Brief Definitive Report

EFFECT OF INTERLEUKIN 1 ON HUMAN THYMOCYTES AND PURIFIED HUMAN T CELLS*

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Recent studies on thymus-derived lymphocyte (T cell) activation in humans and experimental animals have independently demonstrated accessory cell (monocyte) dependence for optimal initial mitogenesis (1). Monocyte augmentation of lymphocyte proliferation has been documented with cells grown in suspension or soft agar culture, and cells stimulated with either mitogen or antigen (2–11). These data, demonstrating the need for both a stimulating mitogen and a growth-supporting accessory cell, suggest a possible requirement for multiple independent signals for optimal T cell proliferation. The role of the monocyte appears to be modulated, at least in part, through the production of lymphocyte-activating factor (Interleukin 1, IL-1) (12). Data derived from rat and mouse systems have indicated that macrophage (Mϕ)-derived conditioned media may function to augment both thymocyte and mature T cell mitogenesis (13–19). Studies in the murine system have further indicated that semipurified IL-1 may replace the macrophage in respect to the induction of T cell mitogenesis, and that this may occur through subsequent T cell production of Interleukin 2 (IL-2) (18, 20).

In the present work, we have focused our attention on the human system. Purified IL-1 has been prepared from human cells and subsequently tested on human thymocytes and highly purified peripheral blood T cells. These studies further reinforce the results in experimental animal systems and demonstrate that obligatory Mϕ signals are needed for the initial commitment to mitogenesis.

Materials and Methods

Cell Purification. T lymphocytes and monocytes were purified from peripheral blood mononuclear cells (PBL) as previously described (7). Briefly, T cells were derived from the plastic nonadherent mononuclear population following E-rosette formation and subsequent nylon wool fractionation. Monocytes were derived from the plastic adherent population following a total incubation time of 24 h. T cells and monocytes prepared as described above were >98% pure as determined by E rosette, erythrocyte antibody complement rosette, surface immunoglobulin, and nonspecific esterase techniques (7). The monocyte contamination of the T cell population was routinely <0.5%. Thymocytes were prepared from fragments of human thymus obtained at cardiac surgery of 4–6-mo-old children. The tissue, aseptically removed, was immediately placed in Hanks' balanced salt solution containing 200 U/ml penicillin, and 200 μg/ml streptomycin (Irvine Scientific, Santa Ana, Calif.). Each fragment was finely minced

* Supported in part by grant CA 21927 from the National Institutes of Health, and grant AM08054 from the National Institutes of Arthritis, Metabolism and Digestive Diseases.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/81/02/0470/06 $1.00
Volume 153 February 1981 470–475

and passed through a sterile 50-mesh stainless steel screen. The minced material was placed in cold RPMI-1640 (Irvine Scientific), containing 10% heat-inactivated fetal calf serum (FCS; Irvine Scientific), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 0.1 mM nonessential amino acids (Grand Island Biochemical Co., Grand Island, N. Y.), 1 mM sodium pyruvate, and 5×10^{-5} M β-mercaptoethanol. Thymocytes were then dispersed by repeated pipetting and passage through a 200-mesh stainless steel screen. Erythrocytes were lysed by exposure to 0.83% ammonium chloride. The human thymocytes, after final washes in RPMI-1640 medium, were ≥95% viable as determined by trypan blue exclusion.

Preparation of Purified IL-1. IL-1 was prepared according to the procedures of Lachman (21, 22). Briefly, crude conditioned media were initially prepared by culturing PBL in minimal essential medium, containing 2 mM glutamine, 10 mM Hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, 5% allogeneic (Grand Island Biochemical Co.) or autologous human serum, and 10^{-5} M β-mercaptoethanol. *Escherichia coli* lipopolysaccharide (LPS) at a concentration of 20 μg/ml (LPS, 0.55:BS or 0.27:B8, Difco Laboratories, Detroit, Mich.) was also included in the incubation media. The cells were cultured for 24 h at 37°C, at which time the crude medium was clarified by centrifugation. The crude medium was "diafiltered" (model DC2, Amicon Corp., Scientific Sys. Div., Lexington, Mass.) with 0.15 M NaCl, 0.05 M Hepes, pH 7.4, using a 50,000-mol wt cut-off cartridge. The diafiltrate was further purified by ultrafiltration through a 50,000-mol wt cartridge and this ultrafiltrate was then concentrated using a YM-10 10,000 mol wt cut-off membrane (Amicon Corp.). The concentrated ultrafiltrate was isoelectrically focused with a 5–50% sucrose gradient and the fraction found at pH 6.8–7.2 was used as the source of semipurified IL-1. Before use in mitogenic assays, the samples were dialyzed against 0.15 M NaCl using 3,500-mol wt cut-off membranes. The sample in the presence of 10% FCS was sterile-filtered and stored at −20°C until use. The isoelectrically focused IL-1 material is hereafter referred to as IEF-purified IL-1.

Further purification of the 6.8–7.2 isoelectric point IL-1 activity was accomplished by gel electrophoresis (21). The material found in the 6.8–7.2 fractions was collected and dialyzed against Tris-glycine, pH 8.9. 7% acrylamide disc gels, under nondenaturing conditions, were next used for isolation of that material which electrophoresed with an R_f value of 0.36. The IL-1 was recovered from the appropriately located gel slice by electrophoretic extraction with subsequent dialysis against 0.15 M NaCl using 3,500-mol wt cut-off membranes. After the addition of 10% FCS and sterile filtration, the IL-1 was stored at −20°C until use. This material is hereafter referred to as preparative gel-purified IL-1.

Preparation of Mϕ-conditioned Media. For the preparation of crude conditioned media from highly purified Mϕ, cells were grown in RPMI-1640 medium supplemented with 2% FCS or 2% human AB serum. Monocytes were used at concentrations of $0.5–1.0 \times 10^6$/ml media. Monocytes were cultured at 37°C for 24 h. The samples were subsequently clarified by centrifugation, dialyzed overnight against RPMI-1640 medium, and stored at 4°C until use.

Mitogenic Assays. T cells were routinely cultured in tubes (Falcon 3033, Falcon Labware, Div. of Becton Dickinson and Co., Oxnard, Calif.) with RPMI-1640 medium. The medium was supplemented with FCS (10% vol/vol), penicillin, streptomycin, L-glutamine, and 5×10^{-5} M β-mercaptoethanol. Thymocyte medium was further supplemented with 0.1 mM nonessential amino acids and 1 mM sodium pyruvate. 1×10^6 T cells/ml were stimulated with phytohemagglutinin-M at a concentration of 0.75–1.0%. Thymocytes, at 5×10^6 cells/ml were stimulated with concanavalin A at 5 μg/ml (23). At the times indicated in the text, the cells were labeled as shown in table legends. The cells were subsequently harvested and the acid-insoluble material was collected on Whatman GF/C filters (Whatman, Inc., Clifton, N. J.) and counted in Scintiverse cocktail (Fisher Scientific Co., Pittsburgh, Pa.) using a Beckman LS8100 liquid scintillation spectrometer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). All experimental determinations were performed in duplicate or triplicate. Standard errors of the mean for thymocytes in the presence of IL-1 and lectin were routinely 15%. Standard errors of T cells in the presence of IL-1 and lectin were 19%.

Immunologic Marker Determinations. These assays were performed as previously described (7).

Results

IEF-purified IL-1 had a protein concentration of <1 ng:25 μl. This material was mitogenic for murine thymocytes at a final dilution of 1:100. Preparative gel-purified

IL-1 was mitogenic for mouse thymocytes at a dilution of at least 1:32. Neither of these materials could be shown to support the long-term growth of cytotoxic T cells and were therefore both considered free of IL-2 activity.

The IEF-purified IL-1 was tested for lectin-augmenting activity on human thymocytes and purified T cells. Data from representative experiments can be seen in Table I. The IL-1 demonstrated human thymocyte lectin-augmenting activity that diminished upon serial dilution, although some activity was still apparent at 1:240. Highly purified human T cells also responded to IL-1, in the presence of lectin, with dilutional characteristics similar to those seen with the thymocytes. The mean T cell mitogen response in the presence of IEF-purified samples of IL-1 compared to the

TABLE I

Effect of IEF-purified IL-1 on Human Thymocytes and T Cells

	[^{3}H]Tdr incorporation*	
	− Con A	+ Con A
Thymocytes‡	2,150	14,941
Thymocytes + IL-1		
1/30	3,027	53,201
1/60	3,854	51,075
1/120	3,105	35,769
1/240	3,209	26,671
	− PHA	+ PHA
T cells§	105	5,428
T cells + Mϕ§	421	24,676
T cells + IL-1		
1/15	421	21,152
1/30	227	28,458
1/60	157	9,437
1/120	285	1,847

* 18–20 h before harvest the cells were labeled with 1 μCi/ml [^{3}H]Tdr (6.7 Ci/mMol). The results represent the mean of multiple determinations from a representative experiment.

‡ Thymocytes were cultured for a total of 72 h at a concentration of 5×10^6 cells/ml.

§ T cells were cultured for 96 h at a concentration of 1.0×10^6/ml. Monocytes, where added, were used at 0.2×10^6/ml. The IL-1 material was diluted with RPMI-1640 medium and added at the initiation of culture for both the thymocytes and the T cells.

TABLE II

Effect of Macrophage-conditioned Media on Human T Cell Proliferation

	[^{3}H]Tdr incorporation*	
	− PHA	+ PHA
T cells	263	8,351
T cells + Mϕ	683	39,851
T cells + conditioned media‡		
(Mϕ) 0.5×10^6/ml	469	15,135
(Mϕ) 1.0×10^6/ml§	374	28,652

* 18–20 h before harvest the cells were labeled with 1 μCi/ml [^{3}H]Tdr (6.7 Ci/mMol). The results represent the mean of multiple independent experimental determinations ($n = 3$–7).

‡ The conditioned media were prepared by culturing purified Mϕ at 0.5 or 1.0×10^6 cells/ml. The cells were incubated 24 h at 37°C. The conditioned media were utilized as 20% of the final volume in the mitogenic assay as preliminary data indicated this concentration to yield optimum mitogenesis.

§ The Mϕ-derived conditioned media were initially tested on murine thymocytes (23). The mean Δ counts per minute of thymocytes exposed to lectin in the presence of conditioned media was 34,853.

TABLE III

Effect of Gel-purified IL-1 on Human Thymocytes and T Cells

	[^{3}H]Tdr incorporation*	
	− Con A	+ Con A
Thymocytes	2,150	14,941
Thymocytes + IL-1		
1/20	2,830	45,220
1/40	3,669	44,354
1/80	3,453	51,785
	− PHA	+ PHA
T cells	292	7,968
T cells + Mϕ	274	30,398
T cells + IL-1		
1/10	NT‡	16,111
1/20		16,365
1/40		19,295
1/80		28,164

* The thymocytes were cultured for 72 h. 18 h before harvest, the cells were labeled with 1 μCi/ml [^{3}H]Tdr (6.7 Ci/mMol). The T cells were grown for 96 h. 3 h before harvest, the cells were labeled with 2 μCi/ml [^{3}H]Tdr (20 Ci/mMol). The results represent the mean of duplicate or triplicate determinations at each point from representative experiments.

‡ NT, not tested.

proliferation with an intact Mϕ was 57.0 ± 19% (n = 5). The T cell response with IEF-purified IL-1 (<1 ng protein:25 μl added sample) compared favorably with the responsiveness seen using crude IL-1 containing conditioned media prepared from highly purified macrophages (Table II). For these experiments, monocyte cell number was varied during conditioned media preparation so that addition of this media as 20% (vol/vol) of the final mitogenic assay would mimic the effect of optimal T cell to monocyte ratio of 10:1 to 5:1.

Experiments using preparative gel-purified IL-1 can be seen in Table III. Again, both the human thymocytes and the human T cells responded to the presence of IL-1 with augmentation of lectin-stimulated mitogenesis. Furthermore, these experiments underscore the sensitivity of the lymphocytes to small concentrations of monokines as was seen above with IEF-purified samples.

Discussion

These results demonstrate the ability of IL-1 purified from human PBL to effectively augment lectin-stimulated mitogenesis with both the human thymocyte and the human T cell. This represents the first demonstration of such activity where all elements were produced and tested in the human system.

Results with experimental animal systems have demonstrated that the monocyte or monocyte-derived soluble products are necessary for T cell-dependent production of IL-2 (18, 20, 24). These studies have recently included a demonstration of IL-1 mitogenic augmentation of murine splenic T cells and lymph node cells (18, 19). The implication of these studies is that IL-1 modulates T cell proliferation by regulating the production of IL-2 (18). IL-2 has therefore been proposed as the ultimate mitogen regulating cell division (25). Studies are currently underway attempting to correlate the production of IL-2 in the human system, and the subsequent induction of cell

division to determine whether this lymphokine and lymphocyte proliferation are invariably linked.

The apparent ability of relatively small amounts of highly purified preparations of IL-1 to augment lectin responsiveness is significant in respect to the role of physical contact in the genesis of lymphocyte proliferation. Previous experimentation has indicated that lymphocyte-macrophage cell to cell interaction represents a component of lectin- and antigen-stimulated proliferation (7, 26, 27). Studies have indicated that lymphocyte blastogenesis is frequently observed with the T cell bound either directly to the macrophage or to another lymphocyte which is itself bound to the macrophage. This close approximation of the two cell types would ensure relatively high concentration gradients of both monokines and lymphokines, which may subsequently control proliferation. The contact between the cells could also amplify feedback control mechanisms for the termination of either IL-1 or IL-2 production and/or release. The present data indicate that IL-1 purified to a high degree of specificity may function in concentrations that could possibly be achieved within a physiological environment of lymphocyte-macrophage contact.

Summary

Human Interleukin 1 (IL-1) purified by molecular weight fractionation, isoelectric focusing, and gel electrophoresis has been tested on human thymocytes and highly purified human T cells. IL-1 prepared in this manner could not support the long-term growth of T cells yet would augment lectin-stimulated mitogenesis. The IL-1 preparations were shown to possess this lectin-augmenting activity at dilutions containing <1 ng of measurable protein. These data are in agreement with the model that IL-1 stimulates production of IL-2 from lectin-stimulated lymphocytes.

We would like to thank Sherrie Heston and Stella Page for their excellent technical assistance. We would also like to thank Dr. Ruppert for help in preparing human thymocytes. We also thank Catherine Johns for preparation of the manuscript.

Received for publication 14 October 1980.

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