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Human Alveolar Macrophages Augment Natural Killer Cell Stimulatory Factor (Interleukin-12)-inducible Killer Activity from Autologous Blood Lymphocytes

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Interleukin-12 (IL-12), also known as natural killer cell stimulatory factor (NKSF), was found to induce cytotoxic activity from human blood T cells and NK cells. The present study was undertaken to examine the effect of human alveolar macrophages (AM) on induction by IL-12 cytotoxic cells from blood lymphocytes. AM were obtained by bronchoalveolar lavage from healthy donors. Highly purified lymphocytes (>99%) and monocytes (>90%) were also isolated by centrifugal elutriation from peripheral blood of the same donors. Cytotoxicity of lymphocytes was measured by 4-h ^{51}Cr release assay. IL-12 stimulated blood lymphocytes to produce interferon γ (IFNγ) and tumor necrosis factor α (TNFα), and this effect was augmented by co-cultivation with monocytes or AM. AM-upregulated induction of cytotoxic lymphocytes was stimulated with IL-12, and this effect was significantly abrogated by addition of antibodies against IFNγ and TNFα. Induction by IL-12 of IFNγ production and cytotoxic activity of CD8$^+$ cells was also augmented by co-cultivation with monocytes or AM. AM were more effective than monocytes in augmenting the cytotoxic activity of IL-12-stimulated lymphocytes and CD8$^+$ cells. These observations suggest that *in situ* induction of IL-12-stimulated cytotoxic cells in the lung may be regulated by complex cytokine networks, depending on participation of monocytes and alveolar macrophages.

Key words: Alveolar macrophage — IL-12 — Cytotoxicity — Lymphocyte — CD8$^+$ cell

Interleukin-12/natural killer cell stimulatory factor (IL-12/NKSF) is a recently described heterodimeric cytokine with ability to augment natural killer (NK) activity and induce interferon γ (IFNγ) production.[1] These actions have been mainly defined using assays of freshly isolated peripheral blood lymphocytes or purified populations of NK cells.[2,3] Both T and NK cells also produce message for IFNγ following overnight stimulation in the presence of IL-12,[4] and the induction of IFNγ production requires accessory cells.[5]

NK cells and cytotoxic T lymphocytes (CTL) constitute the major effector cell populations of the immune system involved in the control of tumor cells and virally infected cells, most probably by lysis of infected and transformed cells.[6] IL-12 enhanced cytotoxicity of both human NK and T cells against tumor cells.[7] Moreover, IL-12 augmented the cytolytic activity of blood lymphocytes from patients with malignant diseases.[8] Although the enhancement by IL-12 of NK activity of CD56$^+$ cells does not require accessory cells,[9] little is known about the regulatory mechanism of IL-12-activated killer cell induction from human blood lymphocytes, particularly from CD8$^+$ T cells. Recently, we found that the ability of CD8$^+$ cells to proliferate and to generate cytotoxicity activity in response to IL-12 depended on the presence of peripheral blood monocytes.[10]

Human alveolar macrophages (AM) play a critical role not only as cytotoxic effectors, but also as regulatory cells of lymphocyte functions in defense of the lung against primary and/or metastatic neoplasmas.[11] There is accumulating evidence showing that IL-12 has potent antimetastatic and antitumor activity in a number of murine tumor models.[12] For example, Brunda *et al.* demonstrated that antimetastatic and antitumor effects of IL-12 administered *in vivo* were mediated by CD8$^+$ T cells.[13] Because AM are supposed to be an important regulator for *in situ* induction of IL-12-activated killer cells in the lung, we examined whether AM obtained by bronchoalveolar lavage from healthy donors affected the responses to IL-12 of peripheral blood lymphocytes and CD8$^+$ T cells, to generate cytotoxic activity. Here we report that full generation of cytotoxic activity of blood lymphocytes and CD8$^+$ cells in response to IL-12 required the autologous AM as well as blood monocytes.

MATERIALS AND METHODS

Subjects Cells were obtained from healthy male volunteers (21–28 years old). These subjects had no evidence by history or physical examination of infectious disease, and were not taking medication. They all gave informed consent to participate in the experiments.

Cell lines Cell lines of human Burkitt lymphoma (Daudi) and human lung small cell carcinoma (N-291)

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were obtained from the American Type Culture Collection (Rockville, MD). A human lung adenocarcinoma cell line (PC-9) and human lung small cell carcinoma cell line (H-69) were kindly supplied by Dr. Y. Hayata (Tokyo Medical College, Tokyo) and Dr. Y. Shimosato (National Cancer Center Research Institute, Tokyo), respectively. Cell lines were maintained in culture in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO, Grand Island, NY) and gentamicin (Schering-Plough, Osaka), designated as CRPMI 1640, at 37°C in a humidified atmosphere containing 5% CO_2. For cytotoxicity assays, cultured target cells were used in the exponential growth phase.

Reagents Recombinant IL-12 (NKSF) was supplied by the Genetics Institute (Tokyo). Anti-human IFNγ antibody (Ab) was a polyclonal rabbit antiserum prepared against purified human IFNγ and was kindly supplied by Dr. Y. Ohmoto (Otsuka Pharmaceutical Co., Tokushima). A monoclonal antibody (mAb) specific to tumor necrosis factor α (TNFα) (IgM type, neutralizing activity 2.2×10^5 U/ml) was a gift from Hayashibara Institute (Okayama). None of these materials contained endotoxins, as judged by *Limulus amoebocyte* assay (sensitivity limit, 0.1 ng/ml) (Seikagaku Kogyo Co., Tokyo).

Harvesting and preparation of human AM Bronchoalveolar lavage was performed as described in detail elsewhere.[14, 15] Briefly, after anesthetizing the oral cavity and the upper airway with lidocaine spray, the tip of an Olympus fiberoptic bronchoscope (Model BF-1T20; Olympus Co., Tokyo) was wedged into one of the segments of the right or left lobe. The lung was washed with 50 ml of sterilized saline (0.9% NaCl) prewarmed to 37°C, and the fluid was gently sucked out with a 50 ml syringe. This process was repeated 3 times. A total of 150 ml of saline was instilled, of which about 65% was recovered. The yield of human AM from normal volunteers was approximately 1.8×10^7 viable cells per wedge segment (>93% viable as determined by trypan blue dye exclusion test). Differential counts established that >89% of the lavaged cells were AM (staining for nonspecific esterase). The other cells were either small mononuclear cells or neutrophils, which were eliminated during subsequent washing.

Isolation and culture of human peripheral blood monocytes Leukocyte concentrates were collected from peripheral blood (200 ml) of healthy donors in an RS-6600 rotor of a Kubota KR-400 centrifuge, and mononuclear cells were separated from the leukocyte concentrates in lymphocyte separation medium (Organon Teknika Co., Durham, NC). Then monocytes were separated from the mononuclear cell samples by centrifugal elutriation in a Hitachi SRR6Y elutriation rotor.[16, 17] A fraction containing more than 95% of the total monocyte population was obtained at a speed of 3000 rpm and flow rate of 30–36 ml/min. More than 90% of these cells were monocytes as determined by nonspecific esterase staining and morphological examination, and more than 97% were viable, as judged by the trypan blue dye exclusion test. This fraction was washed twice with phosphate-buffered saline (PBS), and resuspended in CRPMI 1640 medium, at a concentration of 5×10^5 monocytes per ml. These cells were then plated for 1 h in 96-well Microtest III plates (Falcon, Oxford, CA), and then, non-adherent cells were removed by washing twice with warm RPMI 1640 medium. At this point, the purity of the monocytes was >99% as judged from their morphology and nonspecific esterase staining.

Isolation of $CD8^+$ cells by using Ab-coated immunomagnetic beads Anti-CD8 Ab adsorbed on uncoated Dynabeads M-450 were obtained from Dyna Inc., Great Neck, NY, as described previously.[18] Sterile Ab-coated immunomagnetic beads were washed twice in ice-cold CRPMI 1640, using a magnet to pellet the beads. The beads pellet (3×10^7/ml) was then suspended in 5 ml of the cell mixture (1×10^7 cells/ml) and incubated on ice with gentle agitation every 5 min for 30 min. After the incubation, the tube was inverted to resuspend the pellet and placed in a magnetic holder for 2 min. The supernatant was pipetted off and the pellet was resuspended in 25 ml of cold CRPMI 1640 and replaced in the magnet. This wash was repeated. The pellet was suspended in 50 ml of CRPMI 1640 at 37°C for 16 h. The beads were then removed from the cultures by harvesting each plate with vigorous agitation with a Pasteur pipette and placing the mixture in a magnetic holder. After 2 min, the supernatant was pipetted off and saved for replating. This separation method allowed us to isolate more than 99% of $CD8^+$ cells, as judged by FACScan analysis (data not shown). The mixture of $CD8^+$ cells with or without monocytes or AM was plated at 10^5 cells/well in a 96-well Microtest III plate (Falcon, Oxnard, CA) and incubated for 4 days at 37°C in medium with or without IL-12.

Cytotoxicity assay Cell-mediated cytotoxicity was assayed by measuring ^{51}Cr release in a 4-h test as described previously.[14] Lymphocytes or $CD8^+$ cells (10^5) were incubated in CRPMI 1640 with IL-12 in the presence or absence of monocytes or AM in 96-well plates for 4 days. In some experiments, anti-TNFα mAb or anti-IFNγ antiserum was added. After 4 days the supernatants were removed and frozen for measurement of cytokines. Remaining cells were washed and their cytotoxicity against ^{51}Cr-labeled Daudi cells was measured at an effector/target ratio (E/T) of 10:1, unless otherwise described. Preliminary experiments indicated that at this time there was no difference in the numbers of lymphocytes incubated with and without IL-12 (data not shown).

Percent cytotoxicity was calculated as follows:

$$\% \text{ cytotoxicity} = 100 \times \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximum cpm} - \text{spontaneous cpm}}$$

Results are presented as mean ± SD of triplicate cultures.

Enzyme immunoassays (EIAs) of human IFNγ and TNFα EIAs for human IFNγ and TNFα were performed as described in detail previously.[19] Briefly, microtitration plates (Nunc, Naperville, IL) were coated with mAb against stated cytokines in 100 μl/well of PBS, pH 7.4. After overnight incubation at 4°C, the wells were blocked with a solution of 1% skimmed milk in PBS for at least 1 h at room temperature and washed 3 times with PBS containing 0.05% Tween 20. This buffer was used for washing at all steps. Volumes of 200 μl of test samples in PBS containing 0.1% bovine serum albumin (BSA) were added to duplicate wells. The plates were incubated at 37°C for 2 h then washed 3 times, and 100 μl of mAbs against stated cytokines (diluted 1:1000 with PBS containing 0.1% BSA) were added to appropriate wells. The plates were incubated for 2 h at 37°C, then washed 3 times, supplemented with 100 μl of peroxidase-labeled anti-mouse or anti-rabbit IgG (diluted 1:1000 with PBS containing 0.1% BSA), and incubated at room temperature for 2 h. Finally, the plates were washed 5 times, 100 μl of enzyme substrate (1 mg/ml of *o*-phenylenediamine in 0.1 *M* sodium citrate buffer, pH 5.0) was added to each well and the plates were incubated at room temperature for 5 min. The reaction was stopped by adding 100 μl of H_2SO_4 to each well, and the absorbance at 492 nm was determined, using a Titertek Multiskan. Sensitivity limits of the EIAs for IFNγ and TNFα were 20 pg/ml.

Statistical analysis The statistical significance of differences between groups was analyzed by means of Student's *t* test (two-tailed).

RESULTS

Effect of AM density on induction of cytotoxic lymphocytes by IL-12 We examined the effect of addition of AM on induction of cytotoxic lymphocytes by IL-12. For this, blood lymphocytes with or without different densities of AM were incubated for 4 days in medium with or without IL-12 (100 U/ml), and their cytotoxic activity against Daudi cells were measured. The results are shown in Fig. 1. Even in the absence of IL-12, incubation of blood lymphocytes for 4 days with AM at densities of 20×10^3 cells/well resulted in slight induction of cytotoxic lymphocytes. Moreover, induction of cytotoxic lymphocytes by IL-12 was augmented by addition of AM in a density-dependent manner. In a parallel experiment, blood monocytes augmented the significant induction of killer cells by IL-12 (data not shown).

We also examined the dose-response relation of IL-12 for augmentation of IL-12-induced cytotoxic lymphocytes by AM. For this, blood lymphocytes (10^5) were incubated for 4 days in medium with different concentrations of IL-12 in the presence or absence of autologous AM (10^4 cells). Significant induction of cytotoxic lymphocytes in the presence of AM was caused by IL-12 at concentrations of more than 10 U/ml (data not shown).

Antitumor spectrum of IL-12-induced cytotoxic lymphocytes Next we examined the antitumor activity of IL-12-induced cytotoxic lymphocytes against various human lung cancer cells. For this, lymphocytes with or without autologous monocytes or AM at a ratio of 10:1 were incubated for 4 days in medium with IL-12 (100 U/ml) before addition of various target cells. The results are given in Table I. Lymphocytes that had been cocultured with or without monocytes or AM and without IL-12 were significantly cytotoxic to H-69 and N-291 lung cancer cells. In the presence of monocytes or AM, IL-12 induced cytotoxic activity of lymphocytes against all the target cells examined. Induction by IL-12 of lymphocyte-mediated cytotoxicity against all the lung cancer cell lines was augmented by AM to a greater extent than by monocytes.

In a parallel experiment, we also examined whether cell-to-cell contact between AM and lymphocytes was

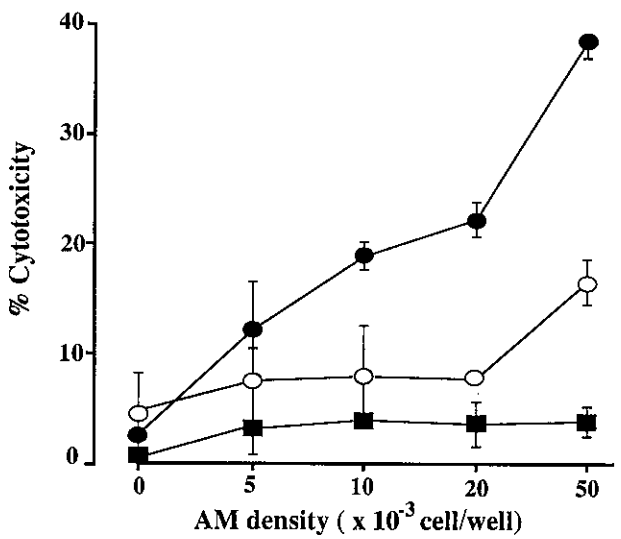

Fig. 1. Effect of AM densities on induction of cytotoxic cells by IL-12. Blood lymphocytes (10^5/well) were incubated for 4 days in the presence of autologous AM at the indicated densities with (●) or without IL-12 (100 U/ml) (○), and their cytotoxic activities against Daudi cells were assayed at an E/T ratio of 10:1. As a control experiment, the cytotoxicity of AM alone was examined (■). Data are representative of three separate experiments. Bars show SDs of means for triplicate cultures.

Table I. Antitumor Spectrum of IL-12-stimulated Lymphocytes against Various Human Lung Cancer Cells

Target cell	Addition of IL-12 (100 U/ml)	% Cytotoxicity[a]		
		Lymphocytes alone	Lymphocytes plus monocytes	Lymphocytes plus AM
PC-9	−	1.3±1.6[b]	3.3±0.2	2.8±0.6
	+	6.0±0.4	12.5±1.5[c]	18.3±1.2[c]
H69	−	10.8±1.8	19.9±0.6[c]	31.2±0.7[c]
	+	33.0±2.6	56.6±0.5[c]	63.4±0.1[c]
N291	−	12.7±2.9	15.2±3.9[c]	31.0±2.4[c]
	+	33.3±0.1	48.6±1.6[c]	58.4±1.3[c]

a) Lymphocytes (10^5/well) with or without monocytes or AM (10^4/well) were incubated in medium with or without IL-12 (100 U/ml), and then their cytotoxic activities were assayed on the indicated target cells at an E/T ratio of 10:1, as described in "Materials and Methods."
b) Mean±SD for triplicate cultures.
c) Significantly different from the corresponding value for lymphocytes alone ($P<0.05$).

Table II. Requirement of Cell-to-cell Contact for Augmentation by AM of IL-12-inducible Killer Lymphocytes

Experimental condition[a]	% Cytotoxicity against Daudi cells	
	Medium	IL-12
Coculture	4.2±2.4[b]	18.3±1.6
Separate culture	0.1±1.2	4.7±2.8[c]

a) Blood lymphocytes (2×10^6/well) were incubated in medium with or without IL-12 (100 U/ml) under conditions of cocultivation with autologous AM (2×10^5/well) or in double chambers separated from AM by a cell-impermeable transmembrane at a lymphocyte/AM ratio of 10:1. After 4 days, the Transwell inserts were carefully removed and the cytotoxic activities were assayed on Daudi cells at an E/T ratio of 10:1.
b) Mean±SD for triplicate cultures.
c) Significantly different from that for lymphocytes cocultured with AM in the presence of IL-12 ($P<0.05$).

Table III. Effect of AM Density on IFNγ and TNFα Productions by IL-12-stimulated Lymphocytes

AM density (cells/well)	IFNγ production (pg/ml)		TNFα production (pg/ml)	
	Medium	IL-12	Medium	IL-12
No AM	<20	61	<20	<20
5×10^3	<20	593	<20	<20
10×10^3	<20	1111	<20	<20
20×10^3	<20	1603	22	30
50×10^3	<20	6580	54	87

Lymphocytes (10^5/well) mixed with the indicated numbers of AM were incubated in the presence or absence of IL-12 (100 U/ml) for 4 days, and the culture supernatants were harvested for measurement of IFNγ and TNFα as described in "Materials and Methods."

required for augmentation of IL-12-induced killer activity from lymphocytes. For this, we performed experiments using the Millicell-HA system (Millipore Products Division, Bedford, MA). Data are given in Table II. Cytotoxicity against Daudi cells of lymphocytes cocultured with AM in the presence of IL-12 was significantly higher than that without IL-12. When lymphocytes and AM were incubated while separated by a cell-impermeable membrane, IL-12 could not induce the full expression of lymphocyte-mediated cytotoxicity, thus indicating that cell-to-cell contact between lymphocytes and AM may be essential for the full generation of cytotoxic properties of IL-12-stimulated lymphocytes.

IFNγ and TNFα production by lymphocytes stimulated with IL-12 in the presence of AM IL-12 was previously reported to induce IFNγ production by NK cells and T cells.[2] We examined whether IL-12 could induce cytokine production by blood lymphocytes in the presence or absence of AM (Table III). Lymphocytes with or without autologous AM at various densities were incubated for 4 days in medium with or without IL-12 (100 U/ml), and then the supernatants were harvested for quantitative measurements of IFNγ and TNFα. IFNγ and TNFα were significantly produced by lymphocytes stimulated with IL-12, depending on the numbers of AM added. Even in the absence of IL-12, addition of AM to cultures of lymphocytes resulted in significant production of both cytokines.

Effect of anti-IFNγ antiserum and anti-TNFα Ab on induction by IL-12 of cytotoxic lymphocytes Lymphocytes were incubated with AM for 4 days in medium with various concentrations of IL-12 in the presence or absence of anti-IFNγ antiserum or anti-TNFα Ab before addition of labeled target Daudi cells. The results are shown in Fig. 2. Cytotoxic lymphocytes in the presence

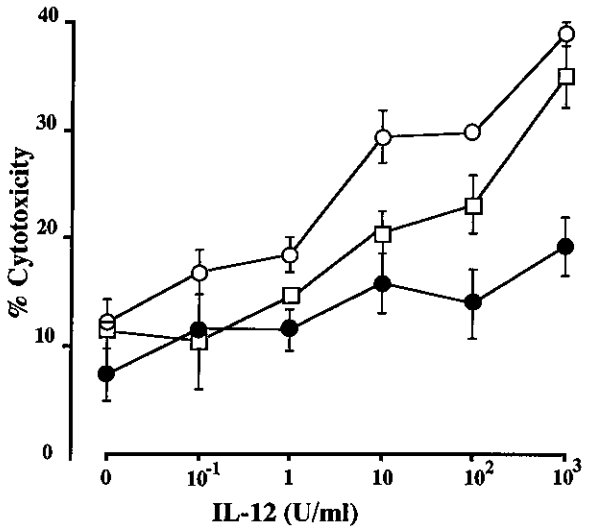

Fig. 2. Effect of addition of anti-IFNγ antiserum or anti-TNFα Ab on induction by IL-12 of cytotoxic lymphocytes in the presence of AM. Blood lymphocytes (10^5/well) with AM (10^4/well) were incubated in medium with the indicated concentrations of IL-12 in the absence (○) or presence of anti-human IFNγ antiserum (finally diluted 1:1000) (□) or monoclonal anti-human TNFα Ab (●). After 4 days, their cytotoxic activities against Daudi cells were assayed at an E/T ratio of 10:1. Bars show SDs of means for triplicate cultures. Data are representative of two independent experiments.

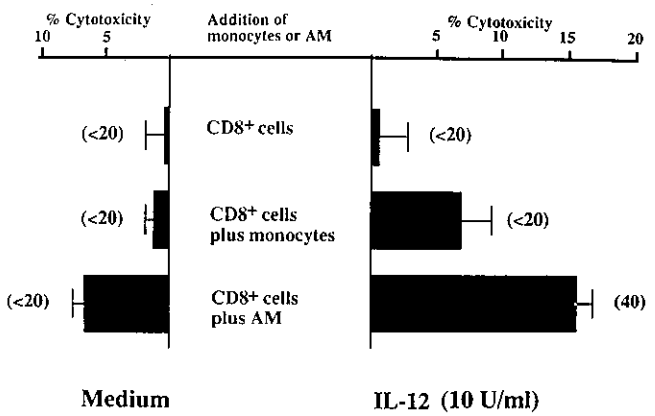

Fig. 3. Effect of IL-12 on induction of cytotoxic CD8$^+$ cells and their IFNγ production in the presence of monocytes or AM. Blood CD8$^+$ cells (10^5/well) were incubated for 4 days in the presence of IL-12 (10 U/ml) with or without autologous monocytes or AM (10^4/well), and their culture supernatants were harvested for quantitative measurement of IFNγ. Cytotoxic activities of the remaining cells were assayed on Daudi cells at an E/T ratio of 10:1. Bars show SDs of means for triplicate cultures. Values in parentheses show amount of IFNγ (pg/ml). Data are representative of three separate experiments.

of autologous AM were induced by IL-12 in a dose-dependent mannar. Under the same experimental conditions, addition of anti-TNFα Ab to the cultures of lymphocytes and AM with IL-12 resulted in almost complete suppression of cytotoxic cell induction. Similarly, anti-IFNγ serum also caused a significant reduction in IL-12-induced cytotoxic activity of lymphocytes in the presence of autologous AM. Anti-TNFα Ab inhibited the IL-12-induced cytotoxic activities more potently than did anti-IFNγ serum.

Up-regulatory effect of AM on induction of cytotoxic CD8$^+$ activity Various studies have shown that functional expression of CD8$^+$ T cells is influenced by monocytes and macrophages.[10, 18] We examined whether AM affected the induction of cytotoxic CD8$^+$ cells by IL-12. For this, CD8$^+$ cells were incubated for 4 days in medium containing IL-12 (10 U/ml) in the presence or absence of autologous monocytes or AM obtained from the same normal donor. The resultant cells and supernatants were used for measurements of cytotoxic activity and IFNγ production, respectively. AM augmented induction of cytotoxic CD8$^+$ cells by IL-12 much more than that by monocytes (Fig. 3). Similarly, addition of AM to the cultures of CD8$^+$ cells plus IL-12 resulted in a significant increase in IFNγ production.

DISCUSSION

The present findings clearly demonstrate that resting human blood lymphocytes fail to respond to IL-12 alone; the presence of AM as well as monocytes was required for full generation of IL-12-induced lymphocyte-mediated cytotoxicity. When human blood lymphocytes were stimulated with IL-12 in the presence of AM or monocytes, they were nonspecifically cytotoxic to all the allogeneic lung cancer cell lines used as target cells (Table I). Interestingly, AM enhanced the cytotoxic activity of lymphocytes stimulated with IL-12 more potently than did blood monocytes.

The present findings showed that addition of large numbers of AM (50×10^3/well) to the culture of blood lymphocytes (10^5) without addition of exogenous IL-12 resulted in induction of low levels of lymphocyte-mediated cytotoxic activity (Fig. 1). This induction might be due to the combined action of endogenous cytokines such as TNFα and IL-12 that had been secreted constitutively in very low amounts by AM through their interaction with lymphocytes. Such endogenous IL-12 may be responsible for secondary IFNγ production by lymphocytes (NK and/or T cells). This possibility is supported by the following findings; 1) we previously found that human AM freshly obtained from healthy volunteers were in a "stimulated and/or activated" state in terms of their TNFα production and

tumor cytotoxicity,[15,20] and 2) monocyte-macrophages were demonstrated to be the primary cell type capable of producing IL-12.[21,22]

IL-12 can affect the functional state of cytotoxic effector cells independently of its ability to stimulate proliferation.[3,12] In the present study we showed that human AM augmented the cytotoxic properties of autologous peripheral blood lymphocytes by IL-12, and that lymphocytes stimulated with IL-12 in the presence of monocytes or AM were significantly cytotoxic to allogeneic lung cancer cells (Table I). Moreover, induction by IL-12 of cytotoxic $CD8^+$ cells was also augmented by addition of autologous AM (Fig. 3). Interestingly, the present finding showing upregulation by AM of IL-12-inducible killer activity was in contrast to our previous observation that AM suppressed IL-2-activated killer cell induction from blood lymphocytes,[14] suggesting differential roles of IL-12 and IL-2 in macrophage-dependent killer induction. Moreover, IL-12 failed fully to induce killer activity when lymphocytes and AM were separately cultured in a double chamber system, suggesting that augmentation by human AM of IL-12-induced killer activity requires a cell-to-cell contact between blood lymphocytes and AM.

IFNγ and TNFα are known to be required for induction of cytotoxic T cells by IL-2.[23,24] This fact suggests that the effect of IL-12 on the induction of cytotoxic cells in the presence of AM and monocytes may be mediated by other induced cytokines. The present findings clearly showed that IL-12-stimulated lymphocytes produced IFNγ and TNFα depending on the cell density of AM added, although IFNγ production was much greater than that of TNFα. Moreover, we obtained evidence for roles of both IFNγ and TNFα in IL-12-induced cytotoxic activity involving blood lymphocytes and AM, because the enhancing effect was partially abrogated by anti-human IFNγ antiserum. Anti-TNFα antibody also almost completely abrogated this effect. Although the source of these cytokines was not identified in the present study, T cells and NK cells might be the primary cells. Interestingly, IL-12-stimulated $CD8^+$ T cells in the presence of monocytes were previously found to produce only IFNγ, but not TNFα.[10] IL-12 also stimulates resting NK cells to produce TNF.[9] Again, the present findings suggest that IL-12 stimulates blood lymphocytes in the presence of AM to produce endogenously TNFα and IFNγ, which may be required to augment a cell-to-cell interaction between lymphocytes and AM for differentiation of the killer precursors to the cytotoxic effector cells.

Recently, much attention has been paid to the usage of IL-12 in combination with IL-2 for therapy of cancer patients. Indeed, IL-12 was found to restore defective NK activity of blood mononuclear cells from patients with metastatic cancer and to enhance the cytolytic function of blood mononuclear cells from patients receiving low-dose IL-2 *in vivo*.[8] In addition to IL-2,[14,25] other cytokines such as IL-4,[26] IL-6[27] and IL-7[28] have also been found to potentiate human CTL responses. Further studies on the regulations of IL-12-induced cytotoxic lymphocytes may contribute to our understanding of the complex cytokine networks and allow us to generate more potentially cytotoxic NK and $CD8^+$ T cells for immunotherapy of primary and metastatic pulmonary cancer in humans.

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