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Interleukin-2 and lymphocyte-induced eosinophil proliferation and survival in asthmatic patients

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Background: Eosinophils are increased in the airways and blood of asthmatic patients.

However, the mechanism of regulation of eosinophilia is incompletely understood.

Methods: To study the potential effect of asthmatic lymphocytes on eosinophils, lymphocytes from the blood of asthmatic patients in exacerbation, or from healthy subjects, were isolated and cultured in medium alone (LC-CM) or with interleukin-2 (IL-2-CM) (125 U/ml), and the effect of supernatant obtained from these cultures on eosinophil proliferation from progenitors and survival was studied.

Results: IL-2-CM from asthmatic patients significantly increased eosinophil colony formation from asthmatic blood but had no effect on colony formation from the blood of healthy subjects. IL-2-CM from asthmatic patients also significantly prolonged the survival of eosinophils. IL-2 alone and IL-2-CM from healthy subjects had no effect on eosinophil proliferation and survival. Asthmatic lymphocytes had more IL-2 receptors (CD25) than normal lymphocytes, and this difference persisted even after culture in IL-2. However, upregulation of the CD25 receptor on normal lymphocytes by incubation with concanavalin A led to the production of IL-2-CM, which did not increase eosinophil proliferation from progenitors.

Conclusions: Lymphocytes from asthmatic patients but not from normal blood can significantly increase eosinophil proliferation and survival. The effects on eosinophil proliferation do not seem to be directly related to the presence of increased CD25 expression on lymphocytes.

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Key words: IL-2, lymphocyte conditioned medium, eosinophil, asthma

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Asthma is a chronic disease characterized by reversible airway obstruction and increased airway responsiveness. The histologic finding of airway inflammation in association with the late airway response of asthmatic patients to inhalational challenge with allergen, and the link between late responses and airway hyperresponsiveness, have led to the hypothesis that inflammation is responsible for hyperresponsiveness.^{1,2} Among the inflammatory cells, eosinophils seem to have an important role in asthma.^{3,4} These cells are present in increased numbers in the blood, lung lavage fluid, and bronchial biopsies of asthmatic patients.⁵⁻⁸ Eosinophils are activated in asthmatic patients and also can release enzymes and membrane-

derived mediators that have been shown to affect airway responsiveness.^{9, 10}

Little is known of the factors that lead to eosinophil survival and proliferation in asthmatic patients. Several studies have suggested a potential role for lymphocytes in eosinophil upregulation. In vitro, interleukin-3 (IL-3), interleukin-5 (IL-5), and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are released mostly by lymphocytes, have been shown to modulate eosinophil proliferation from progenitors as well as activation and release of membrane-derived mediators.¹¹⁻¹³ Because lymphocytes are also increased in bronchial and lung biopsies of asthmatic patients,^{14, 15} they have the potential to affect the presence and function of eosinophils in the airway by secreting specific cytokines. We have shown that the administration of interleukin-2 (IL-2) or T-cell growth factor to rats can increase airway responsiveness to not only inhaled methacholine but also to antigen challenge in a dose that does not affect responsiveness to methacholine.^{16, 17} In vivo administration of IL-2 caused an increase in eosinophils around the airways of rats,^{16, 17} suggesting the possibility that the effects of IL-2 may be mediated by the eosinophil. The administration of IL-2 also causes peripheral blood eosinophilia in humans.¹⁸⁻²⁰ Our hypothesis is that asthmatic lymphocytes contribute to the eosinophilia in asthmatic patients as well as the increased state of activation of eosinophils. To test this hypothesis, nonadherent mononuclear cells (NAMNCs) were isolated from the blood of asthmatic patients in exacerbation and healthy subjects. The effect of supernatant from these cells, cultured with or without IL-2, on eosinophil proliferation from progenitors and eosinophil survival was tested. Because asthmatic NAMNCs have increased receptors for IL-2,²¹ we investigated the possibility that upregulation of the IL-2 receptor CD25 may cause normal NAMNCs to secrete cytokines that affect eosinophils.

MATERIAL AND METHODS

Subjects

Blood samples (105 ml) were obtained from 19 asthmatic patients in acute exacerbation and 14 healthy subjects (mean age, 31.5 ± 5 and 38 ± 6 years for asthmatic and healthy subjects, respectively) after written informed consent was obtained. The protocol was approved by the ethics and research committee of St-Luc Hospital (Montreal, Canada). Atopic or nonatopic asthmatic subjects were defined as having reversible airway obstruction (increase in peak expiratory flow [PEF] by 20% or more after a bronchodilator) without a significant smoking history (<5 pack/year). Asthmatic subjects were recruited in the emergency rooms of St-Luc and Charles LeMoyne Hospitals (Montreal, Canada). PEF, blood leukocyte count, and total eosinophil count were

Abbreviations used

IL-2:	Interleukin-2
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL-2-CM:	Interleukin-2 stimulated lymphocyte conditioned medium
LC-CM:	Lymphocyte conditioned medium
CFU-Eo:	Colony-forming unit of eosinophils
NAMNC:	Nonadherent mononuclear cells
PEF:	Peak expiratory flow

determined before specific treatment was instituted. Asthmatic PEF was 208 ± 104 L/min, range from 100 to 400 L/min. Leukocyte count was $9.1 \pm 3.1 \times 10^6$ cells/ml for asthmatic subjects and $4.9 \pm 1.9 \times 10^6$ cells/ml for healthy subjects. Total eosinophils were higher in asthmatic subjects than in healthy subjects ($0.624 \pm 0.46 \times 10^6$ cells/ml vs $0.056 \pm 0.058 \times 10^6$ cells/ml, $p < 0.01$). Asthmatic patients were being treated with bronchodilators ($n = 19$) or inhaled steroids ($n = 4$), but none was receiving any systemic steroids or undergoing hyposensitization at the time of blood retrieval. Most patients had undergone skin testing previously, but when patients were stable, prick skin tests to 11 common allergens were repeated on the forearm to evaluate atopic status. From these tests it was determined that 14 asthmatic subjects were atopic and five were nonatopic. Healthy controls had no symptoms of respiratory or allergic diseases, were not receiving any medication, and had normal spirometry as well as negative skin prick tests before blood retrieval.

Isolation of blood lymphocytes and preparation of lymphocyte conditioned medium

Peripheral blood lymphocytes were separated on Ficoll-Paque (Pharmacia AB, Uppsala, Sweden) from 30 ml of heparinized blood. After washing three times with RPMI 1640, cells were resuspended in 40 ml of enriched RPMI 1640 medium (RPMI 1640 supplemented with 100 U/ml of penicillin, 100 μ g/ml of streptomycin, 10% heat inactivated fetal calf serum, 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acid) and adhered in a plastic flask (Falcon Plastics, Oxford, Calif.) for 2 hours at 37° C in 5% CO₂. NAMNCs were collected and resuspended in enriched RPMI 1640 medium at 1×10^6 cells/ml and cultured with or without 125 U/ml of IL-2 in 24-well tissue culture plates (Falcon) at 1 ml/well. The culture medium, which was presumed to include cytokines produced by the lymphocytes, was collected at day 5, filter sterilized, and kept at -70° C. The supernatant of cells cultured in IL-2 is referred to as IL-2 conditioned medium (IL-2-CM), and the supernatant of cells cultured without IL-2 is referred to as lymphocyte conditioned medium (LC-CM). To upregulate the IL-2 receptor (CD25) on lymphocytes, concanavalin A (ConA)-stimulated IL-2-CM was prepared by incubating NAMNCs with ConA (5 μ g/ml) for 12 hours then washing

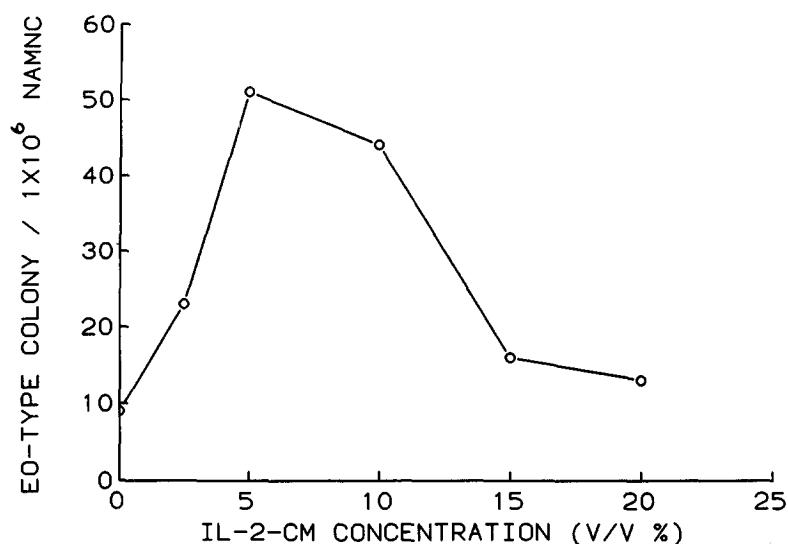


FIG. 1. Dose-response curve of eosinophil-type colony production induced by IL-2-CM from asthmatic subjects. IL-2-CM and NAMNCs were obtained from the blood of two asthmatic subjects in exacerbation who had a high level of eosinophil progenitors in their peripheral blood. NAMNCs were cultured in the presence of different concentrations of IL-2-CM from asthmatic subjects. Eosinophil-type colonies obtained from 1×10^6 NAMNCs in methylcellulose culture were counted on day 14.

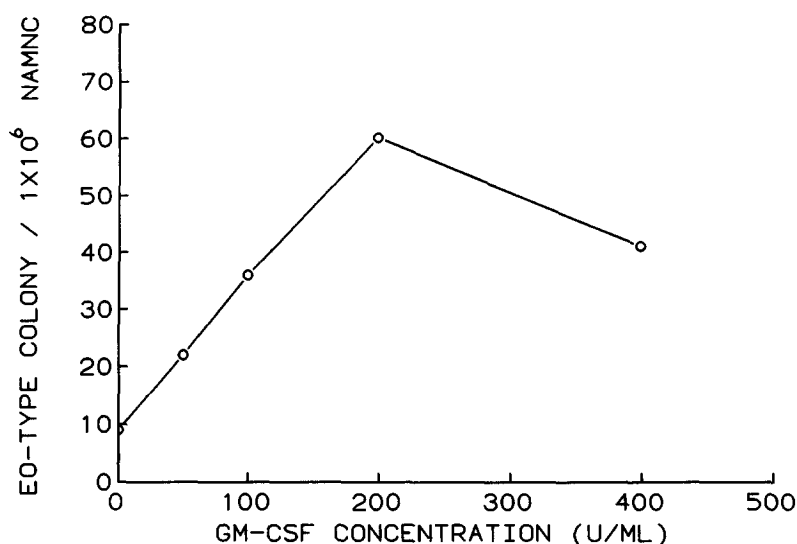


FIG. 2. Dose-response curve of eosinophil-type colony production induced by GM-CSF. NAMNCs from two asthmatic patients in exacerbation who had a high level of eosinophil progenitors in their peripheral blood were cultured with different concentrations of GM-CSF. The total number of eosinophil-type colonies obtained from 1×10^6 NAMNCs in methylcellulose culture were counted on day 14.

twice with RPMI 1640. After adjusting cell concentration to 1×10^6 cells/ml, the cell suspension was replated into a 24-well culture plate and incubated with or without IL-2 (125 U/ml) for a total of 5 days as described above.

Isolation of eosinophils

Eosinophils were isolated on a discontinuous metrizamide density gradient from 75 ml of heparinized blood with the technique described by Owen et al.²² In brief, leukocyte

buffy coats recovered from dextran sedimentation of heparinized venous blood were mixed with an equal volume of isolation medium (enriched RPMI-1640 medium plus 45 U/ml of deoxyribonuclease-1 and 1 mmol/L HEPES) and centrifuged at 250 g for 10 minutes at 4°C. Then, the mixed leukocyte preparations were resuspended in isolation medium at a concentration of 3×10^7 cells/ml and layered on metrizamide gradients (18%, 20%, 22%, 23%, 24%). The densities were standardized by their refractive indexes

TABLE I. Effect of IL-2 and conditioned medium on eosinophil-type of colony growth

	Asthma*			Healthy		
	Stimuli No.	Mean*	SE	No.	Mean†	SE
IL-2	12	1.25	3.56	9	0.00	0.00
IL-2CMAS	19	16.4	5.04	14	1.21	0.65
IL-2CMNS	18	0.67	2.91	6	0.08	1.04
LC-CMAS	7	0.29	4.66	8	1.19	0.90
LC-CMNS	6	1.83	5.04	6	1.42	1.04
Medium	17	1.00	2.99	14	0.61	0.68
CM-CSF	10	15.0	3.90	7	3.64	0.96

IL-2CMAS, IL-2-CM from asthmatic subjects; *IL-2CMNS*, IL-2-CM from normal (healthy) subjects; *LC-CMAS*, LC-CM from asthmatic subjects; *LC-CMNS*, LC-CM from normal (healthy) subjects. No., Number of experiments.

*ANOVA, $F = 3.265$, $p < 0.0063$.

†Mean of total eosinophil-type colony production from 1×10^6 NAMNCs.

($r = 1.3620, 1.3650, 1.3680, 1.3695, 1.3710$, for the 18%, 20%, 22%, 23%, and 24% metrizamide layers, respectively). After centrifugation at 1000 g for 45 minutes at room temperature, the interfaces were collected, and the cell suspensions recovered from different layers were diluted with equal volumes of enriched medium and centrifuged at 1000 rpm for 10 minutes at 4° C. Contaminant erythrocytes were removed by hypotonic lysis, and the cell pellet was resuspended in enriched medium. The cells at the 22%/23% and 23%/24% gradient interface were pooled with those cells that pelleted to the bottom of the gradient and were considered as normodense eosinophils. The purity of metrizamide purified normodense eosinophils was $92\% \pm 8\%$ (mean $\pm$ SD, $n = 24$) as assessed by May-Grünwald-Giemsa staining. Cell viability was always greater than 95% as assessed by trypan blue dye exclusion.

Eosinophil culture

To study the survival of eosinophils, freshly isolated normodense eosinophils at concentrations of 0.25 to 0.5×10^6 cells/ml enriched medium were plated into a 24-well tissue culture plate containing optimal concentrations of cytokines or conditioned medium. Dose-response curves were performed in pilot studies to determine the optimal concentration of media. These were 125 U/ml of IL-2, 200 U/ml of GM-CSF, and 10% (vol/vol) of IL-2-CM. LC-CM was added at a concentration of 10% (vol/vol). Then cells were cultured at 37° C for 7 to 14 days in a humidified atmosphere of 5% CO₂ in air. Culture media were replaced at 2- to 3-day intervals.

Assessment of eosinophil survival

Eosinophil survival was assessed at days 3, 5, and 7. Eosinophils were recovered from culture, washed and centrifuged at 1000 rpm for 10 minutes at 4° C, and resuspended in 1 ml of enriched medium. Ten microliters of the cell suspension were diluted in an eosinophil count solution,²³ and cell number was determined with a hemocytometer. Cell viability was judged by trypan blue dye exclusion. After a

5-minute incubation at 37° C the eosinophil survival rate was calculated by use of the following formula: $100 \times (\text{number of eosinophils recovered} \times \text{percent of cells excluding trypan blue}) / (\text{original number of eosinophils plated})$.

Colony-forming unit assay

The colony-stimulating activity of conditioned media was assayed with use of a methylcellulose culture technique.²⁴ In brief, peripheral blood NAMNCs from asthmatic subjects and healthy volunteers were resuspended in Iscove's modified Dulbecco's medium and plated at a concentration of 1×10^6 cells per ml in 35×10 mm tissue culture dishes (Falcon) in supplemented Iscove's modified Dulbecco's medium containing 20% fetal bovine serum, 1% penicillin, 1% streptomycin, 5×10^{-5} mol/L 2-mercaptoethanol, 0.9% methylcellulose, and optimal concentrations of conditioned medium or cytokines. After 14 days of culture at 37° C in a humidified atmosphere containing 5% CO₂ in air, colonies (defined as any aggregation of more than 40 cells) and clusters (8 to 39 cells) were counted by inverted microscopy and classified into two types by their morphologic appearance. Eosinophil-type colonies (CFU-Eo) appeared as tight, compact aggregations of round, refractile cells and showed varying combinations of eosinophilic, basophilic, and mixed eosinophilic-basophilic cells on May-Grünwald-Giemsa staining. Neutrophil-macrophage type colonies had a diffuse polymorphic appearance of loose irregular cells and contained more than 95% of neutrophils and macrophages.

Immunochemical staining for the CD25+ lymphocyte subset

NAMNCs were suspended in enriched RPMI 1640 at a concentration of 1×10^6 cells/ml. Slides were prepared by centrifuging 100 μ l of the cell preparation for 5 minutes at 700 rpm in a Shandon Cytospin-3 cytocentrifuge (Shandon Scientific Ltd., Cheshire, England). Slides of NAMNCs were fixed in acetone for 10 minutes. A streptavidin-biotin system (Zymed Laboratories Inc., Camarillo, Calif.) was

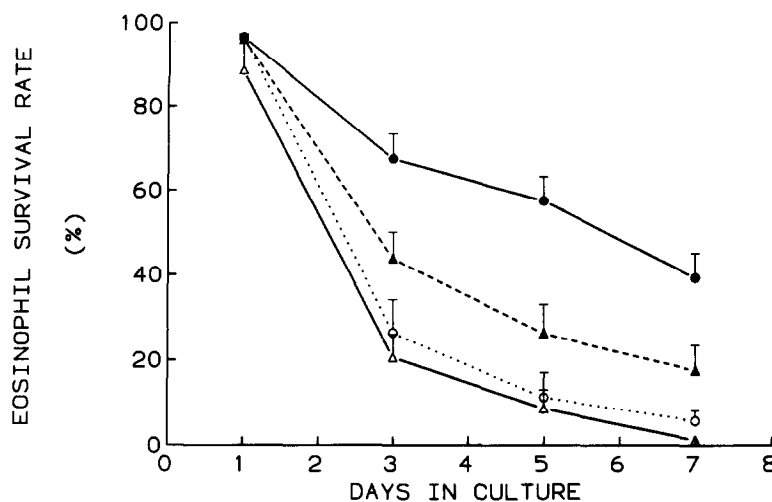


FIG. 3. Asthmatic eosinophil survival. Normal density eosinophils from atopic asthmatic subjects were cultured with IL-2 (○), IL-2-CM from atopic asthmatic subjects (●), LC-CM from atopic asthmatic subjects (▲), or medium alone (△). Survival was measured by trypan blue dye exclusion. The results are expressed as mean $\pm$ SE of 13 experiments performed.

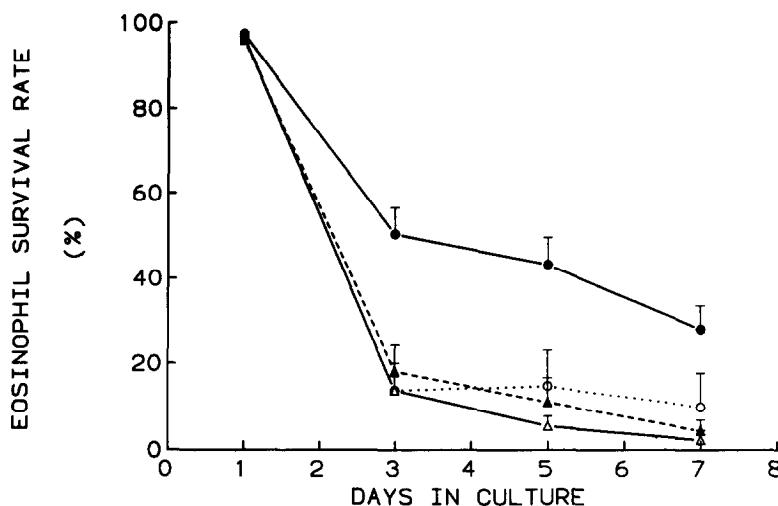


FIG. 4. Normal eosinophil survival: Normal density eosinophils from nonatopic healthy subjects were cultured with IL-2 (○), IL-2-CM from atopic asthmatic subjects (●), LC-CM from atopic asthmatic subjects (▲), and medium alone (△). Survival was measured by trypan blue dye exclusion. The results were expressed as mean $\pm$ SE of nine experiments performed.

used for immunohistologic staining. For this, mouse anti-human CD25 monoclonal antibody (Becton Dickinson, Mountain View, Calif.) was diluted at a concentration of 1:20 and incubated on slides for 1 hour at room temperature. A total of 400 lymphocytes was counted on two slides.

Reagents

Gelatin, L-serine, 2-mercaptoethanol, deoxyribonuclease-1, were obtained from Sigma Chemical Co. (St. Louis, Mo.); D-glucose and dextran molecular weight 150,000 to 200,000 were obtained from BDH Laboratory Supplies (Poole, England); fetal bovine serum, penicillin, strepto-

mycin, L-glutamine, RPMI medium 1640, Iscove's modified Dulbecco's medium, McCoy's 5A medium, nonessential amino acid, HEPES, and trypan blue were obtained from Gibco Laboratories (Long Island, N.Y.); other reagents were Ficoll-Paque (Pharmacia AB, Uppsala, Sweden); metrizamide (Nyegaard & Co., Oslo, Norway), and methylcellulose (Dow Chemical Co., Midland, Mich.).

Lymphokines

Recombinant human IL-2 was kindly provided by Biogen, Inc. (Cambridge, Mass.) as a gift. IL-2 activity was 3.1×10^6 U/mg determined by [^{3} H] thymidine uptake with

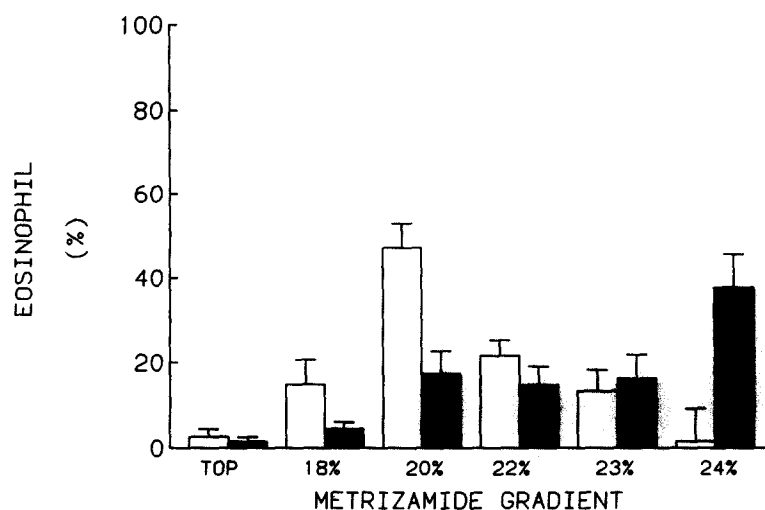


FIG. 5. Comparison of eosinophil density distribution between atopic asthmatic subjects ($\square$ $n = 9$) and healthy subjects ($\blacksquare$ $n = 10$). Eosinophils were centrifuged on metrizamide gradients, and the amount of eosinophils presented in each layer is expressed as a percentage of total eosinophils counted in the gradients.

the CTLL-2 cell line. Ultrapure human GM-CSF was supplied by Endogen Inc. (Boston, Mass.), GM-CSF activity was 10,000 CFUs/ml.

Statistical analysis

The unpaired Student's *t* test was used to compare the difference between two different conditioned media. Analysis of variance (ANOVA) was performed when several conditioned media were compared. Significance was accepted when the *p* value was less than 0.05. Results are presented as mean $\pm$ SEM.

RESULTS

Effect of IL-2 and IL-2-CM on eosinophil-type colony growth

Dose-response curves of medium versus colonies were obtained to determine the optimal amounts of IL-2-CM and GM-CSF for maximal stimulation of eosinophil progenitors. Results for the two asthmatic patients with the highest baseline CFU-Eo and highest response to stimulation with IL-2-CM or GM-CSF are shown in Figs. 1 and 2. From these results we chose a concentration of 10% for IL-2-CM and 200 U/ml for GM-CSF. Some asthmatic NAMNCs had no colony formation in medium alone and were unresponsive to every concentration of IL-2-CM or GM-CSF tried.

The effects of IL-2 and various conditioned media on the growth of eosinophil-basophil progenitors are presented in Table I. IL-2-CM from asthmatic subjects significantly increased the formation of eosinophil colonies from asthmatic blood but not from the blood of healthy subjects. The addition of IL-2 directly to the

culture had no effect on progenitor growth. LC-CM at a concentration of 10% (vol/vol) and medium alone had no effect on colony formation. IL-2-CM from healthy subjects was unable to induce eosinophil colony formation in NAMNCs from the blood of asthmatic subjects and healthy subjects.

The same significant changes were found for the effects of different conditioned media on eosinophil-type cluster formation. No difference was found in the stimulation of eosinophil colonies and clusters from blood of asthmatic patients by IL-2-CM from asthmatic subjects and by that of optimal concentrations of GM-CSF (for clusters: IL-2-CM = 12.1 ± 1.5 colonies vs GM-CSF = 7.7 ± 2.7 colonies, $p > 0.05$). The eosinophil colony-stimulating activity of IL-2-CM from asthmatic subjects was the same whether the asthmatic subjects were atopic or not (15.6 ± 6 vs 18.5 ± 10 colonies produced on stimulation with atopic asthmatic ($n = 14$ experiments) and nonatopic asthmatic ($n = 5$) IL-2-CM, respectively). The same changes were noted for cluster formation.

Effect of IL-2 and IL-2-CM on eosinophil survival

When compared with IL-2 and medium alone, IL-2-CM from asthmatic subjects significantly prolonged the survival of eosinophils isolated both from the blood of asthmatic subjects (Fig. 3) and healthy subjects (Fig. 4) at 3, 5, and 7 days. However, eosinophils from asthmatic subjects survived longer than eosinophils from healthy subjects when stimulated with IL-2-CM from asthmatic subjects. LC-CM from asth-

TABLE II. Effect of IL-2 on lymphocyte CD25 expression

	No.	CD25 + lymphocytes (%)	
		Before	After IL-2
Asthma	6	4.8 ± 1.27*	17.8 ± 4.67**
Normal (healthy)	6	0.5 ± 0.29	7.30 ± 7.30

CD25 + lymphocytes measured by immunocytochemistry staining of nonadherent mononuclear cells before and after 5 days of culture in IL-2 (125 U/ml).

* $p < 0.002$.

** $p < 0.004$ vs normal lymphocytes.

matic subjects also increased the survival of asthmatic eosinophils.

Effect of IL-2 and IL-2-CM on eosinophil density

Fresh eosinophils isolated from the blood of asthmatic subjects had a significantly lower density (Fig. 5). Sixty-four percent of eosinophils were low density in asthmatic subjects compared with 21% in healthy subjects ($p < 0.002$). Culture of normal density eosinophils in IL-2 or IL-2-CM did not significantly affect their density when compared with culture in media alone.

Effect of IL-2 on lymphocyte CD25 expression

Significantly more CD25 positive cells were found in asthmatic NAMNCs than in NAMNC of healthy subjects (Table II). After incubation with IL-2 (125 U/ml) for 5 days both asthmatic and normal NAMNCs had an increased CD25 expression; however, the CD25 expression was still significantly higher in asthmatic NAMNCs.

Effect of conA stimulation on CD25 expression and eosinophil progenitor growth in different culture media

After incubation with ConA for 12 hours, NAMNCs from both healthy and asthmatic subjects showed an increased CD25 expression (mean, 28% for asthmatic subjects and 30% for healthy subjects' NAMNCs). However, IL-2-CM prepared from these activated cells did not significantly increase eosinophil progenitor growth (Table III).

DISCUSSION

Asthma is a disease characterized by systemic and intrabronchial eosinophilia. Because activated eosinophils release mediators that can affect airway re-

sponsiveness,^{7, 25} a decrease in the number and state of activation of eosinophils could be an important factor in the treatment of asthma. We have been interested in the role of lymphocytes in initiating or perpetuating eosinophilia. It has recently been shown that administration of IL-2 to human subjects causes systemic eosinophilia.¹⁸⁻²⁰ Furthermore, the eosinophils isolated from these subjects are of low density, as described in asthmatic subjects.²⁶ We have found, in the Brown Norway rat, that the subcutaneous injection of IL-2 increases airway responsiveness to ovalbumin while increasing eosinophil number in airways.¹⁷ These results suggest that lymphocytes and IL-2 (also called T-cell growth factor) may be involved in eosinophil proliferation, survival, and activation in asthmatic subjects. In this study we have shown that peripheral blood NAMNCs from asthmatic subjects in exacerbation could be stimulated with IL-2 to secrete mediators that affect eosinophil proliferation from progenitors and survival. These effects were not a direct effect of IL-2 because IL-2 itself was unable to affect eosinophil survival and eosinophil progenitor growth. Because IL-2 is released by helper T lymphocytes when antigen is presented,²⁷ this lymphokine may be involved in eosinophil regulation in asthmatic subjects.

Eosinophils and basophils originate from common progenitors that are present in human hemopoietic tissue and cord blood and are increased in the peripheral blood of patients with asthma and those with atopy.²⁸⁻³¹ In agreement with previous observations we also found that asthmatic subjects had more eosinophil progenitors than healthy subjects.²⁶ Ten of 19 asthmatic subjects had more than 10 colonies/10⁶ NAMNCs when cultured in IL-2-CM or GM-CSF, whereas only 1 of 14 healthy subjects had more than 10 colonies/10⁶ NAMNCs when cultured in the same media. IL-2-CM from asthmatic subjects and GM-CSF failed to induce eosinophil colony formation from NAMNCs of healthy subjects and of some asthmatic subjects, probably because eosinophil progenitors are absent from the peripheral blood of these persons. Although dose-response curves were not performed in every subject, a high concentration inhibition of colony proliferation by cytokines seems unlikely because eosinophil-type colonies were not generated in some experiments where different concentrations of IL-2-CM-AS and GM-CSF were used. The presence of a cell in NAMNCs from healthy subjects or from some asthmatic subjects that secretes suppressors of eosinophil growth is also possible.

IL-2 conditioned medium from healthy subjects was unable to induce eosinophil-type colony formation from the blood of asthmatic subjects and healthy sub-

TABLE III. Effect of ConA stimulation on eosinophil-type colony growth

	IL-2CM	No.	Mean	SE	<i>p</i> Value
Colonies	Asthma	9	13.89	4.62	0.1239
	Normal (healthy)	13	6.73	3.85	
Clusters	Asthma	9	6.56	2.32	0.2626
	Normal (healthy)	13	4.58	1.96	

NAMNCs from asthmatic subjects in exacerbation were cultured with IL-2-CM obtained from ConA (5 μ g/ml for 12 hours) stimulated NAMNCs of asthmatic subjects or healthy subjects. Data are presented as mean of eosinophil-type colonies or clusters obtained from 1×10^6 NAMNCs.

jects. Our results concur with those of other studies that used normal human bone marrow and cord blood as a source of progenitor for eosinophils.^{32, 33} In these studies supernatant from cultured asthmatic mononuclear cells increased eosinophil colony formation, whereas supernatant from normal mononuclear cells had no effect. Furthermore, the stimulation of normal mononuclear cells with IL-2 does not lead to the production of a supernatant that increases eosinophil progenitor growth.

IL-2 conditioned medium from atopic and non-atopic asthmatic subjects increased eosinophil-type colony formation from asthmatic NAMNCs. This increase could be explained by the presence of more cells bearing CD25 (IL-2 receptors) on asthmatic NAMNCs even after incubation in IL-2. However, increasing CD25 by incubating normal and asthmatic NAMNCs with ConA did not lead to the generation of increased colony-stimulating activity. Although CD25 positive cells represented 30% of normal NAMNCs, conditioned medium obtained from these cells did not significantly increase eosinophil progenitor growth. It thus seems that CD25 expression is not directly associated with colony-stimulating activity in IL-2-CM. It is possible that only a subset of receptor positive lymphocytes are responsible for the colony-stimulating activity and that these cells are already maximally stimulated in asthmatic subjects in exacerbation. Nonspecific lectin stimulation of uncloned normal T lymphocytes would thus cause preferential stimulation of cells whose cytokines do not support, or might inhibit, eosinophil growth.

Eosinophil survival and activation are certainly important in eosinophil-associated inflammation. Eosinophils have been considered as short lived cells that remain alive for only a few days in culture.³⁴ Recent studies demonstrated that eosinophil survival can be prolonged by IL-3, IL-5, and GM-CSF. Our results show that IL-2-CM from asthmatic subjects also can prolong eosinophil survival. These effects are indirect

because IL-2 by itself is unable to prolong eosinophil survival. It is also impossible to conclude whether the increased eosinophil survival is related to atopy or to asthma because the experiments were only performed on supernatant obtained from atopic asthmatic mononuclear cells. Lymphocyte conditioned medium from asthmatic subjects also tended to increase asthmatic eosinophil survival. In a recent study Loretta et al.³⁵ reported that supernatant from cultured, unstimulated mononuclear cells from atopic subjects could increase eosinophil survival. The cells responsible for these effects seemed to be monocytes. Walker et al.³⁶ also showed that T-cell conditioned medium from asthmatic subjects could increase eosinophil survival. In their study lymphocyte conditioned medium was used at a concentration of 50% (vol/vol). We used only a 10% concentration of lymphocyte conditioned medium, which could explain why we only found a trend for increased eosinophil survival. We have confirmed a previous report that asthmatic eosinophils comprise more lower density eosinophils than normal eosinophils.²⁶

As a whole, our results suggest that stimulation of asthmatic lymphocytes with IL-2 leads to an increase in the production of cytokines that affect eosinophils. Further studies are necessary to determine whether one or several cytokines are responsible for the effects described on eosinophils and whether this effect is of physiologic importance. Several cytokines, secreted by lymphocytes, could have explained the results. In similar studies Enokihara et al.³⁷ found that IL-5 was responsible for most of the eosinophil colony-stimulating activity in IL-2-CM obtained from blood lymphocytes of eosinophilic patients. Burke et al.³⁵ reported that the major factor that enhances eosinophil viability in mononuclear cell supernatant was GM-CSF. When the effect of supernatant obtained from pleural fluid lymphocytes of patients with cancer was examined, the effects on eosinophils were more related to a combination of cytokines (IL-3, IL-5, and

GM-CSF).³⁸ We focused our attention on multiple functional effects of lymphocytes but not on the cytokines that caused the effects.

In summary, we have shown that stimulation of NAMNCs from asthmatic subjects but not from healthy subjects, with IL-2, produces mediators that can increase eosinophil progenitor growth and prolong eosinophil survival. These effects are not related directly to IL-2 nor to the lectin-induced increase of CD25 on NAMNCs. Whether this is a genetic or an acquired difference in asthmatic subjects remains to be determined.

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