

Eosinophil apoptosis caused by theophylline, glucocorticoids, and macrolides after stimulation with IL-5

Tetsuya Adachi, MD,^a Shinji Motojima, MD,^b Akira Hirata, MD,^b
Takeshi Fukuda, MD,^b Norio Kihara, MD,^a Akinori Kosaku, PhD,^c
Hideki Ohtake, PhD,^d and Sohei Makino, MD^b Tokyo and Mibu, Japan

Background: Glucocorticoids have long been used as the most potent drugs in the treatment of bronchial asthma. Data reported recently have led to the proposal that theophylline and macrolides have antiinflammatory effects.

Objective: We examined the abilities of theophylline, glucocorticoids, and macrolides to counteract the prolongation of eosinophil survival caused by IL-5.

Methods: Purified guinea pig eosinophils were cultured in the presence or absence of human IL-5 and with or without the aforementioned drugs at various concentrations. The percentage of cells alive after 3 days in culture was determined.

Results: Aminophylline (AM), methylprednisolone (MP), erythromycin (EM), and clarithromycin (CAM) suppressed the IL-5 induced prolongation of eosinophil survival in a dose-dependent manner. The effects of these drugs on eosinophil survival were significantly greater at low concentrations of IL-5 than at high concentrations of IL-5. When eosinophils were cultured in the presence of IL-5 (1 ng/ml) with physiologic concentrations of MP (10^{-6} mol/L), AM (10^{-4} mol/L), and either EM or CAM (both 10 µg/ml), the effect of IL-5 was almost completely abolished, and the morphologic changes in eosinophils observed by electron microscopy were consistent with apoptosis. DNA extracted from eosinophils cultured with IL-5 and each of the drugs was definitely fragmented.

Conclusions: One mechanism of the effectiveness of these drugs is induction of eosinophil apoptosis. Some combination of these drugs may be useful in the treatment of bronchial asthma. (*J Allergy Clin Immunol* 1996;98:S207-15.)

Key words: Apoptosis, bronchial asthma, eosinophil, glucocorticoid, guinea pig, interleukin-5, macrolide, theophylline

Eosinophils play an important role at the site of inflammation in allergic diseases, including bronchial asthma.¹ Eosinophilopoietic cytokines, such as IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-5 are released from many kinds of cells. They activate eosinophils and prolong their survival.²⁻⁴ These cytokines are present in the airways of patients with bronchial

Abbreviations used

AM:	Aminophylline
CAM:	Clarithromycin
EM:	Erythromycin
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
MP:	Methylprednisolone

From ^athe Department of Respiratory Medicine, Kihara Hospital, Tokyo, and ^bthe Department of Medicine and Clinical Immunology, ^cthe Laboratory of Clinical Science, and ^dthe Department of Physiology, Dokkyo University School of Medicine, Tochigi.

Supported by Dokkyo University School of Medicine.

Reprint requests: Shinji Motojima, MD, Department of Medicine and Clinical Immunology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/0/76959

asthma⁵ and can greatly contribute to eosinophil activation, which results in epithelial damage.⁶

Glucocorticoids have long been used therapeutically for their antiinflammatory effects in diseases such as bronchial asthma. They cause eosinopenia in normal subjects, decrease the number of circulating eosinophils in patients with eosinophilia, and reduce eosinophil influx at sites of inflammation. The clinical effects of glucocorticoids are mediated

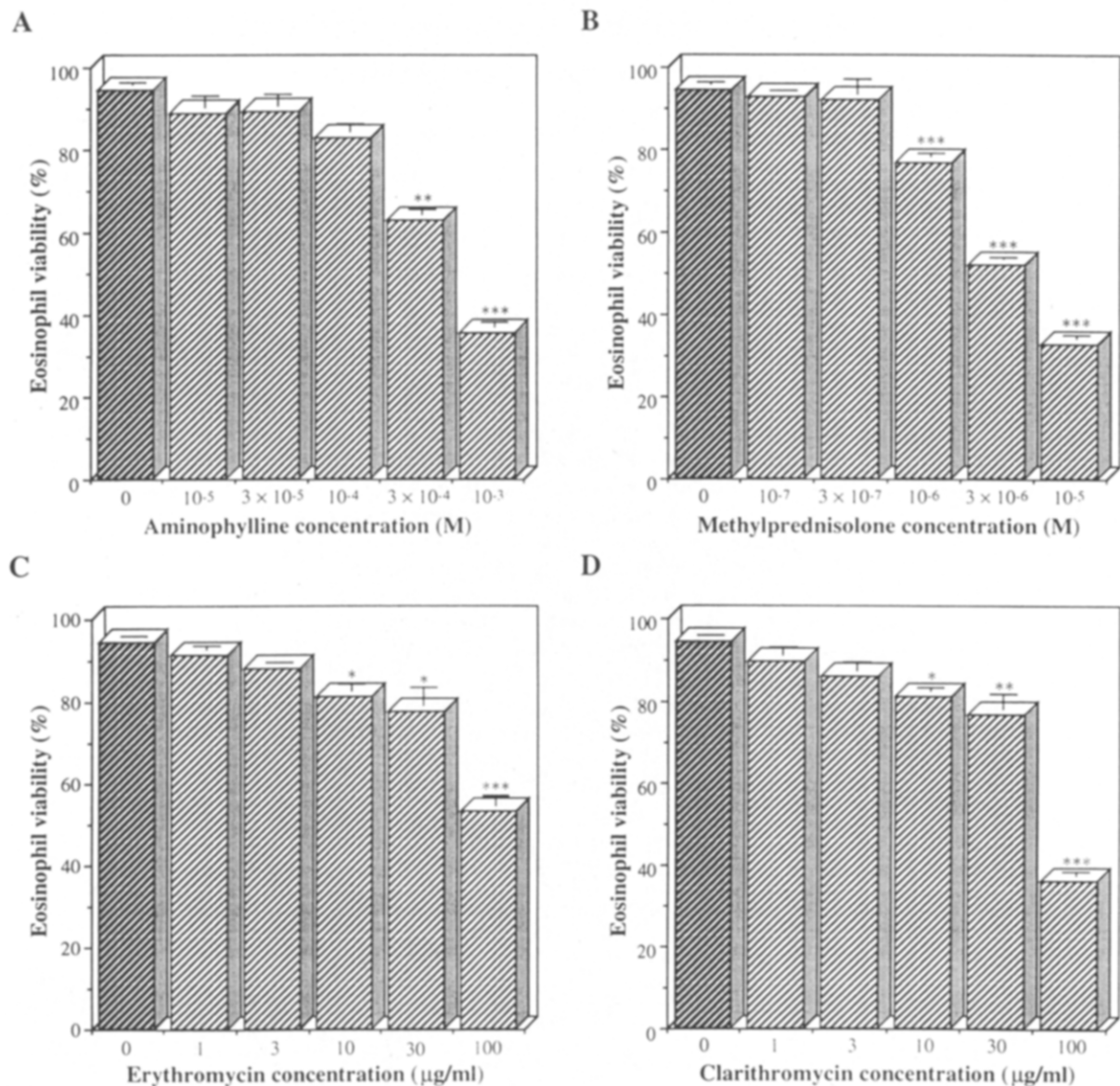


FIG. 1. Effects of AM (A), MP (B), EM (C), and CAM (D) on survival of eosinophils stimulated with 1 ng/ml IL-5. Data are expressed as means $\pm$ SEM ($n = 4$), and were analyzed by one-way analysis of variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

in part by inhibition of the release of eosinophil-active cytokines.⁷ Moreover, glucocorticoids directly inhibit cytokine-mediated eosinophil survival.⁸⁻¹²

Recently reported data have led to the proposal that theophylline and macrolides have antiinflammatory effects. Theophylline significantly inhibits the late asthmatic response, not the immediate response, after the allergen challenge at the sub-bronchodilating level, suggesting that theophylline is likely to modulate bronchial inflammation.¹³ As for macrolides, long-term treatment with erythro-

mycin has already been established for managing chronic airway diseases, such as diffuse panbronchiolitis.^{14, 15} Erythromycin has also been shown to decrease bronchial hyperresponsiveness in patients with bronchial asthma as measured by PC₂₀ in histamine challenge tests.¹⁶ However, the mechanism of antiinflammatory effects of theophylline and macrolides has not been clarified.

Therefore we studied whether theophylline and macrolides inhibit the survival of eosinophils stimulated by IL-5 as glucocorticoids do, and we also examined the effects of various combinations of

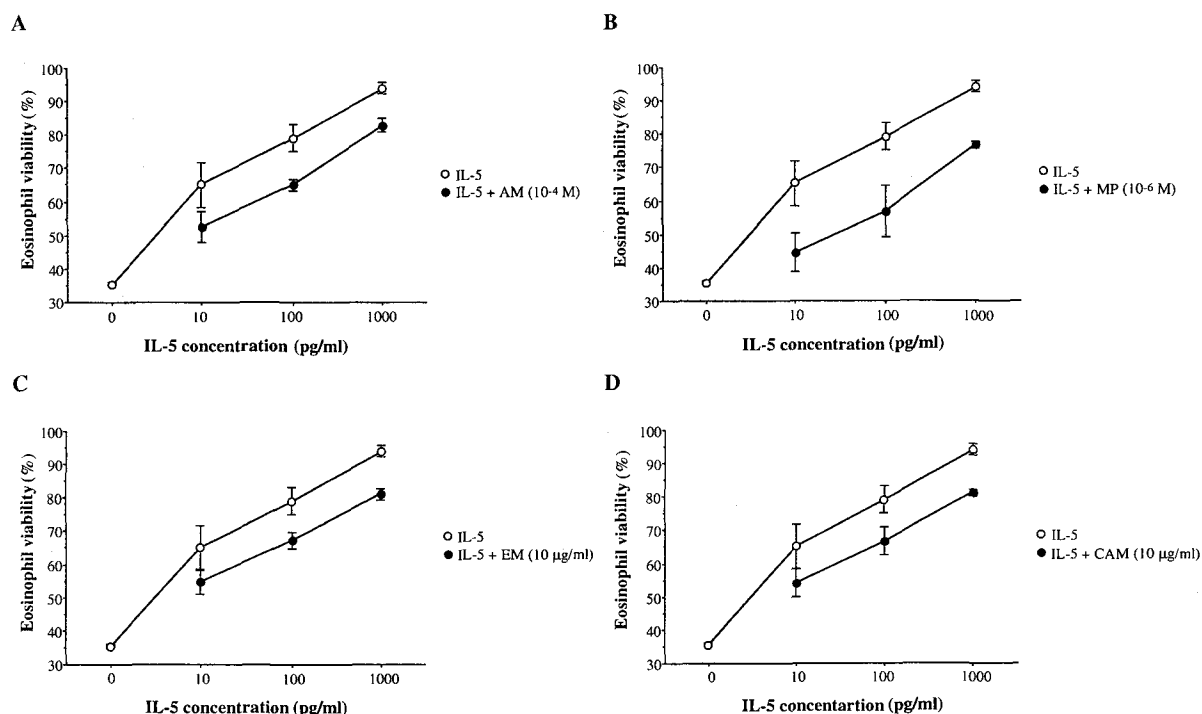


FIG. 2. Effects of AM (A), MP (B), EM (C), and CAM (D) at physiologic levels on survival of eosinophils stimulated with IL-5 at various concentrations. Data are expressed as mean $\pm$ SEM ($n = 4$).

these drugs. In addition, we tried to observe eosinophil apoptosis induced by these drugs with an electron-microscopic study and an analysis of DNA fragmentation.

METHODS

Purification of eosinophils

Eosinophils were purified as reported previously.⁵ In brief, eosinophils in peritoneal lavage fluid obtained from guinea pigs sensitized with horse serum were isolated by centrifugation on a discontinuous Percoll density gradient. Purified eosinophils were suspended at 2.5×10^5 cells/ml in Hybricare medium (American Type Culture Collection, Rockville, Md.) supplemented with 10% fetal bovine serum with 100 µg/ml piperacillin. The purities and the viabilities of the eosinophils were >95%. Guinea pig eosinophils cross-react with human IL-5 and GM-CSF.⁵ We have also found, with a binding assay, that guinea pig eosinophils have receptors that bind to human IL-5 (approximately 2000 sites/cell, dissociation constant = 4.8 nmol/L) and human GM-CSF (approximately 1600 sites/cell, dissociation constant = 2.7 nmol/L).¹⁷

Eosinophil survival assay

Recombinant human IL-5 was purchased from Genzyme (Cambridge, Mass.). The eosinophil survival assay was performed as reported previously.⁵ In brief, 200 µl of eosinophil suspension was put into a well of a 96-well

culture plate with IL-5 and with or without aminophylline (AM), methylprednisolone (MP), erythromycin (EM), or clarithromycin (CAM) at various concentrations and incubated with 5% carbon dioxide in air at 37° C for 3 days. To measure eosinophil viability, the cells were first stained with fluorescein diacetate and propidium iodide and then counted with a hemacytometer and an epifluorescent microscope. Eosinophil viability was $35.0 \pm 1.7\%$ ($n = 4$) after 3-day culture with the medium alone.

Electron-microscopic analysis

Cultured eosinophils (1×10^6 cells) were fixed in 2.5% glutaraldehyde, further double-fixed with 1% osmium tetroxide followed by dehydration, and routinely embedded. Thickly cut (1 µm) sections were stained with methylene blue, and the presence of the eosinophils was confirmed by light microscopy. The sections were further cut (500 nm) by a glass knife, were double-stained with uranyl acetate-lead citrate, and then were examined with an electron microscope (JEX 100, JEOL, Tokyo, Japan).

Analysis of DNA fragmentation

DNA was extracted from guinea pig eosinophils with a DNA extractor WB kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacture's instructions. In brief, the cultured eosinophils (1×10^6 cells) were washed with phosphate-buffered saline; lysed

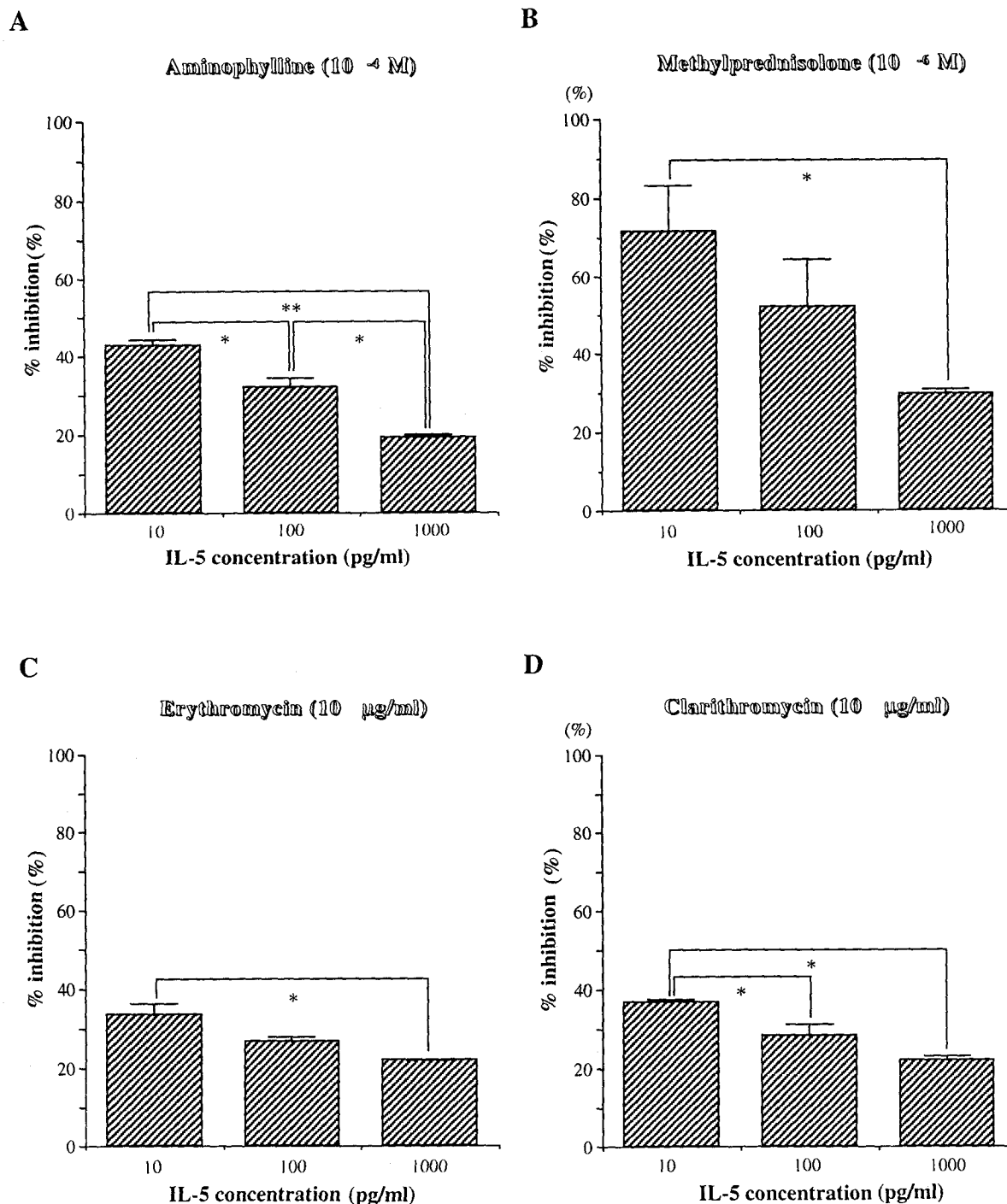


FIG. 3. Percentage inhibition of survival of eosinophils stimulated with IL-5 at various concentrations. AM, MP, EM, and CAM were used at physiologic concentrations. Percentage inhibition was calculated as $(V_{IL-5} - V_{drug}) / (V_{IL-5} - V_{control}) \times 100$, where V_{IL-5} is viability in the presence of IL-5 alone; V_{drug} is viability in the presence of IL-5 and drug; and $V_{control}$ is viability with medium alone. Data are expressed as mean $\pm$ SEM ($n = 4$) and were analyzed by one-way analysis of variance. * $p < 0.05$; ** $p < 0.01$.

with a nonionic surfactant, polyoxyethylene 10-oxyphenyl ether; and then briefly centrifuged. The nuclear fraction was treated with protease in the presence of 1% sodium dodecyl sulfate for 1 hour. The DNA was

extracted with sodium iodide and precipitated with isopropanol. Each sample was electrophoresed on 2% agarose gel with TBE buffer (90 mmol/L Tris-HCl, 90 mmol/L borate, 2.5 mmol/L ethylene diamine tetraacetic

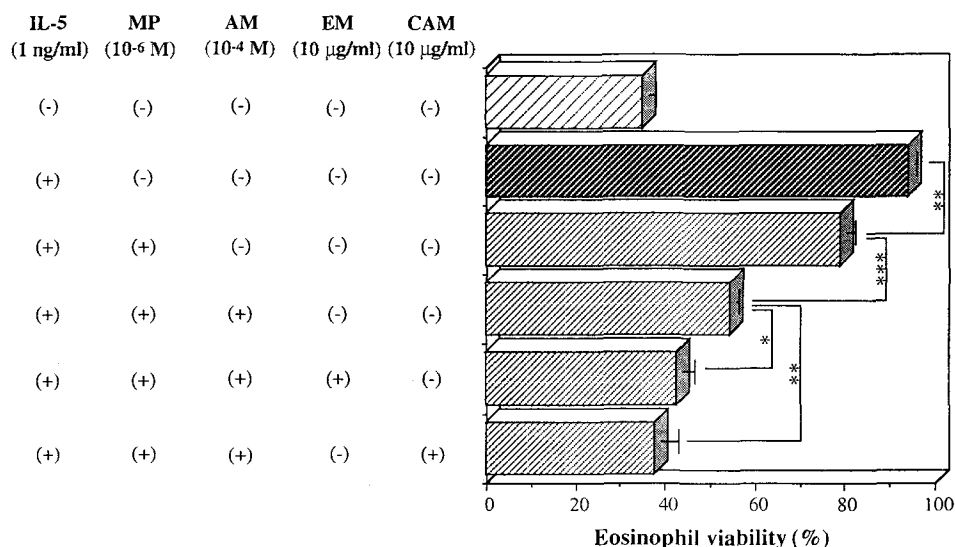


FIG. 4. Effects of various combinations of AM, MP, and EM or CAM at physiologic concentrations on survival of eosinophils stimulated with 1 ng/ml IL-5. Data are expressed as mean $\pm$ SEM ($n = 4$) and were analyzed by one-way analysis of variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

acid) containing 0.5 μ g/ml ethidium bromide, and the stained DNA was observed under ultraviolet light.

Statistical analysis

Data are expressed as means $\pm$ SEM and were analyzed by one-way analysis of variance.

RESULTS

Eosinophil survival assay

The effects of AM, MP, EM, and CAM on the survival of eosinophils stimulated with 1 ng/ml IL-5 are shown in Fig. 1. When AM was added at concentrations from 10^{-5} to 10^{-3} mol/L, eosinophil viability was suppressed in a dose-dependent manner (Fig. 1, A). When MP was added at concentrations from 10^{-7} to 10^{-5} mol/L, eosinophil viability was also suppressed in a dose-dependent manner (Fig. 1, B). Similarly, EM and CAM at concentrations from 1 to 100 μ g/ml inhibited, in a dose-dependent manner, the survival of eosinophils stimulated with IL-5 (Fig. 1, C and D). We did not observe any significant differences between eosinophil viability cultured with these drugs in the absence of IL-5 and with medium alone, suggesting that the effects of these drugs on eosinophils do not constitute cytotoxicity.

Next, the effects of physiologic concentrations of these drugs on eosinophil survival after stimulation with various concentrations of IL-5 were studied. Eosinophils were cultured with or without the drugs in the presence of 10, 100, or 1000 pg/ml IL-5. As shown in Fig. 2, all drugs reduced the survival of eosinophils stimulated with IL-5 at all

concentrations of IL-5 tested. The percentage inhibition of eosinophil survival was also computed for each drug (Fig. 3). For all drugs, the percentage inhibition was significantly greater at the lowest concentration of IL-5 than at the highest concentration. MP had the strongest inhibitory effect.

Combinations of these drugs were tested with the concentration of each drug in its therapeutic range (Fig. 4). The viability of eosinophils cultured with 1 ng/ml IL-5 only was $94.2\% \pm 0.2\%$ ($n = 4$). When 10^{-6} mol/L (corresponding to 0.5 μ g/ml) MP was added, eosinophil viability was $79.2\% \pm 1.8\%$ ($n = 4$), which was significantly lower than the value for IL-5 alone ($p < 0.01$). When 10^{-4} mol/L (corresponding to 34 μ g/ml) AM was included with MP, eosinophil viability was $54.5\% \pm 0.3\%$ ($n = 4$), which was significantly lower than the value for IL-5 with MP ($p < 0.001$). Moreover, when eosinophils were cultured with MP (10^{-6} mol/L), AM (10^{-4} mol/L), and either EM or CAM (both 10 μ g/ml) in the presence of IL-5 (1 ng/ml), their viabilities were $42.6\% \pm 2.4\%$ ($n = 4$) and $37.6\% \pm 4.0\%$ ($n = 4$), respectively. The latter two values were significantly lower than the values for IL-5 with MP and AM ($p < 0.05$ and $p < 0.01$, respectively). The combination of MP, AM, and either EM or CAM almost completely abolished the effect of IL-5. When eosinophils were cultured without IL-5 but with MP (10^{-6} mol/L), AM (10^{-4} mol/L), and either EM or CAM (both 10 μ g/ml), they were no less viable than when they were cultured with medium alone.

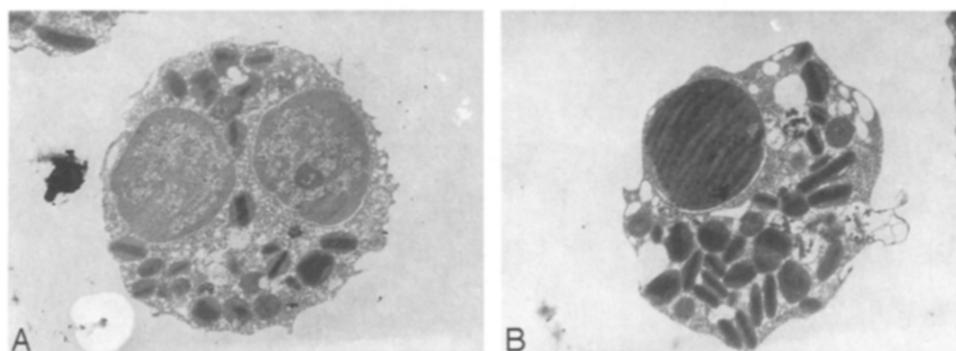


FIG. 5. Electron-microscopic findings of cultured eosinophils. Eosinophils were cultured with 1 ng/ml IL-5 for 3 days in the absence (**A**, 9400 $\times$ original magnification; viability 95%) or presence (**B**, 9400 $\times$ original magnification; viability 43%) of MP (10^{-6} mol/L), AM (10^{-4} mol/L), and EM (10 μ g/ml).

Electron-microscopic analysis

To test whether the observed reductions in the viability of eosinophils incubated with the drugs are the result of apoptosis, we observed the cultured eosinophils by electron microscopy. Most of the eosinophils, which had been cultured for 3 days with IL-5 (1 ng/ml) and with MP (10^{-6} mol/L), AM (10^{-4} mol/L), and EM (10 μ g/ml), revealed microscopic changes consistent with apoptosis (i.e., condensation of nuclear chromatin and surface membrane swelling), whereas these changes were rarely observed in eosinophils cultured with IL-5 alone (Fig. 5).

Analysis of DNA fragmentation

DNA fragmentation was observed to determine whether each drug is capable of inducing eosinophil apoptosis. Eosinophils (1×10^6 cells) were cultured for 3 days in the medium alone or in the presence of IL-5 (1 ng/ml) with or without the drugs. The concentration of each drug was an order higher than its physiologic concentration: 10^{-3} mol/L of AM, 10^{-5} mol/L of MP, and 100 μ g/ml of EM and CAM, each of which was enough to decrease the viability of eosinophils without any other drugs. As shown in Fig. 6, DNA fragmentation was rarely observed in the sample to which 1 ng/ml IL-5 had been added. However, the DNA extracted from samples cultured with the combination of IL-5 and AM, MP, EM, or CAM was definitely fragmented.

DISCUSSION

One of the rapidly developing areas in the fields of allergy and immunology is the study of programmed cell death, namely apoptosis. Apoptosis

is characterized by condensation of nuclear chromatin, shrinkage of the cell, separation of both nucleus and cytoplasm into multiple apoptotic bodies, and destruction of the cell by phagocytic cells. At the same time, cellular DNA is destructed by endonuclease, which cleaves the internucleosomal regions, forming a "ladder" of DNA.¹⁸ In vitro, cultured eosinophils are known to die within several days as a result of apoptosis, and their survival can be prolonged when incubated with eosinophilopoietic cytokines such as IL-3, GM-CSF, and IL-5.²⁻⁴ IL-5 inhibits eosinophil apoptosis with induction of new RNA and protein synthesis, but the details are unclear.¹⁹ Recent data have shown that Fas antigen can modify eosinophil survival by inducing apoptosis and that its pathway is independent of the survival-promoting effect of IL-5.²⁰

Glucocorticoids, which are among the most useful drugs for managing bronchial asthma, inhibit eosinophil chemotaxis and adhesion and thereby cause eosinopenia and decrease the local accumulation of eosinophils. They also suppress the release of eosinophil-active cytokines from T lymphocytes or eosinophils themselves.⁷ Several groups have shown that glucocorticoids can inhibit cytokine-mediated eosinophil survival⁸⁻¹² and that the decrease in the survival is the results of apoptosis.²¹ However, the molecular mechanism by which glucocorticoids induce eosinophil apoptosis and antagonize the effect of eosinophil-activating cytokines is unknown. As for the signaling of eosinophilopoietic cytokines in eosinophils, activation of several tyrosine kinases are involved. Pazdrak et al.²² reported that Lyn is phosphorylated after the activation of IL-5 receptor and then the signal is propagated via Ras-Raf 1-MEK-

microtubule-associated protein (MAP) kinase pathway. Microtubule-associated protein kinases activate many cytosolic proteins, including *c-fos* and *c-jun*.²³ Recent work relating the suppressive effect of glucocorticoids on cytokine gene transcription, such as IL-2, has shown that glucocorticoid receptor complex binds to the transcriptional factor activator protein-1 (AP-1), which is a heterodimer of *c-jun* and *c-fos*.²⁴ AP-1 forms a DNA-protein complex with nuclear factor of activated T cells (NFAT), and the transcriptional activation of IL-2 gene is induced by the complex. In contrast, the involvement of NFAT-related factor, which is distinct from NFAT, for the regulation of IL-5 gene has been suggested.²⁵ Although the interaction of AP-1 and NFAT-related factor in IL-5 gene transcription remains to be solved, it is possible that glucocorticoids inhibit the survival-prolonging effect of IL-5 in eosinophils in the same manner as for IL-2.

Theophylline is a potent bronchodilator and has long been used to treat bronchial asthma. In addition to its effects on airway smooth muscle, antiinflammatory effects have also been noted. Theophylline withdrawal is associated with a significant increase in asthma symptoms and a decrease in spirometry %FEV_{1.0} in patients with asthma treated with long-term theophylline and high-dose inhaled corticosteroids. This phenomenon is accompanied by a significant decrease in activated CD4⁺ and CD8⁺ T cells in peripheral blood and an increase in CD4⁺ and CD8⁺ T cells in bronchial mucosa.²⁶ In vitro, theophylline inhibits the synthesis of IL-2 in T cells and tumor necrosis factor- α in mononuclear cells.^{27, 28} Methylxanthines inhibit the expression of interferon- γ -inducible or tumor necrosis factor- α inducible nitric oxide synthase and vascular cell adhesion molecule-1 in vascular endothelial cells.²⁹ Several reports have also shown the effects of theophylline on eosinophils. The number of EG2⁺ eosinophils in bronchial mucosa after allergen challenge is significantly decreased in patients with atopic asthma treated with oral theophylline compared with that for pretreatment.³⁰ Treatment with theophylline 24 and 3 hours before antigen challenge or before exposure to platelet-activating factor can significantly reduce the increase in the number of eosinophils in bronchoalveolar lavage fluid.³¹ Theophylline can strongly inhibit IgG-induced or secretory IgA-induced degranulation of human eosinophils, which is measured by the release of eosinophil-derived neurotoxin.³² Ohta et al.³³ showed that theophylline causes apoptosis of eosinophils stimulated with IL-5, a finding similar to

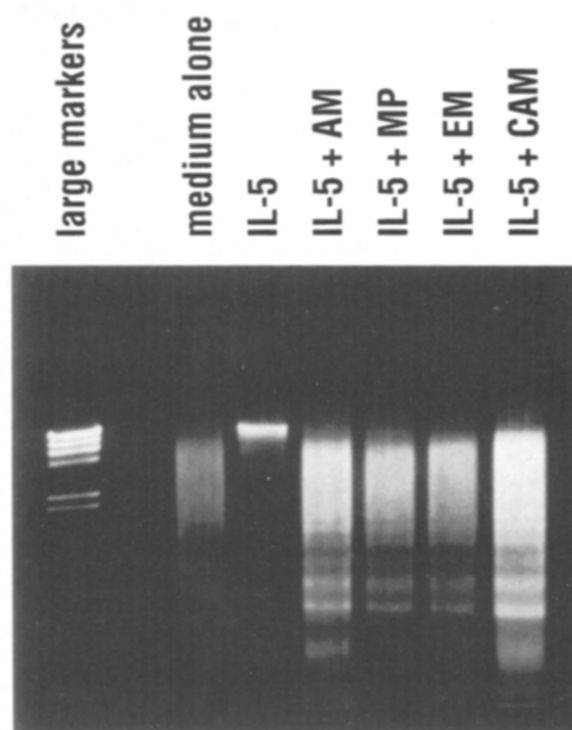


FIG. 6. Results of agarose gel electrophoresis used to detect apoptosis of eosinophils. Eosinophils were cultured for 3 days with medium alone or in the presence of IL-5 (1 ng/ml) with or without AM (10^{-3} mol/L), MP (10^{-5} mol/L), EM (100 μ g/ml), or CAM (100 μ g/ml).

the current results. Although the mechanism by which theophylline causes apoptosis of eosinophils is unclear, the increase in the level of intracellular cyclic adenosine monophosphate may be important.

Chronic airway diseases, including diffuse pan-bronchiolitis, were the first to be treated with EM. EM impairs neutrophil functions, such as chemotaxis, the release of elastase, and IL-8 production.^{14, 15} It also reduces bronchial hyperresponsiveness in patients with bronchial asthma, but no difference is found in the improvement of bronchial hyperresponsiveness between atopic and nonatopic patients.¹⁶ In vitro, EM inhibits T-cell proliferation induced by IL-2,³⁴ and roxithromycin inhibits IL-5 production from T cells.³⁵ EM and CAM suppress IL-6 expression by bronchial epithelial cells.³⁶ FK506, an immunosuppressive macro-lide, inhibits IL-2 gene transcription in activated T cells by blocking the phosphatase activity of calcineurin, for which NFAT is a potential target.^{37, 38} Rapamycin binds to the same receptor site as FK506, but rapamycin and FK506 antagonize each other.³⁹ In contrast, EM does not antagonize but rather potentiates the biologic activity of FK506, which suggests that EM does not competitively

bind to FK506-binding protein.³⁴ We found that EM and CAM cause apoptosis of eosinophils stimulated with IL-5, but the mechanism of action of these macrolides is unknown. EM probably acts through a pathway distinct from those used by FK506 and rapamycin.

Troleandomycin is sometimes used to treat steroid-dependent asthma.⁴⁰ It inhibits glucocorticoid clearance *in vivo*⁴¹ and decreases the elimination of theophylline.⁴² Although such data indicate that troleandomycin may be effective in the treatment of severe asthma, its precise mechanism of action is still unclear. In Europe and the United States, theophylline is no longer used as a first-line drug for treating bronchial asthma. However, the current results show that *in vitro* a combination AM and MP can shorten eosinophil survival more than can MP alone. Moreover, when eosinophils were cultured with AM, MP, and either EM or CAM in the presence of IL-5, the effect of IL-5 on eosinophil survival was almost completely abolished. These results suggest that macrolides can be useful in the treatment of patients with steroid-dependent asthma because they inhibit eosinophilic inflammation.

REFERENCES

- Gleich GJ. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 1990;85:422-36.
- Yamaguchi Y, Hayashi Y, Sugama Y, Miura Y, Kasahara T, Kitamura S, et al. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs *in vitro* survival. *J Exp Med* 1988;167:1737-42.
- Lopez AF, Williamson DJ, Gamble JR, Begley CG, Harlan JM, Klebanoff SJ, et al. Recombinant human granulocyte-macrophage colony-stimulating factor stimulates *in vitro* mature human neutrophil and eosinophil function, surface receptor expression, and survival. *J Clin Invest* 1986;78:1220-8.
- Rothenberg ME, Owen WF, Silberstein DS, Woods J, Soberman RS, Austen KF, et al. Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human interleukin 3. *J Clin Invest* 1988;81:1986-92.
- Adachi T, Motojima S, Hirata A, Fukuda T, Makino S. Eosinophil viability enhancing activity in sputum from patients with bronchial asthma: contributions of interleukin-5 and granulocyte-macrophage colony-stimulating factor. *Am J Respir Crit Care Med* 1995;151:618-23.
- Hirata A, Motojima S, Adachi T, Akutsu I, Fukuda T, Makino S. Rh IL-5 and rh GM-CSF enhance damage to respiratory epithelium induced by IgG-stimulated guinea pig eosinophils [abstract]. *J Allergy Clin Immunol* 1994;93:245.
- Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol* 1994;94:1202-13.
- Lamas AM, Marcotte GV, Schleimer RP. Human endothelial cells prolong eosinophil survival: regulation by cytokines and glucocorticoids. *J Immunol* 1989;142:3978-84.
- Lamas AM, Leon OG, Schleimer RP. Glucocorticoids inhibit eosinophil responses to granulocyte-macrophage colony-stimulating factor. *J Immunol* 1991;147:254-9.
- Wallen N, Kita H, Weiler D, Gleich GJ. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J Immunol* 1991;147:3490-5.
- Cox G, Ohtoshi T, Vancheri C, Denburg JA, Dolovich J, Gauldie J, et al. Promotion of eosinophil survival by human bronchial epithelial cells and its modulation by steroids. *Am J Respir Cell Mol Biol* 1991;4:525-31.
- Hallsworth MP, Litchfield TM, Lee TH. Glucocorticoids inhibit granulocyte-macrophage colony-stimulating factor and interleukin-5 enhanced *in vitro* survival of human eosinophils. *Immunology* 1992;75:382-5.
- Ward AJM, McKenniff M, Evans JM, Page CP, Costello JF. Theophylline: an immunomodulatory role in asthma? *Am Rev Respir Dis* 1993;147:518-23.
- Kadota J, Sakito O, Kohno S, Sawa H, Mukae H, Oda H, et al. A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. *Am Rev Respir Dis* 1993;147:153-9.
- Oishi K, Sonoda F, Kobayashi S, Iwagaki A, Nagatake T, Matsushima K, et al. Role of interleukin-8 (IL-8) and an inhibitory effect of erythromycin on IL-8 release in the airways of patients with chronic airway diseases. *Infect Immun* 1994;62:4145-52.
- Miyatake H, Taki F, Taniguchi H, Suzuki R, Takagi K, Satake T. Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* 1991;99:670-3.
- Adachi T, Motojima S, Hirata A, Fukuda T, Kihara N, Makino S. Guinea pig eosinophils possess receptors capable of binding to human interleukin-5 and human granulocyte/macrophage colony-stimulating factor. *Dokkyo J Med Sci* 1995;22:171-7.
- Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980;68:251-306.
- Yamaguchi Y, Suda T, Ohta S, Tominaga K, Miura Y, Kasahara T. Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 1991;78:2542-7.
- Matsumoto K, Schleimer RP, Saito H, Iikura Y, Bochner BS. Induction of apoptosis in human eosinophils by anti-Fas antibody treatment *in vitro*. *Blood* 1995;86:1437-43.
- Her E, Frazer J, Austen KF, Owen WF. Eosinophil hemopoietins antagonize the programmed cell death of eosinophils: cytokine and glucocorticoid effects on eosinophils maintained by endothelial cell-conditioned medium. *J Clin Invest* 1991;88:1982-7.
- Pazdrak K, Schreiber D, Forsythe P, Justement L, Alam R. The intracellular signal transduction mechanism of interleukin 5 in eosinophils: the involvement of Lyn tyrosine kinase and the Ras-Raf 1-MEK-microtubule-associated protein kinase pathway. *J Exp Med* 1995;181:1827-34.
- Sato N, Sakamaki K, Terada N, Arai K, Miyajima A. Signal transduction by the high-affinity GM-CSF receptor: two distinct cytoplasmic regions of the common β subunit responsible for the different signaling. *EMBO J* 1993;12:4181-9.
- Paliogianni F, Raptis A, Ahuja SS, Najjar SM, Boumpas DT. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference

- with nuclear factors AP-1 and NF-AT. *J Clin Invest* 1993; 91:1481-9.
25. Lee HJ, Masuda ES, Arai N, Arai K, Yokota T. Definition of cis-regulatory elements of the mouse interleukin-5 gene promoter: involvement of nuclear factor of activated T cell-related factors in interleukin-5 expression. *J Biol Chem* 1995;270:17541-50.
26. Kidney J, Dominguez M, Taylor PM, Rose M, Chung KF, Barnes PJ. Immunomodulation by theophylline in asthma: demonstration by withdrawal of therapy. *Am J Respir Crit Care Med* 1995;151:1907-14.
27. Mary D, Aussel C, Ferrua B, Fehlmann M. Regulation of interleukin 2 synthesis by cAMP in human T cells. *J Immunol* 1987;139:1179-84.
28. Endres S, Fülle HJ, Sinha B, Stoll D, Dinarello CA, Gerzer R, et al. Cyclic nucleotides differentially regulate the synthesis of tumor necrosis factor- α and interleukin-1 β by human mononuclear cells. *Immunology* 1991;72:56-60.
29. Bereta M, Bereta J, Georgoff I, Coffman FD, Cohen S, Cohen MC. Methylxanthines and calcium-mobilizing agents inhibit the expression of cytokine-inducible nitric oxide synthase and vascular cell adhesion molecule-1 in murine microvascular endothelial cells. *Exp Cell Res* 1994;212:230-42.
30. Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. *Lancet* 1994;343:1006-8.
31. Lagente V, Moodley I, Perrin S, Mottin G, Junien J-L. Effects of isozyme-selective phosphodiesterase inhibitors on eosinophils infiltration in the guinea-pig lung. *Eur J Pharmacol* 1994;255:253-6.
32. Kita H, Abu-Ghazaleh RI, Gleich GJ, Abraham RT. Regulation of Ig-induced eosinophil degranulation by adenosine 3',5'-cyclic monophosphate. *J Immunol* 1991;146:2712-8.
33. Ohta K, Sawamoto S, Nakajima M, Nagai A, Tanaka Y, Hirai K, et al. Theophylline induce apoptosis in eosinophils surviving with interleukin-5 in vitro [Abstract]. *J Allergy Clin Immunol* 1994;93:200.
34. Keicho N, Kudoh S, Yotsumoto H, Akagawa KS. Antilymphocytic activity of erythromycin distinct from that of FK506 or cyclosporin A. *J Antibiot* 1993;46:1406-13.
35. Konno S, Adachi M, Asano K, Okamoto K, Takahashi T. Anti-allergic activity of roxithromycin: inhibition of interleukin-5 production from mouse T lymphocytes. *Life Sci* 1993;52:PL25-30.
36. Takizawa H, Desaki M, Ohtoshi T, Kikutani T, Okazaki H, Sato M, et al. Erythromycin suppresses interleukin 6 expression by human bronchial epithelial cells: a potential mechanism of its anti-inflammatory action. *Biochem Biophys Res Commun* 1995;210:781-6.
37. McCaffrey PG, Perrino BA, Soderling TR, Rao A. NF-ATp, a T lymphocyte DNA-binding protein that is a target for calcineurin and immunosuppressive drugs. *J Biol Chem* 1993;268:3747-52.
38. Jain J, McCaffrey PG, Miner Z, Kerppola TK, Lambert JN, Verdine GL, et al. The T-cell transcription factor NFATp is a substrate for calcineurin and interacts with Fos and Jun. *Nature* 1993;365:352-5.
39. Dumont FJ, Melino MR, Staruch MJ, Koprak SL, Fischer PA, Sigal NH. The immunosuppressive macrolides FK-506 and rapamycin act as reciprocal antagonists in murine T cells. *J Immunol* 1990;144:1418-24.
40. Wald JA, Friedman BF, Farr RS. An improved protocol for the use of troleandomycin (TAO) in the treatment of steroid-requiring asthma. *J Allergy Clin Immunol* 1986;78:36-43.
41. Szeffler SJ, Brenner M, Jusko WJ, Spector SL, Flesher KA, Ellis EF. Dose- and time-related effect of troleandomycin on methylprednisolone elimination. *Clin Pharmacol Ther* 1982;32:166-71.
42. Weinberger M, Hudgel D, Spector S, Chidsey C. Inhibition of theophylline clearance by troleandomycin. *J Allergy Clin Immunol* 1977;59:228-31.