

Asthma, rhinitis, other respiratory diseases

Montelukast, a leukotriene receptor antagonist, inhibits the late airway response to antigen, airway eosinophilia, and IL-5-expressing cells in Brown Norway rats

Daizo Ihaku, MD, Lisa Cameron, BSc, Masaru Suzuki, MD, Sophie Molet, PhD, James Martin, MD, and Qutayba Hamid, MD, PhD *Montreal, Quebec, Canada*

Background: Asthma is characterized by airflow obstruction, inflammatory cell infiltration, and the synthesis of mediators, such as T_{H2} cytokines and leukotrienes, in the airways. Cysteinyl leukotriene (cysLT) receptor antagonists have recently been associated with clinical improvement of asthma and reduced airway inflammation. Whether the beneficial effects of cysLT antagonists are mediated through the modulation of cytokine expression has not been determined.

Objective: The aim of the study was to determine the presence of eosinophils and IL-5 messenger (m)RNA⁺ cells within the lungs of antigen-challenged Brown Norway rats after treatment with the cysLT₁ receptor antagonist montelukast (MK).

Methods: Ovalbumin-sensitized Brown Norway rats were treated with either MK or saline before ovalbumin challenge. Pulmonary mechanics were monitored for 8 hours. Subsequently, immunocytochemistry and in situ hybridization were used to examine bronchoalveolar lavage (BAL) fluid and lung tissue for cells expressing major basic protein (eosinophils) and IL-5 mRNA, respectively. Simultaneous in situ hybridization and immunocytochemistry was used to phenotype the cells expressing mRNA encoding IL-5.

Results: Animals treated with MK had significantly lower lung resistance and fewer eosinophils and IL-5 mRNA⁺ cells within BAL fluid and lung tissue compared with that found in saline-treated animals. Colocalization studies revealed that the majority of IL-5 mRNA⁺ cells were T cells and that the number of IL-5 mRNA⁺/CD3⁺ or IL-5 mRNA⁺/major basic protein⁺ cells were significantly less within BAL from animals treated with MK than from those treated with saline.

Conclusions: These results indicate that the cysLT₁ receptor antagonist MK can diminish the pulmonary response to antigen, tissue eosinophilia, and the number of cells expressing IL-5 mRNA, suggesting that leukotrienes may also regulate the allergic response through the modulation of inflammation and cytokine synthesis. (*J Allergy Clin Immunol* 1999;1147-54.)

Abbreviations used

BAL:	Bronchoalveolar lavage
BN:	Brown Norway
CysLT:	Cysteinyl leukotriene
ER:	Early response
ISH:	In situ hybridization
LR:	Late response
LT:	Leukotriene
MBP:	Major basic protein
MK:	Montelukast
OVA:	Ovalbumin
R _L :	Lung resistance

Key words: Montelukast, IL-5, antigen-induced late response, Brown Norway rat, leukotriene

Asthma is a chronic inflammatory condition accompanied by variable airway obstruction and hyperresponsiveness. Infiltration of eosinophils and T lymphocytes and the production of T_{H2} cytokines is characteristic of the asthmatic lung.¹ The mechanisms of airway eosinophilia are complex, involving cytokines such as IL-5,² chemokines such as eotaxin,³ and the lipid mediators platelet-activating factor,⁴ 5-oxo-eicosatetraenoic acid,⁵ and leukotrienes (LTs).⁶ The interaction between these various factors is not yet fully understood but may be important.

LTs are derived from the 5-lipoxygenase pathway of arachidonic acid metabolism. After the production of LTA₄ by 5-lipoxygenase, acid metabolism proceeds through the action of LTA₄ hydrolase, which catalyzes the synthesis of LTB₄ or glutathione conjugation by LTC₄ synthase, initiating production of cysteinyl LTs (cysLTs). Both LTB₄ and the cysLTs are found within the lungs of patients with asthma⁷ and have been shown to induce smooth muscle contraction,⁸ enhance mucus production,⁹ increase microvascular leakage,¹⁰ and contribute to the recruitment of eosinophils to the airways.¹¹ Although the principle action of cysLTs is not considered chemotaxis, at least in vitro, there is recent evidence to suggest that cysLTs are involved in eosinophil recruitment. LTD₄ facilitated N-formyl-methionyl-leucyl-phenylalanine-induced eosinophil migration from the

From Meakins-Christie Laboratories and Montreal Chest Institute Research Center, McGill University, Montreal, Quebec.

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Reprint requests: Qutayba Hamid, MD, PhD, Meakins-Christie Laboratories, 3626 St Urbain St, Montreal, PQ, H2X 2P2, Canada.

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airway lamina propria to the lumen,¹² whereas inhaled LTE_4 was associated with an increased number of eosinophils within the asthmatic airway.¹³

Recently, a concerted effort has been made to establish the effectiveness of interrupting pathways of LT production and function. In particular, antagonists for the cysLT_1 receptor have been shown to be active in asthma, resulting in improvement in asthma symptoms¹⁴ and reduction in markers of inflammation.¹⁵ Furthermore, pretreatment of guinea pigs with the LT receptor antagonist pranlukast inhibited LTD_4 -induced lung eosinophilia.¹⁶

Cytokines, such as IL-3, IL-5, and GM-CSF, present within the lungs of atopic asthma individuals¹⁷ have been reported to enhance LT release from inflammatory cells after stimulation by the Ca^{2+} ionophore A23187¹⁸ and to increase LT biosynthesis in the eosinophil cell line HL-60.¹⁹ GM-CSF has also been shown to upregulate the principal enzymes responsible for cysLT synthesis in neutrophils and monocytes.²⁰ Moreover, administration of the anti-IL-5 antibody TRFK-5 before antigen challenge was associated with an inhibition in LTD_4 -induced lung eosinophilia.¹⁶ Although this work indicates that cytokines may regulate LT production, whether these lipid mediators can in turn act on cytokine expression is not yet known.

To study the interaction between cysLT and $\text{T}_{\text{H}2}$ cytokines within the airways, we have used the specific LTD_4 receptor antagonist montelukast (MK) and examined its effect on pulmonary resistance and markers of allergic inflammation in antigen-sensitized and antigen-challenged Brown Norway (BN) rats. We focused particularly on the presence and expression of eosinophils and IL-5 messenger (m)RNA within bronchoalveolar lavage (BAL) fluid and lung tissue. Immunocytochemistry was used to detect major basic protein (MBP), identifying the presence of eosinophils, in situ hybridization (ISH) was used to determine the number of cells expressing IL-5 mRNA, and simultaneous immunocytochemistry-ISH was used to phenotype these cells.

METHODS

Animals and sensitization

We studied BN rats weighing 200 to 300 g (Olac Bicester, Oxon, UK). The animals were actively sensitized by subcutaneous injection in the neck with 1 mg of ovalbumin (OVA; Sigma, St Louis, Mo) in 4 mg of aluminum hydroxide (BHD Chemicals, Poole, UK) and heat-killed *Bordetella pertussis* (kind gift from Dr T. Issekutz, Dalhousie University, Halifax, Nova Scotia) in 0.9% (wt/vol) saline suspension administered intraperitoneally (1×10^9 organisms) as an adjuvant.

Study design

Fourteen days after OVA sensitization, rats were anesthetized with 35 mg of sodium pentobarbital administered intraperitoneally (Abbott Laboratories, Chicago, Ill) and administered either MK (3 mg/kg) or saline intravenously 30 minutes before OVA challenge. Animals were then exposed to an aerosol spray of 5 g/100 mL solution of OVA for 5 minutes, and pulmonary responses were measured every 15 minutes from 5 minutes to 8 hours after the challenge. Blind orotracheal intubation was performed by using a 6-cm

length of polyethylene tubing (PE240; Clay Adams, Parsippany, NJ). The tip of the tracheal tube was inserted into a small Plexiglas box (265 mL in volume). Pulmonary responses were evaluated from lung resistance (R_L), which was obtained from flow, transpulmonary pressure, and the integrated flow signal by fitting the equation of the lung. A Hudson nebulizer was connected to a side port of the box and driven by compressed air to produce an output-generated aerosol spray of OVA with an output of 0.15 mL/min.

Bronchoalveolar lavage

BAL fluid was obtained 8 hours after antigen provocation. Animals were killed by using an intraperitoneal sodium pentobarbital overdose. The lavage was performed in situ by using a tracheal tube to instill and subsequently gently withdraw 5 mL of saline heated to 37°C. This procedure was repeated 5 times for a total of 25 mL of instilled saline solution. Recovery averaged about 80% of the volume instilled. Total cell counts were determined by using a hemacytometer, and cytospin preparations were prepared for differential cell counts performed by counting 300 cells. Granulocytes and mononuclear cells were enumerated by using Giemsa staining. Cytospin slides were prepared by cytocentrifuging (Shandon, Pittsburgh, Pa) the BAL fluid onto poly-L-lysine-coated (Sigma Chemical Co) glass slides. For in situ hybridization, cytospin preparations were fixed in 4% paraformaldehyde for 30 minutes and washed for 5 minutes in PBS; for immunocytochemistry, slides were fixed in an acetone/methanol (60:40) solution for 10 minutes. Cytospin preparations were stored at either -80°C or -20°C , respectively.

Tissue preparation

Lungs were obtained aseptically, as previously described.²¹ Briefly, animals were placed in a laminar flow hood, washed with 70% ethanol, and exsanguinated by sectioning the inferior vena cava. The anterior chest wall was removed, the heart and lungs were excised, and the right ventricle was cannulated. To remove excess blood, the pulmonary circulation was perfused by means of the right ventricle with 20 mL of sterile Ca^{2+} - and Mg^{2+} -free HBSS. The excised heart and lungs were then fixed in 4% paraformaldehyde for 4 hours and washed 3 times in 15% sucrose-PBS solution 1 hour each for 2 washes and overnight for the third wash. The lungs were cut in cross-section and blocked by using optimal cutting temperature medium by placing the cross-sections in isopentane cooled by liquid nitrogen. Blocks were stored at -80°C until use.

Immunocytochemistry

Cytospin preparations and cryostat sections of lung tissue were immunostained by using the alkaline phosphatase-antialkaline phosphatase method.²² To detect eosinophils, we used a murine monoclonal IgG1 antibody against the eosinophilic granule MBP (BMK-13, a kind gift from Dr R. Moqbel, University of Alberta). The use of this antibody to identify rat eosinophils has been previously established.²³

In situ hybridization

To detect cytokine mRNA in cytospin preparations of BAL fluid and within lung tissue, radiolabeled complementary riboprobes (antisense) were used. The complementary (c)DNA sequences for rat IL-5 were inserted into a pGEM vector, grown in *Escherichia coli*, and linearized with the appropriate restriction enzymes. Before ISH, in vitro transcription was performed in the presence of ^{35}S -uridine triphosphate and the appropriate T7 or Sp6 polymerases to generate sense and antisense probes.²⁴ As previously described,²⁴ cytospin preparations and tissue sections were permeabilized with 1 $\mu\text{g/mL}$ proteinase K and fixed in 4% paraformaldehyde. Prehybridization was performed in 50% formamide, and

slides were hybridized with radiolabeled RNA probes complementary to IL-5 mRNA (10^6 cpm/section) overnight in a humid chamber at 42°C . Nonspecific binding was reduced by high-stringency posthybridization washes and elimination of excess probe with a $20\text{ }\mu\text{g/mL}$ RNase A solution. Slides were dipped in Amersham LM-2 emulsion and stored at 4°C for a period of 12 days. The autoradiographs were developed in Kodak D-19 developer and counterstained with hematoxylin, and positive signal was identified as a collection of silver grains overlying the cells. Negative control experiments with sense probes and RNase pretreatment of sections before antisense probe application were performed to confirm probe specificity.

Simultaneous ISH-immunocytochemistry

To identify the phenotype of IL-5 mRNA⁺ cells, colocalization studies were performed on BAL fluid and lung tissue preparations by using a simultaneous in situ hybridization-immunocytochemistry.²⁵ All solutions used were made up in 0.1% diethyl pyrocarbonate-treated double-distilled water to avoid RNase contamination. The specimens were first immunostained by using the alkaline phosphatase-antialkaline phosphatase method, as described above, with mAbs directed against the T-cell receptor (anti-rat CD3; Pharmingen, San Diego, Calif) and MBP. Specimens were then processed for in situ hybridization by using radiolabeled antisense riboprobes specific for IL-5 mRNA.

Data analysis

The magnitude of the early response (ER) was calculated from the highest value of R_L within 30 minutes after challenge. Late responses (LRs) were quantified as the area under the R_L versus time curve from 180 to 480 minutes after OVA challenge of MK- or saline-treated animals. The numbers of cells expressing IL-5 mRNA or MBP immunoreactivity within the subepithelium were obtained in medium-sized airways (basement membrane perimeter = 1.0 to 1.99 mm). Data for airways were counted per 1 mm length of basement membrane. The number of cells expressing IL-5 mRNA or MBP immunoreactivity in cytospin preparations was counted per 1000 total cells. The differences in ER, LR, number of eosinophils, and IL-5 mRNA⁺ cells after OVA challenge of animals that received the MK or saline treatment were analyzed by using the Mann-Whitney *U* test or unpaired *t* test, as appropriate. To avoid observer bias, the slides were coded before analysis and read in a blinded fashion. Statistical significance was accepted as a *P* value of less than .05. Data are expressed as means $\pm$ SEM throughout.

RESULTS

Baseline values of lung resistance (R_L) before OVA challenge were similar in both the saline- and MK-treated groups ($P > .05$). All animals treated with saline and 6 of 7 treated with MK responded with an ER to antigen; there was no difference in the magnitude of the ER as a percentage of the baseline R_L in saline ($145.23\% \pm 24.49\%$) compared with that of MK-treated animals ($147.25\% \pm 25.17\%$, $P > .05$; Fig 1, A). Furthermore, all animals treated with saline and 6 of 7 of those treated with MK also exhibited an LR to antigen. However, animals that received MK treatment had significantly lower area under the curve values ($4.04 \pm 2.18\text{ cm H}_2\text{O}/[\text{mL}\cdot\text{sec}]$) than those treated with saline ($9.83 \pm 4.02\text{ cm H}_2\text{O}/[\text{mL}\cdot\text{sec}\cdot\text{min}]$, $P < .05$; Fig 1, B), indicating that treatment with MK decreased the bronchoconstriction after OVA challenge.

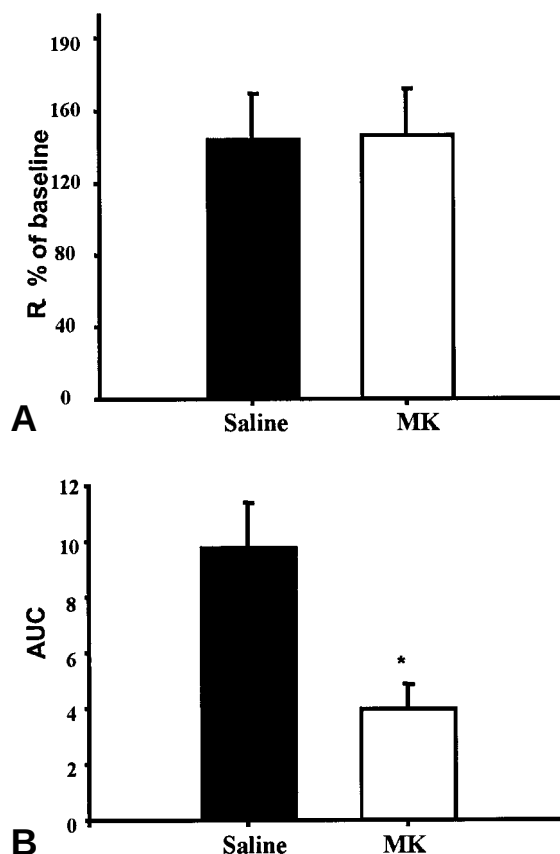


FIG 1. The effect of MK on the ER and LR of sensitized BN rats after OVA challenge. The ER, determined by the percentage of baseline R_L , was similar in saline- compared with MK-treated animals (A; $P > .05$). However, LRs in animals treated with MK, assessed by the area under the curve (AUC) of pulmonary resistance versus time ($\text{cm H}_2\text{O}/[\text{mL}\cdot\text{sec}\cdot\text{min}]$), were significantly lower than those in animals treated with saline (B; $P < .01$).

Cellular composition of the BAL can be seen in Table I. The differences in total cells, alveolar macrophages, and polymorphonuclear leukocytes in MK- compared with saline-treated animals were not significant ($P > .05$). There were, however, fewer mononuclear cells within BAL fluid from animals that received MK treatment ($0.85 \times 10^5 \pm 0.21$) than from those that received saline treatment ($1.73 \times 10^5 \pm 0.30$, $P < .05$). To assess the effects of drug treatment on eosinophils, BAL fluid and lung tissue were immunostained with an mAb against MBP. This method is superior to conventional staining, which is prone to underestimate eosinophil numbers after allergen challenge.²⁶ As determined by immunostaining, there were significantly fewer eosinophils within both BAL fluid (23.83 ± 7.24 ; Fig 2, A and B, and Fig 3, A and B) and lung tissue (11.50 ± 1.73 ; Fig 3, B) obtained from animals treated with MK than were found in those treated with saline (59.83 ± 4.7 and 20.50 ± 3.28 , respectively; $P < .05$).

All BAL fluid and lung tissue specimens showed positive hybridization signal for IL-5 mRNA. BAL from MK-treated animals exhibited significantly fewer IL-5 mRNA⁺ cells (23.25 ± 6.02 cells/1000 cells counted)

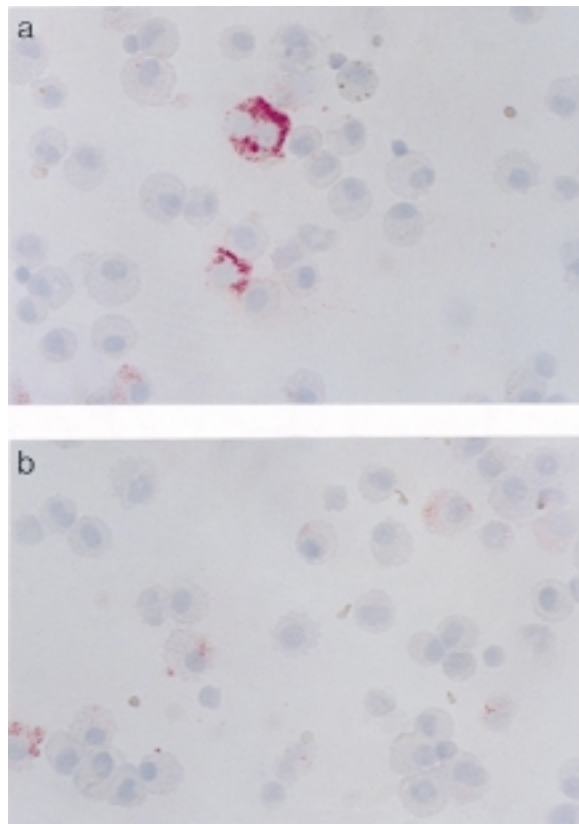


FIG 2. Representative examples of MBP immunoreactivity (eosinophils) within BAL fluid from sensitized BN rats after OVA challenge. Note the prominent red staining of the eosinophil within BAL fluid from saline-treated animals (**a**) and the lesser staining within BAL fluid from MK-treated animals (**b**).

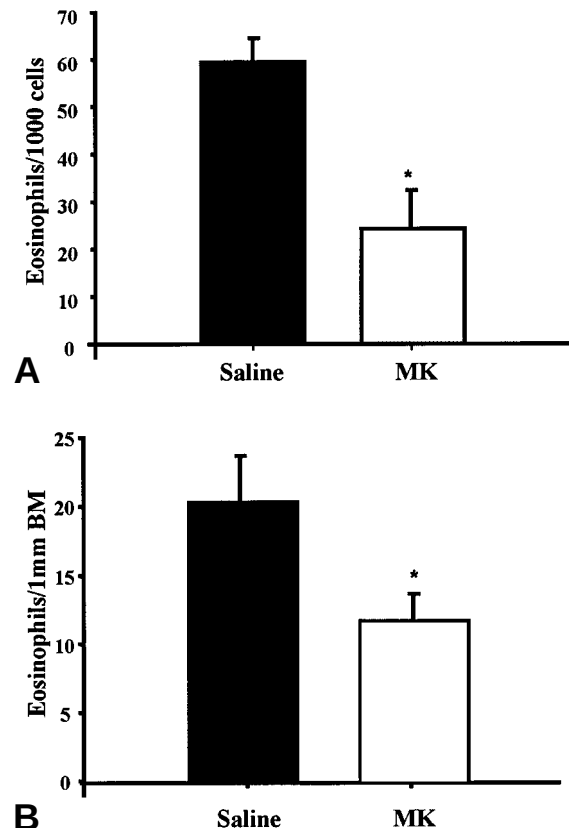


FIG 3. Effect of MK on the influx of eosinophils to the BAL fluid and lung tissue of BN rats after OVA challenge. Fewer eosinophils were observed within both BAL fluid (**A**) and lung tissue (**B**) of animals treated with MK compared with those treated with saline ($P < .05$).

TABLE I. Effect of MK on differential cell counts within BAL fluid at 8 hours after aerosolized antigen challenge

	Total cells ($\times 10^5$)	PAM ($\times 10^5$)	PMN ($\times 10^5$)	MNC ($\times 10^5$)
MK-treated animals (n = 7)	15.7 $\pm$ 0.21	12.87 $\pm$ 1.63	1.90 $\pm$ 0.44	0.85 $\pm$ 0.21*
Saline-treated animals (n = 7)	21.4 $\pm$ 0.28	16.9 $\pm$ 2.4	1.81 $\pm$ 0.43	1.73 $\pm$ 0.30

PAM, Pulmonary alveolar macrophages; MNC, mononuclear cells; and PMN, polymorphonuclear leukocytes.

* $P < .05$; data expressed as means $\pm$ SEM.

than BAL from saline-treated animals (51.50 ± 5.9 , $P < .05$; Fig 4, A-C, and Fig 5, A). In a similar fashion lung tissue obtained from animals treated with MK had fewer inflammatory cells expressing IL-5 mRNA (6.50 ± 1.06) than that found in tissue from animals receiving saline treatment (13.83 ± 2.2 , $P < .05$; Fig 5, B).

To establish whether the observed differences in IL-5-expressing cells reflected an alteration in the number of lymphocytes or eosinophils expressing IL-5 mRNA, we performed simultaneous immunocytochemistry and in situ hybridization to phenotype the cells. Within BAL fluid we observed that MK-treated animals had significantly fewer IL-5 mRNA⁺/CD3⁺ (14.2 ± 2.7) and IL-5 mRNA⁺/MBP⁺ cells (9.0 ± 3.6) per 1000 BAL cells compared with that found in saline-treated animals (34.3 ± 4.2 , 21.2 ± 1.9 ; $P < .01$; Table II). Furthermore, the

majority of IL-5 mRNA⁺ cells within BAL of both MK- and saline-treated animals were either CD3 ($61.7\% \pm 11\%$ and $72.8\% \pm 5.6\%$, respectively) or MBP ($35.3\% \pm 7.5\%$ and $35.8\% \pm 2.7\%$, respectively; Fig 6).

DISCUSSION

This study provides in vivo evidence for the effectiveness of the LTD₄ receptor antagonist MK on eosinophil recruitment and expression of IL-5 within the lungs of antigen-sensitized and antigen-challenged BN rats. We demonstrate that MK treatment was associated with a diminished LR to antigen, fewer eosinophils, and a decrease in the number of inflammatory cells expressing IL-5 mRNA within both lung tissue and BAL fluid. Furthermore, our results indicate that the MK-induced

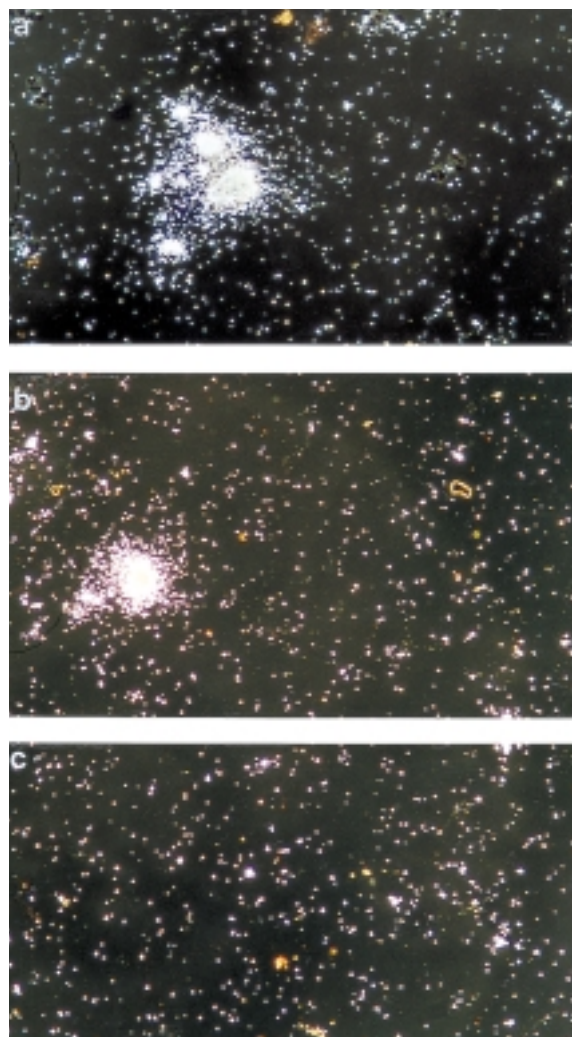


FIG 4. Photomicrograph of in situ hybridization under dark field illumination of IL-5 mRNA⁺ cells by using radiolabeled antisense probes to IL-5 mRNA. A substantial number of IL-5 mRNA⁺ cells were observed within BAL fluid from saline-treated animals (**A**). However, BAL fluid from animals treated with MK exhibited significantly fewer IL-5 mRNA⁺ cells (**B**). As a negative control, sense probes were used, indicating that the observed signal is specific (**C**).

reduction in the number of cells expressing IL-5 mRNA was attributable to both T cells and eosinophils.

MK treatment had no effect on pulmonary resistance during the early airway response to antigen. Although the mast cell is a major source of LTs and thought to determine the magnitude of the ER,²⁷ the contribution of the cysLT to the ER appears to be variable, depending on the animal studied and the conditions of the experiment. Sapienza et al²⁸ have noted a significant attenuation of the ER by MK-571, an earlier generation of cysLT₁ receptor antagonist than montelukast. However, Nagase et al,²⁹ also using BN rats, have suggested that serotonin may be a more important mediator of the ER than LTD₄. Biogenic amines are also important in primates; observing an inhibitory effect of the selective LT receptor antagonist SK&F 104353 on antigen-induced bron-

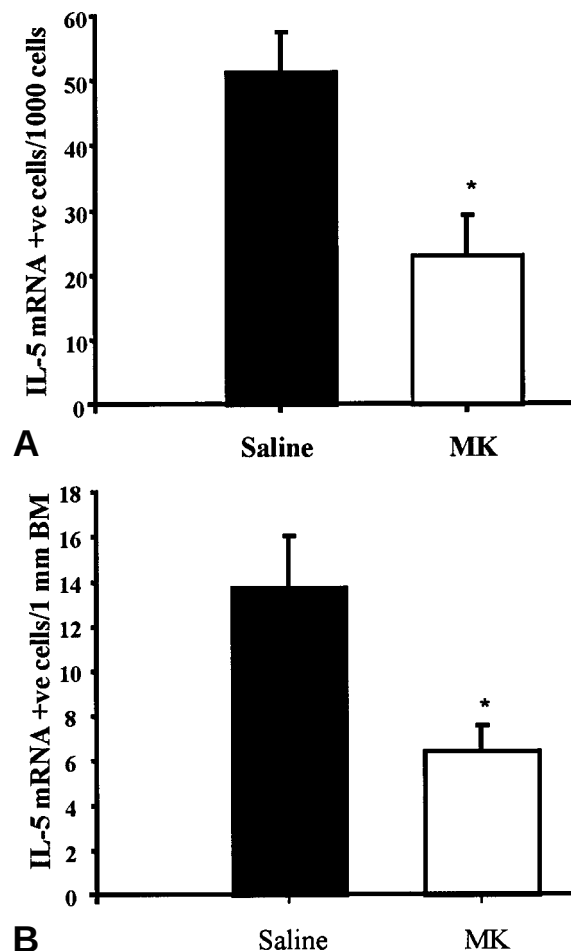


FIG 5. Effect of MK on expression of IL-5 mRNA within the BAL fluid and lung tissue of BN rats after OVA challenge. The number of IL-5 mRNA⁺ cells were significantly less within BAL fluid (**A**) and lung tissue (**B**) of animals treated with MK compared with BAL fluid and lung tissue of animals treated with saline ($P < .01$).

TABLE II. Number of IL-5 mRNA⁺ cells within BAL fluid (per 1000 cells) that coexpress CD3 and MBP

	IL-5 ⁺ /CD3 ⁺	IL-5 ⁺ /MBP ⁺
MK-treated animals (n = 7)	14.2 ± 2.7*	9.0 ± 3.6*
Saline-treated animals (n = 7)	34.3 ± 4.2	21.2 ± 1.9

* $P < .01$; data expressed as means ± SEM.

choconstriction during the ER in cynomolgus monkeys was conditional on pretreating animals with an H1-antagonist.³⁰ Presumably, in the current experiments, mast cell-derived mediators, such as histamine and serotonin, played a more prominent role than cysLTs in the ER, accounting for our finding that the LT receptor antagonist MK could not attenuate this response.

In contrast to the lack of efficacy in inhibiting the ER, animals that received MK treatment experienced a significantly lower LR to antigen, findings consistent with studies of various cysLT₁ antagonists in both animals (sheep, monkeys, and rats) and human subjects.³¹

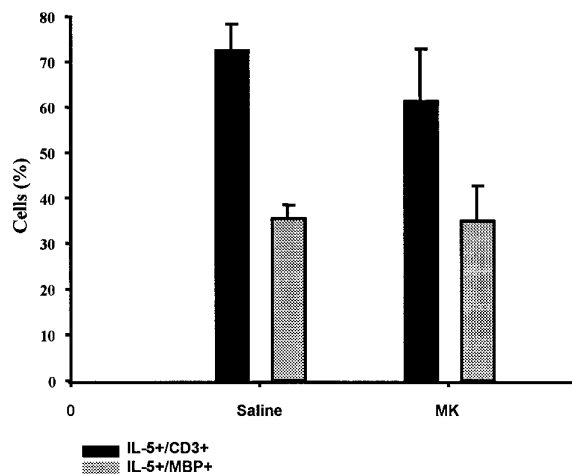


FIG 6. The percentage of IL-5 mRNA⁺ cells that are T cells or eosinophils. Simultaneous immunocytochemistry and in situ hybridization colocalization studies demonstrated that the percentage of IL-5 mRNA⁺ cells coexpressing CD3 or MBP did not differ within BAL from BN rats treated with MK compared with those treated with saline ($P > .05$).

CysLTs are excreted in increased amounts in association with the LR in rats and frequently show a biphasic pattern of excretion, suggesting either the recruitment or activation of cysLT-synthesizing cells at this time point after antigen challenge. The precise cellular source of cysLTs during the LR is as yet unknown. Eosinophils are a potential source in human subjects³² but not in rodents, in which macrophages, and not eosinophils, are the predominant cysLT-synthesizing cells in the lungs.³³

MK treatment was associated with a 60% reduction in the number of eosinophils in BAL fluid and a 43% reduction within the lungs compared with the numbers found in saline-treated animals. These findings are consistent with previous studies associating LTD₄ and LTE₄ expression with lung eosinophilia.^{10,17} Infiltration of eosinophils into the lung has been largely attributed to eotaxin.³⁴ Although recent in vitro studies have suggested that cysLTs induce the expression of this chemokine by endothelial cells,³⁵ a number of mediators have been shown to stimulate eotaxin production.³⁶⁻³⁸ The findings of the present study, that MK did not completely inhibit the number of eosinophils infiltrating the lung, are in accord with this and indicate the importance of other cysLT-independent mechanisms of eosinophil recruitment.

Pretreatment of antigen-challenged BN rats with MK was associated with significantly fewer IL-5 mRNA⁺ cells within both BAL fluid and lung tissue (reductions of 55% and 53%, respectively) compared with numbers found in saline-treated animals. It is possible that a more dramatic effect may be observed with longer pretreatment periods. However, the current data demonstrate that MK does cause significant attenuation with only a 30-minute pretreatment and implies that LTD₄, either directly or indirectly, can regulate the expression of IL-5. Although studies have suggested the effect of cytokines on cysLT expression,^{18,19} to our knowledge this is the

first report to discuss whether these mediators may modulate cytokine synthesis. Previously, Sedgwick et al³⁹ correlated the presence of eosinophils with the concentration of both IL-5 and LTC₄ within BAL fluid after segmental antigen challenge. They suggested that IL-5 induced eosinophil release of LTC₄. However, because cysLTs are reported to act on isolated eosinophils,⁴⁰ it is conceivable that they may affect expression of IL-5. Indeed, our results demonstrating that MK treatment significantly reduced the number of IL-5 mRNA⁺ cells coexpressing MBP support this.

IL-5 is highly important for eosinophil infiltration⁴¹; however, migration of these cells to the target organ is a complex process involving not only cytokine and chemokine expression but also a number of adhesion molecules and other mediators that are not yet fully determined. Nevertheless, we do know that eosinophils express very late antigen 4 and use the endothelial expression of its counter ligand, vascular cell adhesion molecule 1, to pass from the blood into the tissue.⁴² IL-4 and IL-13 have been shown to increase the endothelial expression of vascular cell adhesion molecule 1^{43,44}; however, the effects of cysLTs on this molecule are not known. T cells are an important source of IL-4 and IL-13 in the lung.^{45,46} In the current study we did not examine the expression of these cytokines; however, we did observe that MK was associated with fewer IL-5 mRNA⁺ cells coexpressing CD3. This may be due to abrogation of cytokine synthesis, and as such similar changes may occur in the expression of IL-4 and IL-13. Nevertheless, we cannot exclude the possibility that MK may interfere with T-cell recruitment, and we are presently investigating this issue in explanted rat lung tissue. Furthermore, although T cells are reportedly activated by LTB₄,⁴⁷ it is not known whether they express receptors for cysLT and therefore whether these lipid mediators affect T cells in a direct manner.

Here we show that pretreatment with MK is associated with improved airway caliber and a reduction in the number of eosinophils and inflammatory cells expressing IL-5 mRNA. Recently, there has been much interest in the relative effectiveness of LT receptor antagonists versus the gold standard of asthma treatment, inhaled corticosteroids. The observed effect of MK pretreatment on airway responsiveness is similar to our previous work with dexamethasone pretreatment.⁴⁸ Furthermore, our data indicate that MK may inhibit the synthesis of mRNA coding for IL-5, as steroids have been previously demonstrated to do.⁴⁹

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