% PHA	0
1A5 + 0.1% PHA	0
1A5 + IL-1	0
1A5 + IL-1 + 1% PHA	476
1A5 + IL-1 + 0.1% PHA	513

* Cultures contained 10^6 cells per ml in RPMI-1640/2% fetal calf serum. When present, IL-1 was at 10 units/ml.

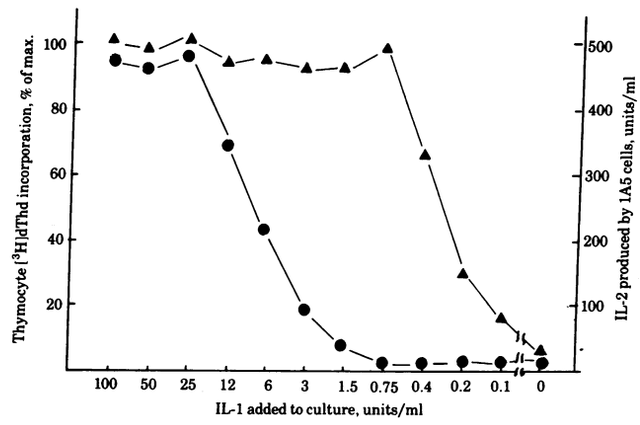

FIG. 1. Dose–response of IL-1 activity as measured by induction of thymocyte mitogenesis (●) and IL-2 production by LBRM-33 1A5 cells (▲).

Fifty percent of maximal IL-1-induced thymocyte proliferation was observed at an IL-1 concentration of approximately 5 units/ml. However, IL-1 at 5 units/ml stimulated 1A5 cells to peak levels of IL-2 production. The capacity of IL-1 to convert 1A5 cells to maximum production of IL-2 did not begin to decline until IL-1 concentrations as low as 0.5 units/ml were tested. In fact, PHA-stimulated 1A5 cells cultured in the presence of IL-1 at 0.2 unit/ml produced a significant amount of IL-2 (165 units/ml). The observation that normal T-cell mitogen-stimulated murine spleen cells produce, under optimal conditions (10^7 cells per ml; concanavalin A at 2.5 μg/ml) only 0.5–1.5 units of IL-2 per ml further substantiates the capacity of low concentrations of IL-1 to convert nonproducer 1A5 cells to high-titer IL-2 production.

We are confident that the effects of IL-1 shown in Fig. 1 were due to the monokine itself as opposed to some unidentified contaminant molecule present in the IL-1 preparation. The IL-1 used in these studies was generated in a four-step purification procedure and was of relatively high specific activity. Additionally, treatment of purified IL-1 with 1% phenylglyoxal totally eliminated not only its capacity to augment thymocyte mitogenesis but also its ability to convert 1A5 cells to IL-2 production

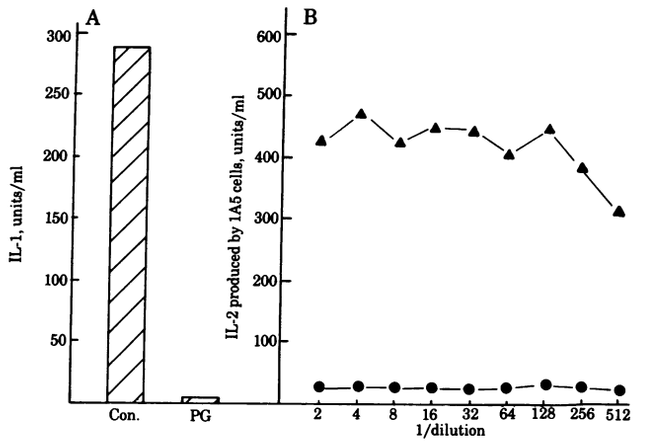

FIG. 2. Phenylglyoxal-mediated inhibition of IL-1 activity. IL-1 (300 units/ml) was exposed to 1% phenylglyoxal for 6 hr (25°C) and then was separated from the phenylglyoxal by Sephadex G-25 gel exclusion chromatography. (*A*) Phenylglyoxal (PG) treatment abolished all IL-1 activity as measured by its capacity to augment thymocyte mitogenesis. (*B*) Capacity of a $\log_2$ dilution series of both control (▲) and phenylglyoxal-treated (●) IL-1 to convert PHA-stimulated 1A5 cells to IL-2 production.

(Fig. 2). Previous studies (22) have shown that phenylglyoxal modification of arginine residues in IL-1 results in destruction of its biologic activity in a number of different biological systems (without altering its mobility on gel exclusion chromatography columns). As a site-specific modifier, it seems unlikely that phenylglyoxal treatment (and destruction of conventionally tested IL-1 activity) would have a similar effect on some other contaminant protein present in the IL-1 population which was responsible for converting 1A5 cells to IL-2 production.

Absorption of IL-1 Activity by LBRM-33 5A4 and 1A5 Cell Lines. It was clear that IL-1 itself was not a sufficient stimulus for IL-2 production in that 1A5 cells exposed to IL-1 in the absence of mitogen did not produce IL-2. In an attempt to dissociate requirements for IL-1 and mitogen sensitization, we tested whether conversion of 1A5 cells to a state in which they were capable of producing IL-2 required the continued presence of IL-1. Multiple cultures were prepared in which 1A5 cells were either treated continuously with both PHA and IL-1 (10 units/ml) or sequentially with IL-1 and then PHA. 1A5 cells were first exposed to IL-1 for 4 hr at 37°C; after exhaustive washing, the cells were cultured in either the presence or absence of additional IL-1 and PHA. Brief exposure of 1A5 cells to IL-1 did not affect their capacity to produce maximal levels of IL-2 upon subsequent exposure to PHA (Table 3).

These results suggested that the interaction of IL-1 with 1A5 cells was relatively rapid in nature. Based on previous studies (3) that demonstrated the capacity of activated T cells to absorb IL-2 from cultures, we questioned whether 1A5 or 5A4 cells possessed a similar cell surface responsiveness (presumably mediated by receptors) for IL-1. To test this hypothesis, large numbers of 1A5 and 5A4 cells were harvested from cultures, washed extensively, and resuspended in the presence of a known amount of IL-1. After a 4-hr incubation at 4°C, the cells were pelleted and the supernatants were tested for residual IL-1 activity (assayed by enhancement of thymocyte mitogenesis as well as by conversion of 1A5 cells to IL-2 production). Absorptions were conducted with two different IL-1 concentrations: 200 units/ml in experiments in which activity was to be tested by conventional IL-1 assay (thymocyte proliferation); and 0.5 unit/ml in experiments in which absorbed supernatants were assayed by their effect on 1A5 cells. Different concentrations of IL-1 were used in the two assay systems because of the significant difference in IL-1 dose–responses noted in the two bioassay systems (Fig. 1).

Both 1A5 and 5A4 cell lines were capable of absorbing IL-1 activity (Fig. 3). As measured by the ability of the residual supernatant IL-1 to enhance thymocyte mitogenesis, 1A5 cells possessed a greater capacity on a per cell basis to absorb IL-1. Perhaps this was predictable from the observation that IL-1 converted the PHA-unresponsive 1A5 cell line (an IL-2 nonproducer) to IL-2 production whereas its effect on a similar cell line already capable of IL-2 production (5A4) was simply to enhance the effect of PHA. We are confident that the absorption shown

Table 3. Conversion of 1A5 cells to IL-2 production does not require continual presence of IL-1

Pretreatment (4 hr)	Subsequent 24-hr culture	IL-2 in 24-hr supernate, units/ml
None	1% PHA	0
None	1% PHA + IL-1	379
IL-1	1% PHA	410
IL-1	1% PHA + IL-1	426

When present, IL-1 was at 10 units/ml. The cells were cultured in RPMI-1640/10% fetal calf serum.

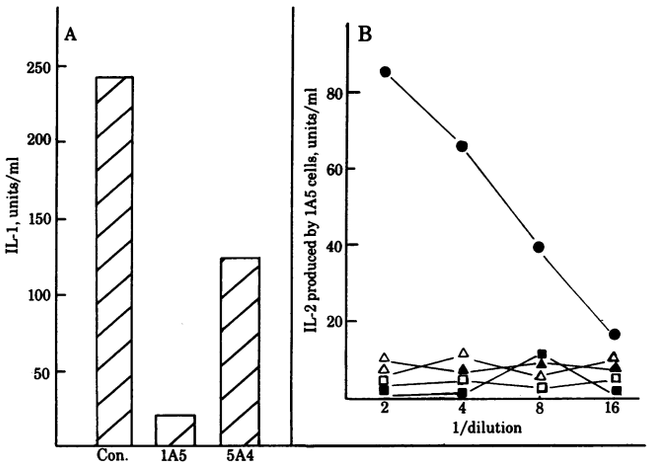

FIG. 3. Absorption of IL-1 by 1A5 and 5A4 cells. (*A*) IL-1 (242 units/ml) was incubated for 4 hr at 4°C either alone (control) or in the presence of 10^8 1A5 or 5A4 cells. Then the cells were removed and the supernatants were tested for residual IL-1 activity by their ability to enhance thymocyte mitogenesis. (*B*) Capacity of these samples to induce IL-2 production by 1A5. ●, Control IL-1 (0.5 unit/ml); ▲,△, IL-1 (0.5 unit/ml) after absorption with live or 2% glutaraldehyde-fixed 1A5 cells; ■,□, IL-1 (0.5 units/ml) after absorption with live or 2% glutaraldehyde-fixed 5A4 cells.

in Fig. 3 was due to expression of a cell-surface responsiveness to IL-1 rather than to an LBRM-33 cell-mediated degradation of IL-1 activity because: (*i*) absorptions were conducted at 4°C, a condition not supportive of high levels of cellular metabolism, and (*ii*) identical absorption characteristics were found when trials were conducted with 2% glutaraldehyde-fixed 1A5 and 5A4 cells (Fig. 3).

DISCUSSION

The experiments presented in this report took advantage of the existence of two cloned T-cell lymphomas of identical lineage (19). One cell line (LBRM-33 5A4) produces large quantities of IL-2 (the interleukin responsible for the sustained proliferation of activated T lymphocytes) whereas the second (LBRM-33 1A5) is incapable of IL-2 production after PHA stimulation. Because several previous investigations have suggested a requirement for the monokine IL-1 in the production of IL-2 by normal murine splenic T cells (12, 14, 17, 18), we used these two lymphoma cell lines in an attempt to dissect the mechanism(s) by which IL-1 participates in the initiation of IL-2 production.

We found that IL-1 has the capacity to modify 1A5 cells in some manner to render them capable of producing IL-2 upon subsequent exposure to a T-cell mitogen (PHA). Such activation occurs after brief exposure to IL-1 at a concentration approximately 1/10th that shown to be effective in the conventional IL-1 assay based on enhancement of thymocyte mitogenesis. This observation is not totally surprising if one considers (on the basis of the experiments detailed in this report) what is truly being measured in a conventional IL-1 assay. It has been well documented that IL-1 *per se* is incapable of triggering the sustained proliferation of activated T lymphocytes (6). This is a property unique to IL-2. Therefore, the enhanced mitogenesis observed when an entire thymocyte preparation is stimulated with PHA and IL-1 must be dependent upon production of the mitogenic factor IL-2. Because only the relatively small, cortisol-resistant population of the thymus (i.e., the mature T-cell compartment) has been shown to produce IL-2 (12), stimulation of an entire thymocyte preparation with mitogen (in the absence of exogenous IL-1) results in poor IL-2 production and concomitant T-

cell proliferation. However in the presence of IL-1, presumably more T cells (perhaps immature thymocytes) are converted to a state in which they can produce IL-2 and, in so doing, markedly augment thymocyte mitogenesis.

Based on such a mechanism of action for IL-1, it is not at all surprising that IL-1 converts 1A5 cells to IL-2 production at a far lower concentration than has been shown to be necessary for thymocyte proliferation. Conversion to IL-2 production as measured on 1A5 cells focuses the effects of IL-1 on a homogeneous cloned cell population whereas the percentage of IL-1-responsive T cells present in an entire thymus preparation is more than likely diminished by the presence of several if not hundreds of distinct T-cell subpopulations at varying levels of IL-1 responsiveness. It is unknown at present which cells in the thymus respond to IL-1 and if those cells (as is the case with 1A5) respond by maturing to a state of potential IL-2 production. However, by using sensitive assay systems which can now be developed due to IL-1 effects on 1A5 cells, it may be possible to isolate and to identify this naturally occurring, IL-1-responsive, thymocyte subset.

To this end, the observation that 1A5 cells and, to a lesser extent, 5A4 cells possess cell surface receptors to IL-1 is extremely important. 1A5 cells were shown to be converted to a state of lectin-inducible IL-2 production after only brief exposure to IL-1. These same 1A5 cells were also shown to be capable of absorbing IL-1. In fact, both live and fixed 1A5 cells exhibited identical absorption of IL-1 activity in 4 hr incubations at 4°C. These results argue in favor of the existence of a 1A5 cell surface responsiveness to IL-1 (presumably via the cell surface receptors). Further evidence in support of the existence of 1A5 cell surface IL-1 receptors comes from recent experiments conducted in our laboratories. Preliminary evidence indicates that biochemically identifiable IL-1 can be eluted from fixed 1A5 cells by heating the cells to 56°C or by washing the 1A5 cell pellet with 0.1% acetic acid. When the material extracted by heat or acid was lyophilized and electrophoresed on two-dimensional Tris-glycinate polyacrylamide/gels, silver-stainable protein bands were detected that were identical in charge and molecular weight to purified IL-1 (unpublished observations).

It should be stressed that previous attempts to use whole thymocyte populations to absorb IL-1 activity consistently have been unsuccessful. Such results might be due to the relatively small percentage of IL-1 responsive cells in the thymus that are converted to a state of potential IL-2 production. It is hoped that, as radiolabeled, purified IL-1 becomes available, 1A5 cells will prove to be a valuable reagent in standardizing assays for IL-1 surface binding and perhaps quantifying numbers of IL-1 cell surface receptors. With such assay systems at hand it would be possible to probe various T cell populations (selected on the basis of either cell surface or functional phenotype) systematically for responsiveness to IL-1 and conversion to IL-2 production.

In any event, it is clear that 1A5 cells will be extremely useful in probing the molecular effects of IL-1 responses. The availability of a cloned cell line that responds after brief exposure to purified monokine allows study of the events that program IL-2 production at the level of gene activation, RNA transcription, and protein synthesis. Additionally, 1A5 cells will be extremely useful in determining the consequences of IL-1 responses in terms of alteration of cell surface phenotype and function. In this regard, it is interesting to note that 1A5 cells contain high levels of cytosol terminal deoxynucleotidyltransferase (unpublished observations). In that this enzyme is a biochemical marker typical of immature T cells, such a 1A5 cell phenotype is (*i*) suggestive that naturally occurring IL-1-responsive cells might be found in the immature compartment of the thymus and (*ii*) predictive that IL-1 should have little effect on mature T cells lacking this transferase (a contention that is well supported by present studies detailing IL-1 action). Finally, by converting 1A5 cells to a state of potential IL-2 production, it appears that the monokine achieves its effect on immune responses via a differentiation/maturation-inducing pathway as opposed to triggering cellular proliferation (an activity previously shown to be associated with IL-2).

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