Lymphokine-Activated Killer Cell Regulation of T-Cell-Mediated Immunity to *Candida albicans*

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Monocytes are important accessory cells in the activation of T cells for specific antigen recognition, yet little is known of their regulation. We demonstrated here that interleukin-2 (IL-2)-induced human lymphokine-activated killer (LAK) cells can inhibit monocyte antigen presentation, depending on the state of differentiation of the monocytes. Adherent monocytes cultured for 4 days in medium or granulocyte-macrophage colony-stimulating factor (GM-CSF) were found to equally process and present intact *Candida albicans* to autologous Percoll gradient-isolated T cells, as measured by [^{3}H]thymidine uptake. However, only the GM-CSF-cultured monocytes were functionally inhibited by autologous 4-day IL-2-induced LAK cells. Even soluble candidal cell wall mannoprotein antigens could not be presented by these monocytes after exposure to LAK cells. Pretreatment of these monocytes with LAK cells for 1 h, followed by subsequent removal of the nonadherent LAK cells, was sufficient to cause significant inhibition, with maximal inhibition observed after 4 h. Northern (RNA) blot analysis indicated that mRNA expression for IL-1α and IL-1β in response to *C. albicans* stimulation was also down-regulated in GM-CSF-cultured monocytes exposed to LAK cells. Interestingly, freshly isolated, Percoll gradient-purified large granular lymphocytes did not suppress antigen presentation in GM-CSF-treated monocytes. Another important finding was the inability of LAK cells to suppress the ability of freshly isolated or gamma interferon-cultured monocytes, which are resistant to LAK cell-mediated lysis, to present antigen to T cells. In contrast, IL-3 was similar to GM-CSF in inducing LAK cell susceptibility in monocytes. Taken together, these results indicated that IL-2 can induce LAK cells to down-regulate antigen presentation function in a select set of monocytes that have been activated by colony-stimulating factor (GM-CSF and IL-3) but not by gamma interferon. LAK cells may therefore play an important role in regulation of monocytes and their function, depending on their differentiation state.

It is now widely accepted that the majority of interleukin-2 (IL-2)-activated killer (LAK) cells are derived from the large granular lymphocytic (LGL), natural killer (NK) subset of lymphocytes with a CD3$^-$ CD16$^+$ CD56$^+$ phenotype (17, 23, 27). These effector cells have demonstrated broad cytotoxic reactivity against a large variety of tumor cell lines as well as fresh, surgically obtained human tumors (16, 21, 29). We have recently demonstrated that IL-2-induced human LAK cells, in addition to having tumoricidal activity, have the capacity to lyse autologous and allogeneic monocytes after coculture for 4 to 6 h, as measured by ^{51}Cr release from radiolabeled target cells (12). The phenotype of the antimonocytic LAK cell fits that of the primary antitumor LAK effector cell. The ability to recognize normal monocytes appears to be specific because granulocytes and nonadherent lymphocytes do not serve as LAK cell targets, whether they are freshly isolated or cultured for 3 to 7 days in vitro. Monocytes, on the other hand, become increasingly more sensitive to LAK cell lysis with in vitro culture. The susceptibility of monocytes to LAK cell lysis may therefore depend on their differentiation state. This inference was further confirmed by the use of monocyte-activating and differentiation factors (2, 3, 13, 14). Differentiation with granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-3 was found to further enhance monocyte susceptibility. On the other hand, gamma interferon (IFN-γ) down-regulated it.

It is also well known that monocytes play an important role as antigen-presenting cells (APC) in initiating an im-

mune response. Antigen presentation is a sentinel process in the immune system. Monocytes take up antigen, process it, and display an immunogenic fragment to lymphocytes, thereby delivering an activation signal. In addition, monocytes may secrete costimulatory molecules, e.g., IL-1, which are important to the activation process (33). The biological significance of LAK cell recognition of monocytes is unknown. Our previous study showed that GM-CSF-treated monocytes, after interacting with LAK cells, were significantly suppressed in their ability to inhibit *Candida albicans* growth (34). On the basis of our studies, we propose that LAK cells may be involved in some level of regulation of monocyte function. It is possible that the host develops a system for the control of immune homeostasis by specifically eliminating or suppressing monocytes in certain stages of differentiation and activation. Those monocytes which have already accomplished their functions and are no longer required must have a method of elimination from the host system.

In this study, we examined whether autologous LAK cells could modulate monocyte antigen presentation to autologous T cells. Monocytes after treatment with autologous LAK cells were assessed for presentation of both heat-killed intact *C. albicans* (HK.CA) yeasts and soluble candidal cell wall mannoprotein (MP-F2) to T cells, as measured by [^{3}H]thymidine incorporation during antigen-specific lymphocytic proliferation (1, 28). Furthermore, the ability of monocytes to generate IL-1α and IL-1β, at both the protein and mRNA levels, was determined before and after treatment with LAK cells. The effect of GM-CSF, IL-3, and IFN-γ was also investigated to determine whether the differentiation of

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monocytes by these cytokines affected their susceptibility to LAK cells. Any effect should be reflected in the modulation of monocyte antigen presentation capacity to T cells.

MATERIALS AND METHODS

Antigens. Whole *Candida* yeasts and MP-F2 were used as antigens throughout this study. *C. albicans* used in this study was a clinical isolate from a patient with chronic mucocutaneous candidiasis and was identified according to the taxonomic criteria established by Lodder (18). The yeasts were grown by weekly transfer onto fresh Sabouraud agar slants and incubation at 28°C. The colony was collected and washed with phosphate-buffered saline (PBS) and then heated at 100°C for 1 h. *C. albicans* MP-F2 was kindly provided by Antonio Cassone, Istituto Superiore di Sanita, Rome, Italy, and was obtained after chromatographic separation of a mannoprotein-rich extract as described elsewhere (1, 28). This material was adsorbed to polymyxin B-sulfate agarose (Sigma Chemical Co., St. Louis, Mo.) for removal of any potentially contaminating endotoxin.

Preparation of human leukocytes. Leukocyte buffy coats, obtained from normal volunteers at Southwest Florida Blood Bank, were isolated by centrifugation on a Ficoll-Hypaque solution (Pharmacia, Piscataway, N.J.) as previously described (3). The peripheral blood lymphocytes were suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% heat-inactivated human AB serum (Flow Laboratories, McLean, Va.), 2 mM L-glutamine, penicillin (10 U/ml), streptomycin (100 μg/ml), 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (GIBCO), and 5×10^{-5} M 2-mercaptoethanol (Sigma), (complete medium). All media and reagents were determined to contain less than 0.1 ng of endotoxin per ml, as determined by the *Limulus* lysate assay (M. A. Biologics, Walkersville, Md.).

Preparation of LGL, LAK, and T cells. PBL were allowed to adhere to tissue culture flasks (Costar, Cambridge, Mass.) for 1 h at 37°C. Nonadherent cells were recovered by vigorous washing of the flask with warm medium. The separation of LGL from T cells was accomplished by use of a discontinuous Percoll density gradient (27). The nonadherent cells were further depleted of adherent cells and B cells by incubation on nylon wool columns for 30 min at 37°C. The cells passing through the columns were then placed on a four-step discontinuous density gradient that varied in 2.5% concentration from 40 to 47.5%. After centrifugation at 550 × g for 30 min at room temperature, the bands of lymphocytes were collected and examined for LGL and T-cell morphology on Giemsa-stained cytocentrifuged slides. Fractions 2 and 3 primarily consisted of LGL, while cells in the pellet below fraction 4 were determined to be T cells. These purified T cells showed no proliferative response to either MP-F2 and HK.CA under culture conditions and concentrations optimal for the proliferation of T cells in the presence of monocytes, confirming that APC had been effectively removed from the T-cell fractions. LGL were further incubated at a concentration of 2×10^6 cells/ml in 5- to 10-ml volumes with 100 U of human recombinant IL-2 (kindly provided by Hoffmann-La Roche, Nutley, N.J.) per ml. Cells were optimally activated with IL-2 for 3 to 4 days in 25-cm² tissue culture flasks, washed twice in medium, readjusted to the original cell concentration, and then added to the monocyte monolayers.

Preparation of monocytes in flat-bottom microwell plates. The flask containing adherent cells after removal of nonad-

herent cells was incubated with 10 ml of cold PBS without Ca^{2+} and Mg^{2+} at 4°C for 20 to 30 min to detach the adherent monocytes. These monocytes were resuspended in medium and adjusted to the concentrations necessary for the monocyte/T-cell ratios to be used. They were then added to triplicate wells of a 96-well flat-bottom microtiter plate (Costar). These adherent populations were found to be greater than 95% pure monocytes, as judged by Wright-Giemsa staining. The trypan blue dye exclusion test for cell viability was also greater than 95%. Human recombinant GM-CSF (generously provided by Immunex Corp., Seattle, Wash.) was added to the monocyte monolayers at 100 U/ml, and the cells were incubated for 4 days at 37°C. In some cases, human recombinant IFN-γ or IL-3 (kindly provided by Genentech, South San Francisco, Calif., or Genetics, Inc., Cambridge, Mass., respectively) was used in place of GM-CSF. The cytokines contained less than 0.1 ng of endotoxin per ml, as determined by the *Limulus* lysate assay (M. A. Biologics).

Treatment of monocytes by LAK cells. After incubation of monocytes with or without cytokines, the culture medium was removed by vigorous washing with warm medium. Immediately thereafter, autologous LAK cells were added to the monocyte monolayers to achieve LAK cell/monocyte ratios of 10:1, 3:1, and 1:1 in 100 μl of medium. The cells were allowed to interact for specified periods of time at 37°C before the nonadherent LAK cells were then gently removed by three washes with warm medium. After complete removal of LAK cells was checked by microscopic examination, the stimulating candidal antigen was added to the monocytes as described below. In some experiments, the monocytes, after treatment with and removal of LAK cells, were incubated for 45 min at 37°C with anti-CD2 plus complement to lyse residual LAK cells. Then, these purified monocytes were stimulated with candidal antigen as described below.

Pulsing of monocytes and T-cell proliferation. For antigen pulsing, monocytes were incubated in the presence of antigen for 2 h, in which the MP-F2 concentration was 100 μg/ml and HK.CA was added to achieve a monocyte/HK.CA ratio of 1:10. After being pulsed with antigen, the monocytes were washed at least three times to remove the unbound stimulant. Finally, 10^5 Percoll gradient-purified T cells were added to triplicate microwells containing control or antigen-pulsed autologous monocytes and were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 6 days. Cultures were pulsed for the last 18 h with 1 μCi of tritiated thymidine per well. The cells were harvested with an automated cell harvester.

The protocol for isolation of LGL, T cells, and monocytes as well as antigen presentation of monocytes to autologous T cells is shown in schematic form in Fig. 1. The mean and standard error of the triplicate cultures was determined, and each experiment was repeated at least thrice. In some cases, to clarify the comparison of the ability of LAK cells to modify monocyte function, the data were also expressed as percent inhibition of monocyte function by LAK cells, calculated as follows: % inhibition of monocyte function = [(T-cell proliferation in control monocytes − T-cell proliferation in LAK cell-treated monocytes)/T-cell proliferation in control monocytes] × 100.

Measurement of IL-1α or IL-1β activity. GM-CSF-cultured monocytes, treated with or without LAK cells for 4 h at 37°C, were pulsed with HK.CA at a monocyte/HK.CA ratio of 10:1 for 48 h at 37°C. After centrifugation to pellet the cells, the supernatants were collected for IL-1 assessment.
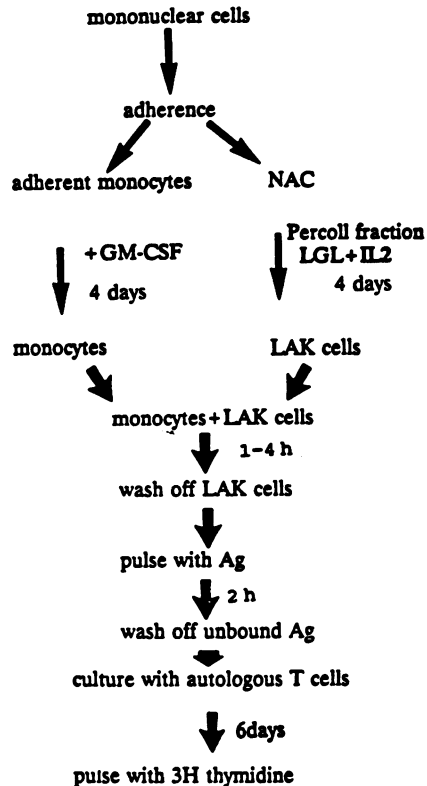
FIG. 1. Protocol for assessment of LAK cell inhibition of monocyte antigen presentation to autologous T cells. Adherent monocytes were cultured in the wells of a microplate for 4 days at 37°C with 100 U of human recombinant GM-CSF per ml or medium control. IL-2-induced LAK cells were added to the GM-CSF-treated adherent monocytes for 4 h at 37°C, the nonadherent LAK cells were carefully washed off, and the LAK cell-exposed monocytes were pulsed for 2 h at 37°C HK.CA yeasts or a soluble MP-F2 obtained from the same yeasts. The uningested antigens (Ag) were removed by thorough washing of the adherent monocyte monolayers. Finally, autologous T cells were added to the variously treated monocytes. The wells containing monocytes and T cells were incubated further for 6 days, with [³H]thymidine added for the last 18 h, before assessment of specific T-cell proliferation to *Candida* antigens presented by autologous monocytes.

For membrane IL-1 measurement, the cell pellets were frozen and thawed four times prior to centrifugation at 13,000 × *g* for 10 min at 4°C. The pelleted membrane fragments were then assessed for the presence of IL-1. IL-1α was measured by a competitive form of enzyme-linked immunosorbent assay (ELISA) by ARI, College Park, Md. IL-1β was measured by a commercial ELISA kit (Cistron Biotechnology, Pine Brook, N.J.).

RNA isolation and Northern (RNA) blot analysis. Autologous LAK cell-treated monocytes were first incubated with anti-CD2 monoclonal antibody plus complement to ensure no T-cell or LAK cell contamination in the monocyte preparations before they were used for RNA isolation as described by Chomczynski and Sacchi (10). Briefly, approximately 10⁷ cells were lysed by a solution of 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol. One-tenth volume of 2 M sodium acetate (pH 4.0) was then added, and the RNA was extracted with an equal volume of acid phenol and 49:1 chloroform-isoamyl alcohol. The RNA was further purified

and precipitated with isopropanol, washed with 70% ethanol, and dissolved in water. From 10⁷ monocytes, we routinely recovered 30 to 40 μg of RNA with this procedure. Twenty-microgram samples of RNA were denatured in a dimethyl sulfoxide-glyoxal mixture and then fractionated on a 0.8% agarose gel in 10 mM sodium phosphate buffer (pH 7.0) with circulation. Fractionated RNA was then transferred to Nytran filter paper by capillary transfer in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), UV irradiated to ensure complete binding of RNA to nylon, and then stained with methylene blue-acetate to determine the presence and integrity of transferred RNA. Prehybridization was performed at 45°C for 2 h, and hybridization was performed at the same temperature for 18 h. Prehybridization solution consisted of 5× SSC, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 0.1% sodium pyrophosphate, 200 μg of salmon sperm DNA per ml, 50% formamide, 10 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES; pH 6.5), and 10 mM EDTA. Hybridization was performed in the same buffer after the addition of a random-primed probe labeled with [³²P]dCTP at a concentration of 1 × 10⁶ to 3 × 10⁶ cpm/ml of solution. The *Xho*I fragment of human IL-1α cDNA and the *Pst*I fragment of human IL-1β cDNA, kindly provided by Genetics Institute, Cambridge, Mass., were used as the probes.

After hybridization, the membrane was washed for 5 min in 2× SSC–0.1% SDS–1 mM EDTA at room temperature and for 30 min in 1× SSC–0.1% SDS–1 mM EDTA at 45°C with an increase of temperature to 60 to 65°C, depending on the signal. The membrane was then exposed to film with an intensifying screen at −70°C.

Cytotoxic assay against monocytes. Monocytes, cultured for 4 days with GM-CSF, were labeled with 400 μCi of sodium [⁵¹]chromate (Amersham Corp., Arlington Heights, Ill.) for 2 h in 0.5 ml of medium as described elsewhere (12). The cells were then washed once and incubated for an additional 30 min in 5 ml of medium. Target cells were then washed twice more and added to IL-2-activated autologous LAK cells at 5 × 10³ cells per well in triplicate wells of a 96-well microplate at effector/target ratios of 20:1, 10:1, and 5:1. After 1 to 6 h of incubation at 37°C, the culture supernatants were harvested and counted in a gamma counter. Maximum isotope incorporated was determined by counting target cells alone, and spontaneous release was measured by counting supernatants of target cells incubated with medium alone. Monocyte targets usually had a spontaneous release of 10 to 20% at the 6-h incubation. The percentage of lysis was calculated by the following formula: % specific lysis = [(experimental cpm − spontaneous cpm/maximal cpm incorporated] × 100. The data were then presented in lytic units per 10⁷ LAK cells, with 1 lytic unit defined as 20% specific lysis.

RESULTS

Autologous LAK cells down-regulate GM-CSF-treated monocytes in HK.CA presentation to T cells. The direct inhibitory effect of autologous LAK cells on monocytes was determined by preincubation of medium-cultured monocytes or GM-CSF-treated monocytes with LAK cells for 4 h prior to addition of T cells from the same individual to the HK.CA-pulsed monocytes (see protocol in Fig. 1). Both medium-cultured and GM-CSF-treated monocytes were found to be capable of antigen presentation to autologous T cells, as measured by [³H]thymidine uptake by the specifically activated proliferating T cells, i.e., 17,739 ± 1,053 and

23,152 ± 957 cpm in T cells incubated with HK.CA-pulsed medium-cultured monocytes and GM-CSF-treated monocytes, respectively (*P* < 0.01 compared with unpulsed T cells). The T cells alone and the antigen-pulsed monocytes by themselves displayed little [³H]thymidine incorporation, i.e., 103 ± 46 and 417 ± 39 cpm, respectively. In comparison with medium-cultured monocytes, GM-CSF-treated monocytes were found to be significantly more sensitive to IL-2-generated LAK cells. When autologous LAK cells, generated with IL-2 from Percoll gradient-purified LGL, were added to the adherent monocytes at an LAK cell/monocyte ratio of 10:1 for 4 h at 37°C and then removed, the antigen-presenting capacity of GM-CSF-treated monocytes, but not that of medium-cultured monocytes, was markedly reduced. Specific T-cell proliferation with LAK cell-treated medium-cultured monocytes was 18,441 ± 341 cpm, but that with LAK cell-treated GM-CSF-differentiated monocytes was 13,018 ± 174 cpm. Further studies therefore concentrated on the effect of LAK cells on GM-CSF-treated monocytes.

Table 1 showed the results of six experiments indicating that GM-CSF-treated monocytes from all donors displayed high capacity to present HK.CA to autologous T cells. T cells alone or monocytes alone showed no proliferative response in any of the donors. Exposure to these monocytes to autologous LAK cells severely depleted their antigen-presenting function in every case. The degree of suppression of monocyte-mediated antigen presentation correlated with an increase in the LAK cell/monocyte ratio from 1:1 to 10:1. Removal of residual LAK cells by anti-CD2 plus complement from the LAK cell-treated monocytes did not affect the outcome of the assay (data not shown). In addition, the supernatants from LAK cells did not have the ability to down-regulate monocyte antigen presentation (data not shown). The effect of freshly isolated autologous LGL NK cells on GM-CSF-treated monocytes was also examined. In contrast to the effective suppression of monocytes by LAK cells, the same monocytes were relatively resistant to inhibition by fresh LGL/NK cells. Treatment with fresh NK cells, even at the high NK cell/monocyte ratio of 10:1, only slightly reduced the monocyte-mediated antigen presentation. These results suggested that fresh LGL NK cells did not significantly alter GM-CSF-differentiated monocyte activity but that IL-2 can induce in LGL the capacity to suppress monocytes in their accessory function.

Autologous LAK cells down-regulate GM-CSF-treated monocytes to present soluble candidal mannoprotein antigen (MP-F2) to T cells. To confirm that antigen presentation of GM-CSF-treated monocytes was inhibited by LAK cells after pulsing not only with whole intact *Candida* yeasts but also with purified soluble mannoprotein from *Candida* yeasts, GM-CSF-treated monocytes, after 4 h of preincubation with LAK cells, were pulsed with MP-F2, at a previously determined optimal dose of 100 μg/ml, for 2 h and washed to remove the unbound glycoprotein before autologous T cells were added. Figure 2 showed that T cells incorporated [³H]thymidine in the presence of monocytes pulsed with MP-F2 but not in the presence of untreated monocytes. More importantly, it shows that monocytes were inhibited by LAK cells in presentation of soluble MP-F2 to T cells. The degree of LAK cell suppression of monocyte function also correlated with an increase in the LAK cell/monocyte ratio from 1:1 to 10:1. T cells alone or monocytes alone showed no ability to respond to any of the doses of MP-F2 (data not shown). This finding also confirms that only LAK cells but not fresh NK cells could interfere

TABLE 1. Effect of IL-2-induced LAK or fresh NK cells on monocyte antigen presentation to autologous T cells

Treatment of monocytes[a]	LAK or NK cell/monocyte ratio	[³H]thymidine incorporation in T cells (cpm ± SE) (% inhibition)					
		Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
None		59,583 ± 1,657	60,181 ± 4,611	23,403 ± 1,380	34,233 ± 4,807	30,245 ± 6,596	67,471 ± 3,262
LAK	10:1	31,485 ± 9,527 (47.1)	24,883 ± 4,527 (58.6)	11,399 ± 1,307 (51.2)	15,332 ± 1,857 (55.2)	19,039 ± 3,519 (37.0)	20,296 ± 1,386 (70.7)
LAK	3:1	36,448 ± 6,745 (38.8)	31,688 ± 3,999 (47.3)	14,705 ± 2,071 (15.8)	13,078 ± 3,900 (61.7)	24,037 ± 3,537 (20.5)	31,226 ± 1,395 (55.0)
LAK	1:1	43,450 ± 1,765 (27.0)	47,536 ± 5,617 (21.0)	20,030 ± 4,245 (14.4)	28,199 ± 3,608 (17.6)	22,464 ± 3,500 (25.7)	48,543 ± 1,690 (30.1)
NK	10:1	50,699 ± 2,286 (14.9)	50,478 ± 2,795 (16.1)	25,831 ± 2,900 (-10.0)	32,429 ± 1,546 (5.2)	ND[b]	59,197 ± 1,150 (14.7)
T cells alone		346	120	586	240	162	115
Monocytes alone		290	142	404	90	87	234

[a] Adherent GM-CSF-cultured monocytes were incubated with autologous fresh NK or LAK cells for 4 h at 37°C at the indicated ratios. After removal of the nonadherent lymphocytic cells, the monocytes were pulsed with *C. albicans* for 2 h at 37°C, and the stimulating yeasts were washed off prior to addition of autologous T cells for another 6 days of culture. T cells alone and GM-CSF-treated monocytes alone, both exposed separately to *C. albicans* for 6 days, were included as controls.
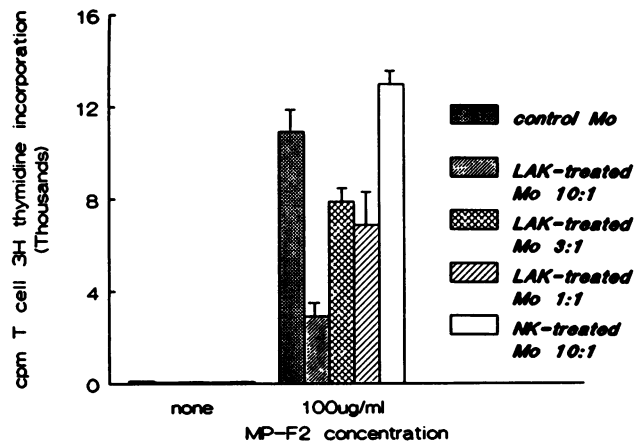
[b] ND, not determined.

FIG. 2. Effect of IL-2-induced LAK cells on monocyte presentation of soluble *Candida* mannoprotein to autologous T cells. Adherent GM-CSF-cultured monocytes (Mo) were incubated with autologous fresh LGL or LAK cells for 4 h at 37°C at the indicated ratios. After removal of the nonadherent lymphocytes, the monocytes were pulsed with 100 μg of candidal MP-F2 per ml for 2 h at 37°C. The unbound soluble mannoprotein was then washed off prior to addition of autologous T cells for another 6 days of culture. T cells alone and the variously treated monocytes alone were pulsed with antigen for controls, and none of these controls showed any [³H]thymidine incorporation (data not shown). Data are expressed as means ± standard errors of the triplicate wells and are from a representative experiment from experiments with three separate donors.
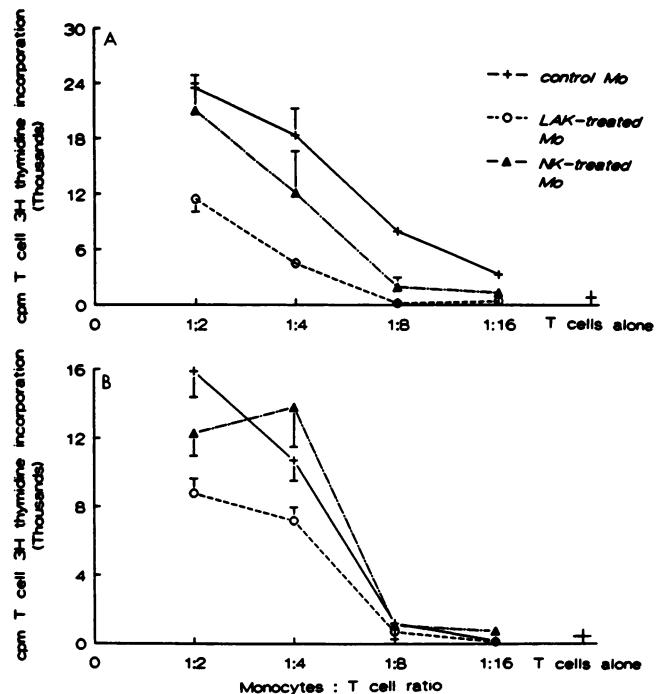

FIG. 3. Effect of LAK cells on optimal and suboptimal antigen presentation by monocytes to T cells. Monocytes were plated at various concentrations in triplicate wells of a 96-well microplate prior to culture for 4 days with GM-CSF. T cells were added to the adherent monocytes (Mo) after 4 h of exposure to LAK or fresh NK cells. The monocytes were pulsed with HK.CA (A) or soluble MP-F2 (B) for 2 h before T cells were added to achieve monocyte/T-cell ratios of 1:2 to 1:16. Results are expressed as means ± standard errors of three separate experiments with different donors.

with monocyte presentation of soluble MP-F2 antigen to T cells.

Effect of LAK cells on optimal and suboptimal monocyte antigen presentation to T cells. To define the optimal number of antigen-pulsed monocytes required for T-cell proliferation, the monocyte/T-cell ratio was varied from 1:2 to 1:16, with the concentration of T cells being held constant. T-cell proliferation was found to be maximal at monocyte/T-cell ratios of 1:2 to 1:4 after culture with either HK.CA-pulsed monocytes (Fig. 3A) or MP-F2-pulsed monocytes (Fig. 3B) for 6 days. The T-cell response was specifically induced by antigen-pulsed monocytes, since T cells alone could not proliferate after pulsing with antigen. The T-cell response to HK.CA was higher, probably because of the larger number of antigenic epitopes on whole yeasts compared with the single mannoprotein moiety. Figure 3 also shows that LAK suppression of intact or soluble antigen presentation by monocytes was still observed at most monocyte/T-cell ratios. In contrast, NK cell-treated monocytes retained both types of antigen-presenting capacity to T cells at either optimal or suboptimal ratios.

Kinetics of LAK cell suppression of monocyte antigen presentation. Initially, 4 h of exposure of monocytes to LAK cells was chosen to examine antigen presentation of monocytes because we consistently detected the ability of LAK cells to lyse monocytes in the ⁵¹Cr release assay at 4 to 6 h. However, we also found that LAK cell inhibition of monocyte function occurred earlier than LAK cell-induced cytoplasmic leakage (34). Because maximal inhibition of antifungal activity in monocytes was seen only after 2 h of exposure to LAK cells, we next determined whether monocyte accessory function, like antifungal activity, was down-regulated prior to cell membrane damage. Therefore, GM-CSF-treated monocytes were incubated with LAK cells for 1 to 6 h at 37°C and washed free of LAK cells before pulsing with

antigen. On the other hand, to further confirm that the suppression of monocyte antigen presentation was specifically mediated by LAK cells, the GM-CSF-treated monocytes were also cultured with fresh LGL/NK cells for 1 to 6 h and washed free of LGL/NK cells before assessment of antigen presentation. The monocytes were then cultured for 6 days with autologous T cells. For comparison, LAK cells were also added to ⁵¹Cr-labeled GM-CSF-treated monocytes for 1 to 6 h to assess the time required for optimal cytoplasmic leakage. As early as 1 h after incubation of monocytes with LAK cells, significant suppression of antigen presentation was observed (Fig. 4A). By 4 to 6 h, maximal suppression of monocytes had taken place. On the contrary, incubation of monocytes with fresh LGL NK cells for up to 6 h did not induce a significant level of suppression of antigen presentation by monocytes. With respect to lysis, no significant ⁵¹Cr release was detected from the monocytes at 1 or 2 h after exposure to LAK cells, and 4 h or longer was required to achieve high levels of lysis (Fig. 4B).

Induction of differentiation by various cytokines changes the susceptibility of monocytes to suppression by LAK cells. The effects of IL-3 and IFN-γ, which are monocyte-activating and differentiation factors, on monocyte susceptibility to LAK cells were examined. In an earlier study, we found that IL-3 was able to increase the susceptibility of monocytes to LAK cells similarly to GM-CSF (34). On the other hand, the effect of IFN-γ was found to be opposite that of GM-CSF or IL-3. Therefore, after adherent monocytes were cultured with IL-3 or IFN-γ for 4 days, their capacity for antigen
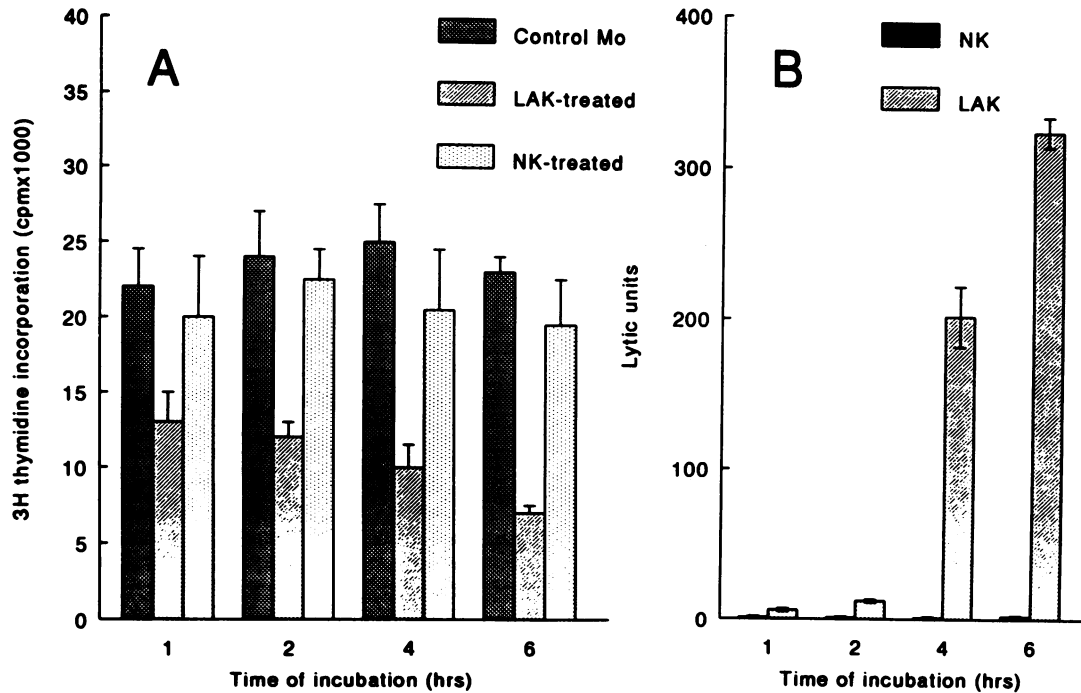
FIG. 4. Kinetics of LAK cell suppression of monocyte antigen presentation. Adherent GM-CSF-treated monocytes (Mo) were exposed to autologous LAK or fresh NK cells for 1 to 6 h at 37°C, the lymphocytes were washed off, and the treated monocytes were pulsed with HK.CA for 2 h before addition of autologous T cells for a further 6-day incubation (A). LAK cells were also added to ^{51}Cr-labeled autologous GM-CSF-treated monocytes for 1 to 6 h at 37°C before the supernatants were collected for measurement of specific lysis (B). Results are expressed as means ± standard errors of three separate experiments with different donors.
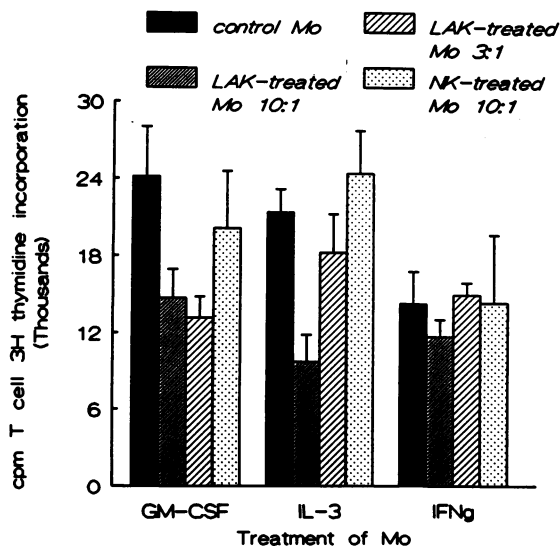

FIG. 5. Effects of GM-CSF, IL-3, and IFN-γ on monocyte susceptibility to LAK cells. Adherent monocytes (Mo) were cultured with 100 U of recombinant human GM-CSF, IL-3, or IFN-γ (IFNg) per ml or with medium alone for 4 days at 37°C prior to 4 h of exposure to LAK or fresh NK cells. The various monocytes were then washed free of lymphocytes and pulsed with HK.CA for 2 h at 37°C before addition of autologous T cells for a further 6-day incubation. Results are expressed as means ± standard errors of three separate experiments with different donors.

presentation was determined by measuring T-cell proliferation. Figure 5 shows that monocytes treated with 100 U of IL-3 per ml were comparable to those treated with 100 U of GM-CSF per ml in their capacity to present antigen to T cells. GM-CSF-treated monocytes were significantly inhibited by LAK cells at ratios of both 10:1 and 3:1 ratios ($P <$ 0.01). In addition, IL-3 had an effect similar to that of GM-CSF on monocytes. Monocytes cultured in IL-3 showed LAK cell susceptibility, which was highly significant at a ratio of 10:1. After LAK cell treatment, IL-3-cultured monocytes showed reduced capacity to present antigen. As with GM-CSF-cultured monocytes, fresh NK cells had little effect on IL-3-cultured monocytes. On the other hand, medium cultured and 100 U of IFN-γ per ml rendered monocytes resistant to the inhibitory effect of LAK cells. The level of T-cell proliferation in the presence of IFN-γ-treated monocytes exposed to LAK cells was not significantly different from that with the same monocytes not exposed to LAK cells. These results suggest that the two classes of cytokines, colony-stimulating factor (CSF) and IFN-γ, can differentially modulate monocyte susceptibility to suppression of LAK cells. Moreover, we found undifferentiated freshly isolated monocytes to be unaffected by LAK treatment at any LAK cell/monocyte ratio. Figure 6 showed that fresh monocytes without cytokine treatment could not be inhibited by LAK cells to present antigen to T cells.

Effect of LAK cells on IL-1 production from GM-CSF-treated monocytes induced by HK.CA at the protein and mRNA levels. It is an established fact that initiation of the immune response is dependent on the capacity of monocytes to process and present a particular antigen that is displayed on the monocyte surface to be recognized by specific T cells. However, this is not sufficient to fully activate most T cells.
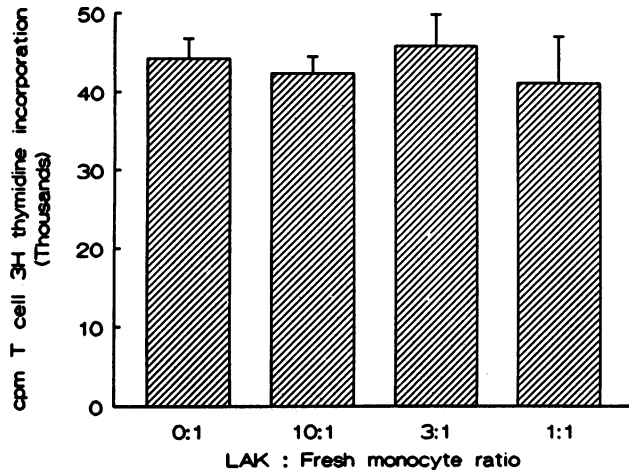
FIG. 6. Resistance of freshly isolated monocytes to LAK cell inhibition. Freshly isolated monocytes were exposed to LAK cells for 4 h at 37°C prior to pulsing with HK.CA for 2 h at 37°C. The monocytes were then washed free of lymphocytes and incubated with autologous T cells for a further 6 days. Results are expressed as means ± standard errors of three separate experiments with different donors.

It is necessary to generate accessory signals or costimulators, such as IL-1, provided by monocytes during the antigen presentation process (33). To determine whether the suppression of antigen presentation of monocytes by LAK cells

was also related to this secondary signal, GM-CSF-treated monocytes, cultured with or without LAK cells for 4 h, were pulsed with HK.CA for 48 h before the supernatants were collected for assessment of IL-1. The amounts of both IL-1α and IL-1β present in the supernatants were tested by using commercial ELISA kits. Figure 7 clearly shows that generation of both forms of IL-1 in LAK-treated monocytes was significantly suppressed compared with that in untreated monocytes, especially at the high LAK cell/monocyte ratio of 10:1. Because monocytes have been reported to produce and utilize extracellular as well as membrane-bound IL-1α for their APC function (11, 31), monocyte membranes were prepared and the concentration of membrane IL-1α induced by HK.CA was assessed. As shown in Fig. 7A, HK.CA could stimulate GM-CSF-cultured monocytes to express membrane IL-1α. After LAK cell treatment, however, the levels of membrane IL-1α on these monocytes were significantly reduced.

Furthermore, to confirm that the LAK cell suppression of IL-1 production also occurred at the mRNA level, Northern blot analysis was carried out. Figure 8 shows that GM-CSF-treated monocytes expressed more mRNA for both IL-1α and IL-1β than did untreated monocytes. However, the GM-CSF-treated monocytes after exposure to LAK cells had significantly decreased IL-1α and IL-1β mRNA expression induced with HK.CA in comparison with monocytes not treated with LAK cells. Almost equivalent amounts of total RNA were isolated from LAK cell-treated and untreated monocytes, indicating that the cells were relatively intact even after LAK cell treatment. Because the RNA was
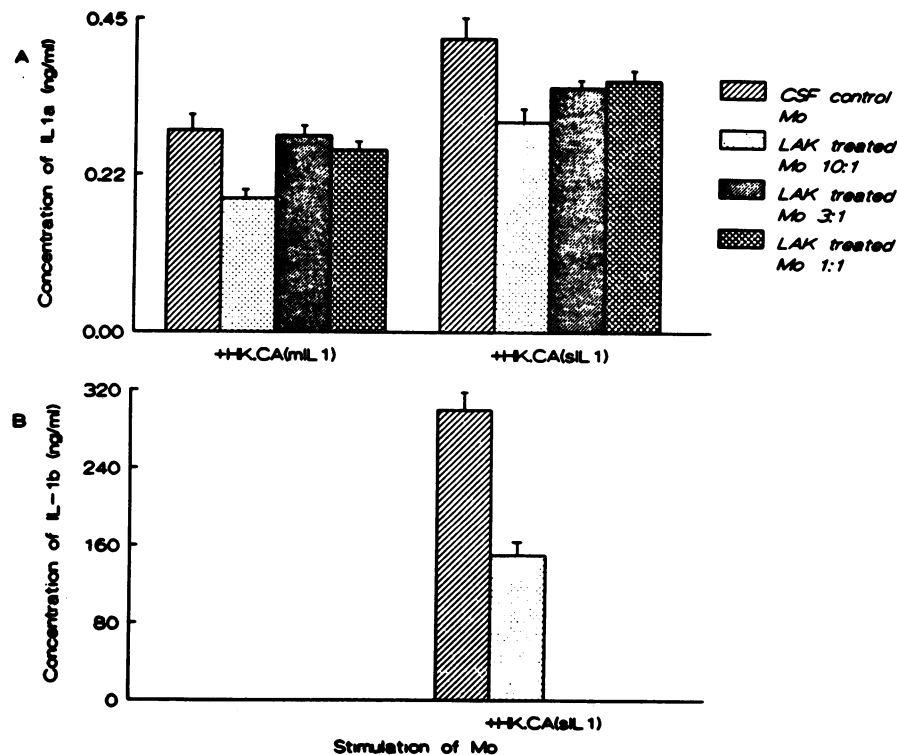

FIG. 7. Effect of LAK cells on IL-1 production from GM-CSF-treated monocytes. Adherent GM-CSF-treated monocytes (Mo) were exposed to LAK cells for 4 h at 37°C. After removal of LAK cells, the monocytes were pulsed with HK.CA for 48 h at 37°C prior to collection of supernatants and cell pellets for determination of soluble IL-1 and membrane-bound IL-1. (A) Soluble and membrane IL-1α (mIL 1 and sIL 1); (B) soluble IL-1β. Data are expressed as means ± standard errors of triplicate wells and are from a representative experiment from experiments with three separate donors.
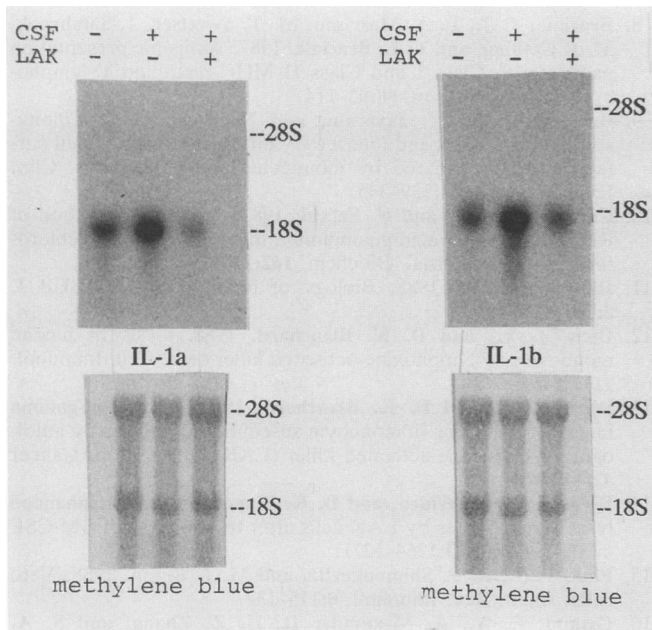
FIG. 8. Northern blot analysis of mRNA synthesis in GM-CSF-cultured monocytes before and after LAK cell treatment. Adherent GM-CSF-treated monocytes with or without 4 h of exposure to autologous LAK cells were pulsed with HK.CA for 2 h and then processed for RNA isolation; medium-cultured monocytes served as an additional control. Hybridization was with human IL-1α cDNA or IL-1β cDNA. Methylene blue staining of the different RNA preparations was also performed to monitor comparable loading of the samples.

intact, we next checked the viability of the monocytes 4 h after LAK cell treatment and found them to be viable by the trypan blue exclusion test. Equal amounts of RNA were then loaded per lane, as shown by methylene blue staining in Fig. 8. These results suggested that LAK cells can interfere with IL-1 induction from monocytes.

DISCUSSION

The central role played by the monocyte in initiation of the primary immune response which results in either production of specific antibodies or cytotoxic T cells is dependent on its ability to process and present antigen associated with self major histocompatibility complex molecules to be recognized by a clonally restricted T-cell receptor complex (8, 25). In the process, the antigen-stimulated monocyte also generates some costimulators, such as IL-1, which is required in the interaction between APC and the T cell (33). The molecular basis for this interaction is now understood in some detail. However, the regulation of this process is still unclear.

The focus of this study was to analyze the possible regulatory role of autologous LAK cells on monocyte antigen-presenting function and generation of IL-1. The results provide clear evidence of the ability of IL-2-induced LAK cells to down-regulate monocyte-mediated antigen presentation. The important observations made here were that freshly isolated LGL did not interfere significantly with monocyte APC function, but that LAK cells could do so effectively in the same monocytes. In addition, we were able to show that monocytes could also be modulated in their susceptibility to LAK cells by monocyte differentiation

factors. Both GM-CSF and IL-3 were equally potent in enhancing the susceptibility of monocytes to LAK cell-mediated inhibition. Inhibition of antigen presentation was detected significantly earlier than cell membrane damage in LAK cell-treated monocytes. In fact, it appears that GM-CSF-cultured monocytes are programmed upon contact with LAK cells to lose their function because these monocytes, after 1 h of pretreatment with LAK cells and subsequent removal of the LAK cells, continue to be inhibited in antigen presentation, at a time when no cytoplasmic leakage or cell membrane damage can be detected. In addition to inhibition of antigen presentation, short exposure to LAK cells also down-regulated monocyte production of IL-1α and IL-1β at both the protein and mRNA levels.

The effect of IFN-γ on monocyte susceptibility was found to be contrary to that of GM-CSF or IL-3 (2, 13). IFN-γ was found to protect monocytes from LAK cell suppression of antigen presentation. When monocytes were cultured for 4 days in the presence of 100 U of IFN-γ per ml, subsequent exposure to LAK cells did not interfere with their APC function. Other investigators have also found that IFN-γ can protect certain tumor cells and fibroblasts from NK/LAK cell lysis (30). The opposing effects of GM-CSF and IFN-γ on monocyte susceptibility to LAK cell functional suppression are equivalent to those previously reported by us on another function of monocytes, i.e., direct antifungal activity against *C. albicans* (34). Thus, both antimicrobial activity and APC function in monocytes are susceptible to inhibition by LAK cells, depending on the state of activation of the monocytes.

Monocytes responding to IFN-γ may serve a slightly different purpose than those responding to GM-CSF, which can be distinctly recognized by LAK cells. Although both cytokines are considered monocyte differentiation and activating factors (9, 20, 22, 32), there are apparently other properties induced by IFN-γ and GM-CSF that differentiate the treated monocytes. These differences become apparent when antimicrobial activity is examined. For example, we have shown that monocytes, placed in culture with medium, progressively lose their ability to inhibit *C. albicans* growth but that GM-CSF or IL-3 can restore this function in aged monocytes whereas IFN-γ cannot (32). In addition, GM-CSF can activate human monocytes harboring intracellular bacteria, such as *Mycobacterium avium-intracellulare*, to kill the microbes, yet IFN-γ has a detrimental effect on the infected monocytes by increasing the survival of the bacteria within the monocytes (4, 6). Other data supporting the view that the susceptibility of monocytes to LAK cell down-regulation depends on the stage of cell differentiation and maturation come from our finding that freshly isolated monocytes and medium-cultured monocytes had relatively high resistance to LAK cells (2–4, 12–14). The results with fresh and medium-cultured monocytes combined with the opposite effects of IFN-γ and GM-CSF or IL-3 on monocytes to LAK cell suppression may reflect an important regulatory pathway that is part of a network of interactive immune cells and cytokines that orchestrate immunologic events.

Some studies have demonstrated that a cellular immune response to an antigen may also be under the influence of a complex series of specific T cells. For example, contrasuppressor and veto cells have been reported to act upon either the helper T or active cytotoxic T cells in order to maintain immune homeostasis or to control the cytotoxic T-cell reaction in order to down-regulate the cellular immune response to an optimal physiological level. This regulatory process has been intensively studied in the murine system

(reviewed in reference 15). However, regulation of the immune response at the APC level is less understood. From the results of this study, we hypothesize that the regulation of LAK cells on monocyte function may represent a system developed to specifically suppress accessory cells at certain stages of activation and differentiation for the control of immune homeostasis. It is possible that the response to a foreign antigen leads to IL-2 induction, followed by LAK cell generation, which might coincide with the induction of GM-CSF at a time that monocytes have presented antigen to T cells and are no longer required. On the other hand, the inhibitory effect of monocytes by LAK cells may serve other purposes. One reported positive effect of LAK cell recognition of monocytes is the elimination of monocytes infected with intracellular pathogens that have established a parasitic state in the phagocytes following ingestion (5, 7). On the negative side, suppression of monocytes by LAK cells could contribute to toxic side effects seen in immunotherapy with IL-2 or LAK cells (19, 24). It has also been reported that human alveolar macrophages are killed by LAK cells (26). Lysis of certain types of monocytes or differentiated macrophages by LAK cells therefore could lead to release of inflammatory proteins that could be deleterious to patients receiving continuous IL-2 or LAK cell administration. We are far from a full understanding of the mechanism of LAK cell regulation of monocytes. Further investigation of the LAK cell-monocyte system should yield better insight into the true role of the various cytokines in monocyte differentiation and provide some clues to the significance of LAK cell recognition of a select subset of differentiated monocytes.

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