

IL-4 and IgE-anti-IgE modulation of 15(S)-hydroxyeicosatetraenoic acid release by mononuclear phagocytes

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Background: IL-4 modulates the synthesis of IgE, the expression of CD23, and the release of 15(S)-hydroxyeicosatetraenoic (15[S]-HETE).

Objective: We evaluated the release of 15(S)-HETE by IL-4-stimulated monocytes and verified whether the observed increase in 15(S)-HETE release after passive sensitization and anti-IgE challenge of IL-4-treated monocytes was secondary to an increased CD23 expression.

Methods: Human monocytes were incubated for 24, 48, and 72 hours with IL-4 (10 ng/mL) with or without an IgE-anti-IgE stimulation. We evaluated CD23 expression by immunocytochemistry and 15(S)-HETE release by HPLC and RIA. To prove that the increase in 15(S)-HETE release was due to the effect of IL-4 on CD23, we performed experiments with an anti-CD23 blocking mAb.

Results: CD23 expression and 15(S)-HETE release were significantly increased by IL-4, reaching a peak after 72 hours ($P < .02$). After passive sensitization with human IgE and anti-IgE challenge, IL-4-stimulated monocytes released higher amounts of 15(S)-HETE than IL-4-unstimulated monocytes ($P < .02$). Pretreatment with the anti-human B-cell CD23 MHM6 mAb caused a dose-dependent inhibition of 15(S)-HETE release.

Conclusions: This study shows that immunologic challenge of IL-4-treated, passively sensitized monocytes results in a CD23-dependent additional increase of 15(S)-HETE release, indicating the presence of a synergistic effect of IL-4 on CD23 expression and 15(S)-HETE production. (*J Allergy Clin Immunol* 1999;103:159-64.)

Key words: Monocytes, asthma, IL-4, IgE, 15(S)-Hydroxyeicosatetraenoic acid

Allergic asthma is essentially mediated by the triggering of IgE-bound mast cells by allergens, leading to the release of vasoactive mediators and to an ongoing inflammatory reaction.¹ Inflammatory cells can respond to allergen stimulation by expressing either the high-affinity or

Abbreviations used

NK:	Natural killer
AAM:	Arachidonic acid metabolite
15(S)-HETE:	15(S)-Hydroxyeicosatetraenoic acid
15-LO:	15 lipoxygenase

the low-affinity (CD23) receptor for IgE. CD23 is expressed by several cells, such as mast cells, eosinophils, and monocytes, and its expression, as well as the synthesis of IgE, can be modulated by inflammatory cytokines. Among these cytokines, IL-4 plays an important role in IgE production² and can increase the expression of CD23 on several inflammatory cells, including mononuclear phagocytes.³

Mononuclear phagocytes are involved in the inflammatory processes underlying asthma.⁴ Monocytes can be activated through an IgE-specific mechanism and can promote the bronchial inflammatory response by releasing numerous mediators, including arachidonic acid metabolites (AAMs).^{5,6} In addition to IgE stimulation, the production of AAMs by human mononuclear phagocytes can be stimulated by IL-4, which is able to induce 15-lipoxygenase (15-LO) activity.⁷ Although either IgE or IL-4 stimulation has been shown to increase AAM production, it is unclear whether the combined IL-4 and immunologic stimulation of monocytes may result in a further increase of the release of AAMs.

Because 15(S)-hydroxyeicosatetraenoic acid (15[S]-HETE) has been hypothesized to play an important role in the pathogenesis of asthma,⁸⁻¹² in this study we evaluated the release of this AAM by monocytes stimulated with IL-4. In addition, because IL-4 is able to modulate the expression of CD23 by monocytes, we examined whether the increased production of 15(S)-HETE after stimulation by IL-4 and anti-IgE was due to an increased expression of CD23.

METHODS

Reagents

Human recombinant IL-4 and polyclonal sheep anti-human IL-4 were obtained from Genzyme (Cambridge, England). Human IgE

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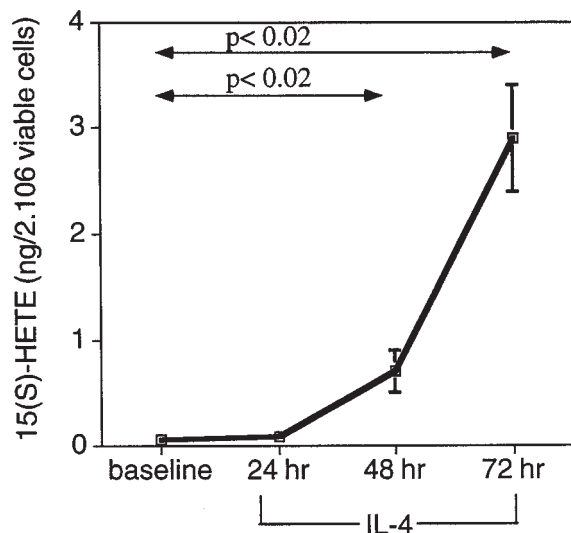


FIG 1. Effect of IL-4 stimulation (10 ng/mL) on release of 15(S)-HETE by normal human peripheral monocytes. Monocytes (2×10^6 cells/mL) were cultured in the presence of IL-4 at the concentration of 10 ng/mL for 24, 48, and 72 hours. Supernatants were harvested and frozen at -20°C for 15(S)-HETE measurement as described in the Methods section. Data are expressed as mean $\pm$ SD of 7 consecutive experiments. Statistical analysis was performed by using the Wilcoxon test.

were obtained from myeloma plasma and were characterized by the presence of a κ light chain. Anti-human-IgE, was an Fc fragment of a mouse mAb (murine isotype: IgG2a-k) highly specific for IgE (100% specificity). Both human IgE and anti-human IgE were from Calbiochem (La Jolla, Calif). All solvents were HPLC grade and were obtained from Merck (Darmstadt, Germany). Nutritive medium came from Whittaker (Verviers, Belgium), and FCS came from Hyclone (Logan, Utah). 15(S)-HETE RIA kit was purchased from Advanced Magnetics (Framingham). CD23 expression was evaluated by using a specific mAb (clone MHM6; Dako, Glostrup, Denmark). The same mAb was used to block CD23, as previously described.¹³

Purification and culture of monocytes

Mononuclear cells were isolated from 7 human buffy coats by density gradient centrifugation with Ficoll Hypaque cushions.⁷ The mononuclear cell band was removed; washed 3 times by centrifugation with PBS containing calcium chloride (CaCl_2) and magnesium chloride (MgCl_2) at final concentrations of 0.5 and 1 mmol/L, respectively; resuspended (20×10^6 cells/mL) in RPMI 1640, 10% heat-inactivated (56°C , 30 minutes) FCS, 1% penicillin-streptomycin solution, and 1 mmol/L L-glutamine (all from GIBCO, Grand Island, NY); and allowed to adhere to 150-mm polystyrene tissue culture dishes for 2 hours at 37°C . More than 96% of the cells stained positive for nonspecific esterase, and the viability assessed by Trypan blue exclusion was greater than 95%.

IL-4 stimulation of monocytes

After the removal of nonadherent cells, the adherent monocytes were harvested by gentle scraping, washed, and resuspended at the concentration of 2×10^6 cells/mL in RPMI 1640, 10% FCS, 1% penicillin-streptomycin solution, and 1 mmol/L L-glutamine. Monocytes were then incubated for 24, 48, and 72 hours with IL-4 at the concentration of 10 ng/mL, which in previous dose-response

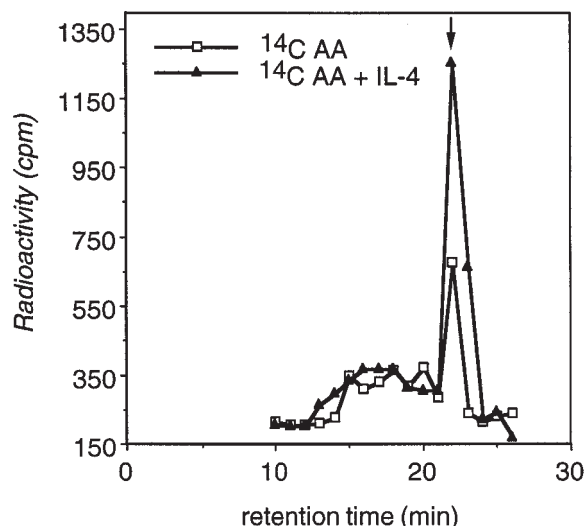


FIG 2. Monocytes cultured with and without IL-4 (10 ng/mL) for 72 hours were incubated with ^{14}C AA (10 $\mu\text{mol/L}$) for 15 minutes. One-minute fractions eluting from reverse-phase HPLC between the retention time of 10 and 25 minutes were collected, and radioactivity was evaluated by scintillation counting. Arrow indicates retention time of standard 15(S)-HETE.

experiments was found to be the optimal concentration. Adherent cells were recovered after a gentle scraping, washed twice, and resuspended at the final concentration of 2×10^6 cells/mL in PBS containing CaCl_2 and MgCl_2 at final concentrations of 0.5 and 1 mmol/L, respectively. In separate experiments the effect of IL-4 incubation on 15(S)-HETE production was assessed on incubation with [^{14}C] arachidonic acid (AA) (10 $\mu\text{mol/L}$) for 15 minutes at 37°C .

Evaluation of CD23 expression by monocytes

CD23 expression was evaluated by using the alkaline phosphatase-antialkaline phosphatase technique¹⁴ on monocytes stimulated for 24, 48, and 72 hours with IL-4. Slides were prepared by using a cytocentrifuge (Shandon Southern Products Ltd, Runcorn, Cheshire, UK), fixed with a dry ice-acetone, and stored at -20°C until analysis. An mAb anti-human B cell, CD23 MHM6 (Dako) diluted 1:50, was used as a primary antibody. Slides were analyzed blindly by 2 independent investigators, and the results were expressed as percentage of positive-stained cells.

To test the specificity of the effects observed, IL-4 stimulation was carried out in the presence of a polyclonal sheep anti-human IL-4 antibody (5 $\mu\text{g/mL}$ to 40 $\mu\text{g/mL}$) for 24, 48, and 72 hours.

IgE-anti-IgE activation of monocytes

Monocytes (2×10^6 cells/mL) were passively sensitized by human IgE (10 $\mu\text{g/mL}$) at 37°C for 60 minutes. Cells were pelleted, washed twice with prewarmed PBS for 5 minutes, and subsequently incubated with the anti-human IgE mAb at the concentration of 10 $\mu\text{g/mL}$ at 37°C for 30 minutes. IgE-anti-IgE activation of monocytes were carried out at baseline and at 24, 48, and 72 hours, either in the absence or presence of IL-4 stimulation.

Inhibition of CD23 activation

We sought to prove that the additional increase in 15(S)-HETE release observed on IL-4 pretreatment and immunologic challenge was connected to the IL-4-dependent increase in CD23 expression. In 6 different experiments, CD23 receptors were blocked by pre-

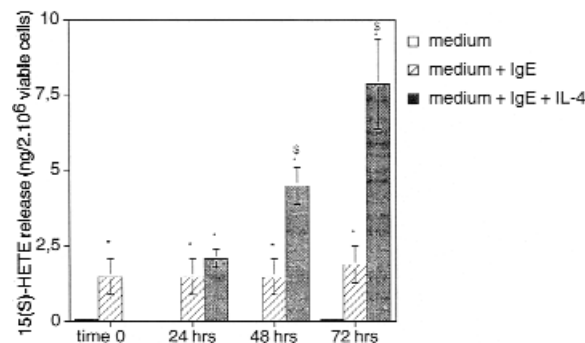


FIG 3. Effect of immunologic stimulation on 15(S)-HETE release. Untreated and IL-4-treated monocytes were passively sensitized with human IgE (10 μ g/mL) for 60 minutes and immunologically activated with anti-human IgE mAb at the concentration of 10 μ g/mL. 15(S)-HETE release is expressed as mean $\pm$ SD of 7 consecutive experiments. Statistical analysis was performed by Wilcoxon test. * P < .02 for statistical comparison between medium + IgE versus medium; \$ P < .02 for statistical comparison between medium + IgE + IL-4 versus medium + IgE.

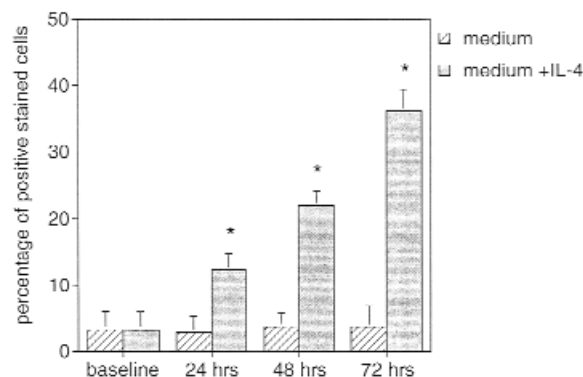


FIG 4. Modulation by IL-4 of CD23 expression on monocytes. CD23 expression was evaluated in monocytes incubated for 24, 48, and 72 hours in absence or presence of IL-4 by using alkaline phosphatase-antialkaline phosphatase technique. Results are expressed as mean $\pm$ SD of the percentage of positively staining cells. Statistical analysis was performed by Wilcoxon test. * P < .02.

TABLE I. 15(S)-HETE release by monocytes stimulated with (+) and without (–) passive sensitization and challenge with anti-IgE mAb after treatment with IL-4 (10 ng/mL) and efficacy of pretreatment (+) with a polyclonal anti-human IL-4 (20 μ g/mL)

Passive sensitization + IgE challenge	Anti-IL-4 (20 μ g/mL)	15(S)-HETE (ng/2 $\times$ 10 ⁶ viable cells)			
		Baseline	24 hours	48 hours	72 hours
–	–	0.1 $\pm$ 0.001	0.1 $\pm$ 0.001	0.7 $\pm$ 0.1	2.9 $\pm$ 0.2
–	+	0.1 $\pm$ 0.005	0.1 $\pm$ 0.3	0.3 $\pm$ 0.003	0.1 $\pm$ 0.007
+	–	1.5 $\pm$ 0.2	2.1 $\pm$ 0.1	4.5 $\pm$ 0.4	7.9 $\pm$ 0.5
+	+	1.4 $\pm$ 0.2	1.1 $\pm$ 0.1	1.4 $\pm$ 0.1	1.1 $\pm$ 0.1

treatment with an anti-CD23 mAb (clone MHM6, Dako), which was previously shown to inhibit IgE binding and passive sensitization.¹³ Unstimulated and IL-4-stimulated monocytes (2×10^6 cells/mL) were resuspended in PBS, CaCl₂, and MgCl₂ and incubated with the mAb anti-human B-cell CD23 MHM6 at different concentrations (5, 10, and 20 μ g/mL) for 60 minutes at 37°C. Monocytes were then washed twice with PBS, CaCl₂, and MgCl₂; passively sensitized with human IgE (10 μ g/mL) for 60 minutes; and subsequently challenged with the anti-human IgE mAb at the concentration of 10 μ g/mL at 37°C for 30 minutes.

Analysis of 15(S)-HETE release

The cell supernatants were harvested and, after the addition of prostaglandin B₂ as an internal standard, were stored at –80°C for further analysis of metabolites by means of HPLC.

15-LO products were analyzed by using a Beckman liquid chromatograph (System Gold) equipped with a guard cartridge (Water Millipore) to protect a Beckman Ultrasphere 5- μ m ODS column connected to a model 168 Beckman diode array UV detector. The column was developed at a flow rate of 1 mL/min by using a gradient from A to 20% B (acetonitrile/acetic acid, 100/0.1 vol/vol) for 0 to 2 minutes and then to 100% B over 18 minutes, with A represented by water/acetic acid (100/0.1 vol/vol). Retention time of 15(S)-HETE was controlled daily by using a radioactive standard, resulting in 21.7 $\pm$ 0.5 minutes. The fractions corresponding to the elution time of 15(S)-HETE were collected (LKB 2112 Redirac fraction collector), dried under N₂, redissolved in RIA buffer, and quantitated by a specific RIA performed

according to the manufacturer protocol (Advanced Magnetics). Results are expressed as nanograms of 15(S)-HETE per 2×10^6 cells.

Statistical analysis

Nonparametric tests were used. The Wilcoxon test was used for unpaired comparisons, and the Spearman rank test was used for correlations between data. Results were expressed as means $\pm$ SD.

RESULTS

Modulation of 15(S)-HETE release by IL-4-stimulated monocytes

15(S)-HETE release by monocytes was significantly increased by IL-4 (10 ng/mL) in a time-dependent fashion, reaching a peak after 72 hours of incubation (P < .02, Wilcoxon test) (Fig 1 and Table I). Reverse-phase HPLC analysis of supernatants from cells incubated with exogenous radiolabeled AA showed increasing amounts of radioactivity eluting at the retention time of 15(S)-HETE on IL-4 treatment (Fig 2), suggesting that the effect of IL-4 on 15(S)-HETE release was mediated by an increased expression of 15-LO, as previously reported.⁷ Pretreatment with a polyclonal anti-IL-4 antibody at the concentration of 20 μ g/mL (found to be the optimal concentration for all time points) resulted in a significant reduction of 15(S)-

TABLE II. Expression of CD23 by monocytes stimulated with IL-4 (10 ng/mL) or a polyclonal anti-human IL-4 (20 µg/mL)

Stimuli	CD23 percent of positive cells			
	Baseline	24 hours	48 hours	72 hours
Medium	1.3 ± 0.4	12.9 ± 0.7	22 ± 0.7	36 ± 1.1
Anti-IL-4	1.3 ± 0.6	1.3 ± 0.4	1.1 ± 0.3	4.4 ± 0.4

HETE release at 48 and 72 hours ($P < .02$, Wilcoxon test) (Table I).

Effect of IgE–anti-IgE stimulation on 15(S)-HETE release by IL-4–pretreated monocytes

In the absence of IL-4, the stimulation of monocytes by IgE–anti-IgE induced a modest but significant increase in 15(S)-HETE release ($P < .02$, Wilcoxon test). On the other hand, passive sensitization and anti-IgE stimulation of monocytes incubated with IL-4 (10 ng/mL for 24, 48, and 72 hours) induced a more pronounced time-dependent increase of 15(S)-HETE release when compared with IL-4–unstimulated monocytes ($P < .02$, Wilcoxon test) (Fig 3). The release of 15(S)-HETE could not be accounted for by simple addition of that observed on IL-4 or immunologic stimulation alone. Coincubation with a polyclonal anti-human IL-4 antibody (20 µg/mL) abolished the effect of IL-4, and the 15(S)-HETE production returned to values observed on challenge of untreated cells ($P < .02$, Wilcoxon test) (Table I).

Modulation of CD23 expression on monocytes by IL-4

Because passive sensitization and anti-IgE stimulation was found to further increase the release of 15(S)-HETE by human monocytes, we examined whether this effect was mediated by the increase of CD23 expression. A limited number of cells expressing CD23 in the absence of IL-4 treatment resulted, and IL-4 (10 ng/mL) caused a time-dependent, significant increase of the percentage of CD23-positive cells, with a maximal expression observed after 72 hours ($P < .02$, Wilcoxon test). Specificity of IL-4 effect was tested by using a polyclonal anti-human IL-4 antibody that significantly reduced the percentage of CD23-positive cells at all time points evaluated (Figs 4 and 5 and Table II).

Inhibition of CD23 activation

The treatment of unstimulated and IL-4–stimulated monocytes with the mAb MHM6 anti-CD23 at different concentrations (5, 10, and 20 µg/mL) did not induce the release of 15(S)-HETE per se. The addition of the anti-CD23 mAb MHM6 to unstimulated and IL-4–stimulated monocytes before passive sensitization and anti-IgE challenge resulted in a significant concentration-dependent inhibition of 15(S)-HETE release (Table III).

DISCUSSION

The results of this study show that IL-4 is able to increase the expression of CD23 and the release of 15(S)-HETE by human monocytes in a time-dependent fashion. In addition, this study demonstrates that the IL-4–induced expression of CD23 represents an additional mechanism, resulting in a significant increase in the production of 15(S)-HETE by passively sensitized and immunologically challenged monocytes.

IL-4 is a cytokine released by T_H2 cells and mast cells,¹⁵ which plays a major role in the pathogenesis of allergic asthma^{16,17} and possibly also of nonallergic asthma.¹⁸ Besides its IgE-regulating effects,^{2,19} IL-4 is responsible for a complex cascade of biologic events. In normal subjects IL-4 has been found to be an inhibitor of the mononuclear phagocyte functions in vitro and ex vivo.^{20,22} This inhibition has been found to be effective at a transcriptional level²² and is not due to a cytotoxic effect because in this study the cell viability remained unchanged. Although these effects suggest a downregulatory activity of IL-4, this cytokine is also involved in a variety of immunologic reactions, which are potentially able to increase the inflammatory processes. In this respect IL-4 stimulates the maturation of blood monocytes²³ and increases the expression of cell membrane markers, such as CD23 and class II MHC antigen, on monocytes.²⁴

The results of this study indicate that in addition to the reported effect on 15-LO expression,⁷ IL-4 directly modulates the AA metabolism by increasing the release of 15(S)-HETE by peripheral human monocytes. Interestingly, this effect is synergistically potentiated by immunologic stimulation and is dependent on the increase of CD23 expression induced by IL-4 itself. The effects of IL-4 on the production of 15(S)-HETE by human monocytes are highly specific because the incubation of the cells with a specific polyclonal antibody anti-IL-4 blocks the release of the mediator. Because IL-4 plays a crucial role in airways inflammation in asthma,^{16,17} the evidence provided in this study can therefore indicate a possible biologic mechanism responsible for the activation of monocytes in this disease.

The increased release of 15(S)-HETE by monocytes stimulated with IL-4 and anti-IgE may be mediated by several mechanisms. Because 15-LO enzyme is specifically induced by IL-4 in monocytes,^{7,25} it is likely that an increased 15-LO expression may contribute to the results of this study, as suggested also by the data obtained with exogenous radiolabeled AA. However, it is interesting to note that the increased release of 15(S)-HETE by immunologically activated monocytes clearly correlated with the IL-4–induced increase in the expression of the low-affinity IgE receptor CD23. This hypothesis is further supported by previous evidence showing that CD23 can function as an important pathway for cellular activation,²⁶ as well as by the results of this study demonstrating a significant concentration-dependent inhibition of 15(S)-HETE release caused by the addition of the anti-CD23 mAb MHM6 to unstimulated and IL-4–stimulated monocytes before passive sensitization

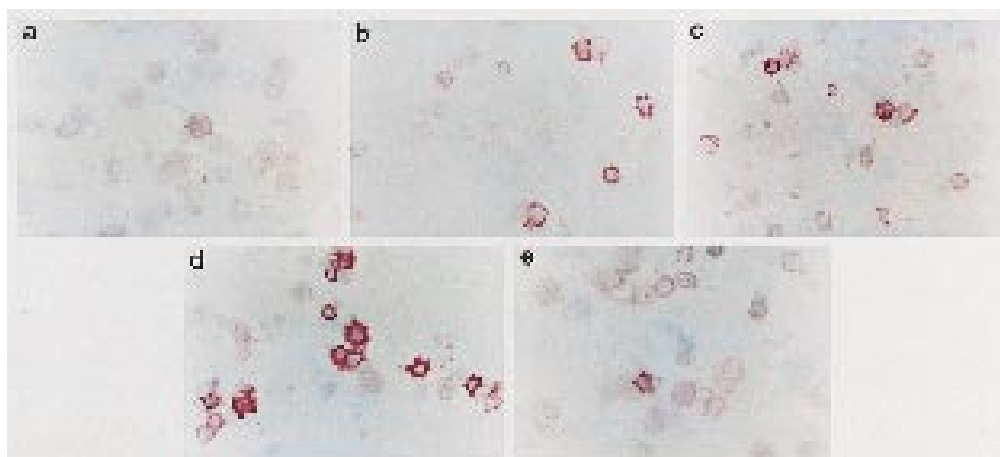


FIG 5. Expression of CD23 receptor in unstimulated monocytes (a) and monocytes stimulated with IL-4 (10 ng/mL) for 24 hrs (b), 48 hours (c), and 72 hours (d). Specificity of IL-4 effect was evaluated by incubating monocytes stimulated with IL-4 for 72 hours with the polyclonal anti-human IL-4 (20 µg/mL) (e).

TABLE III. Inhibition of 15(S)-HETE release with different concentrations of the mAb MHM6 anti-CD23

	15(S)-HETE release*			
	Baseline		IL-4	
	ng/2 × 10 ⁶ cells	Percent inhibition	ng/2 × 10 ⁶ cells	Percent inhibition
Monocytes	0.05 ± 0.01	—	0.2 ± 0.03	—
Monocytes + IgE-anti-IgE	1.9 ± 0.1	—	9.4 ± 2	—
MHM6 (5 µg/mL)	1.6 ± 0.1	5.6	4.5 ± 0.1	47.8
MHM6 (10 µg/mL)	1.4 ± 0.1	16.7	3 ± 0.05	65.2
MHM6 (20 µg/mL)	0.9 ± 0.1	44	1.6 ± 0.1	79.3

*Results are expressed as nanograms per 2 × 10⁶ viable cells.

and anti-IgE challenge. Thus although recent observations show that human monocytes can also express the FcεRI receptor,²⁷ the significant inhibition of 15(S)-HETE release by the anti-CD23 mAb MHM6, particularly after IL-4 treatment, suggests that CD23 plays a major role in the modulated 15(S)-HETE release by monocytes. This is also supported by the evidence that monocytes isolated from healthy subjects express very low levels of FcεRI receptor.²⁷

Several studies have shown that the production of 15(S)-HETE is increased in asthma. First, it has been demonstrated that 15(S)-HETE is the major AA metabolite produced in the lung homogenates,²⁸ as well as in cultured human lung tissue obtained from both asthmatic and normal donors. Second, it has been shown that when lung tissue obtained from asthmatic subjects is subjected to allergen challenge in vitro, 15(S)-HETE production is approximately 100 times greater than that of leukotriene C₄.²⁹ Third, bronchial allergen challenge in atopic asthmatic subjects has been found to determine a 30-fold increase of the concentrations of 15(S)-HETE recovered in bronchoalveolar lavage fluid.¹² Once released, 15(S)-HETE can exert several immunoregulatory functions that may be relevant in the pathogenesis of asthma. 15(S)-HETE has been shown to be a potent mucosecretagogue in the human airway³⁰ and to possess chemotactic activity for neutrophils directly contributing to the recruitment of these

cells in the airways.³¹ It has also been found that 15(S)-HETE can prolong the duration of airway obstruction during the early response, suggesting that it either augments the release of mediators from mast cells or potentiates the effects of other mediators on airway smooth muscle.¹¹ On the other hand, it has been reported that 15(S)-HETE inhibits 5-lipoxygenase,^{32,33} and incorporation of 15(S)-HETE into membrane phospholipids impairs the response of human polymorphonuclear leukocytes to inflammatory stimuli, such as the formylated tripeptide N-formyl-methionyl-leucyl-phenyl-alanine.³⁴ Thus although it is always difficult to relate in vitro evidence to in vivo pathologic situations, the ability of IL-4 to increase the release of 15(S)-HETE by human monocytes either spontaneously or after immunologic stimulation may play an important role in the evolution of the inflammatory response underlying the pathogenesis of asthma.

In conclusion, this study shows that IL-4 is able to directly increase the production of mediators involved in the inflammatory response, such as 15(S)-HETE, in isolated human monocytes, as well as to induce a significant expression of CD23. Immunologic challenge of IL-4-treated, passively sensitized monocytes results in a CD23-dependent additional increase of 15(S)-HETE release, indicating the presence of a synergistic cross-reaction between the effect of IL-4 on CD23 expression and 15(S)-HETE production.

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