**Interleukin-5** (IL-5) has been shown to be a selective eosinophil growth and differentiation factor. In the present study, the effect of recombinant human IL-5 on human eosinophil sulfidopeptide leukotriene production was investigated. IL-5 did not affect leukotriene synthesis in unstimulated eosinophils. However, IL-5 potentiated leukotriene synthesis by eosinophils stimulated with serum treated zymosan (STZ) or the calcium ionophore A23187 by 69% and 135%, respectively. The priming effect of IL-5 was dose dependent, with significant stimulation occurring at 1,000 U/ml for STZ and 100-1,000 U/ml for A23187. Pre-incubation with IL-5 did not increase leukotriene synthesis further.

**Key words**: Eosinophil, Interleukin-5, Leukotriene

---

**Introduction**

Human eosinophils play an important role in the pathogenesis of bronchial asthma. Peripheral blood and sputum eosinophils often accompany asthma, a large number of eosinophils infiltrate the airways during the late asthmatic reaction and mediators released by these cells can affect airway function. Bronchospasmogenic substances such as sulfidopeptide leukotrienes (LT) and platelet-activating factor (PAF), as well as other eosinophil derived mediators appear to play a role in the development of airway hyperreactivity, a main characteristic of bronchial asthma. Eosinophil cytotoxic cationic proteins can damage airway epithelial cells, which may cause airway hyperreactivity. Furthermore, inhaled sulfidopeptide leukotrienes and PAF are able to induce airway hyperreactivity in laboratory animals and humans.

It has been demonstrated that eosinophils from asthmatic patients are hypodense and produce more leukotriene C4 and reactive oxygen metabolites than those from healthy persons. The mechanism causing the eosinophilia and the primed state of eosinophils in asthmatics is unknown. It has been suggested that T-lymphocyte derived cytokines may play an important role in this phenomenon. T-lymphocytes are activated in acute asthma and infiltrate the airways after allergen provocation, thus creating an ideal environment for eosinophil proliferation and activation.

Several cytokines have been shown to induce eosinophil proliferation, chemotaxis, activation and/or priming. Granulocyte macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor (TNF), interleukin-3 (IL-3) and IL-5 have all been shown to regulate one or more eosinophil functions. Of these cytokines, only IL-5 seems to be a selective activator of eosinophils, whereas other cytokines also influence neutrophils. It has been shown that IL-5 induces eosinophil proliferation, chemiluminescence, release of cytotoxic cationic proteins, chemotaxis and cytotoxicity, and enhances adhesion to endothelial cells. In the present study, the influence of IL-5 on serum-treated zymosan (STZ) and calcium ionophore (A23187) induced sulfidopeptide leukotriene production by human eosinophils was investigated.

**Materials and Methods**

Eosinophils were isolated from blood of normal human volunteers (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam and Bloodbank, Utrecht, the Netherlands) as described before in detail. The purity of the eosinophils was 95 ± 3% and the viability was always more than 95% as determined by trypan blue...
exclusion. For the production of leukotriene, 200 μl of eosinophils (3 × 10^6 cells/ml) were incubated (37°C, constant agitation) with or without different concentrations of recombinant human IL-5 (generously provided by Dr C. J. Sanderson, National Institute for Medical Research, Mill Hill, London NW7, UK). The cells were stimulated with either serum treated zymosan (0.5 mg/ml final concentration) for 30 min or with the calcium ionophore A23187 (2.5 μM) for 10 min in a final volume of 250 μl. Incubations were terminated by cooling on ice, and supernatants were collected by centrifugation at 8000 × g for 1 min followed by storage under nitrogen at −80°C until analysis. The amount of leukotriene C_4/D_4/E_4 in the supernatants was determined with a radioimmunoassay kit according to the manufacturer’s instructions (Amersham, Buckinghamshire, UK).

**Results**

Incubation of human eosinophils with IL-5 for 30 min at 10–1 000 U/ml itself induced a small but not significant increase in leukotriene synthesis (Table 1). However, IL-5 (1 000 U/ml) potentiated STZ induced leukotriene synthesis by 69% compared with the production of leukotriene in the absence of the cytokine (Fig. 1). IL-5 (300 U/ml) induced a potentiation of the calcium ionophore induced leukotriene synthesis by 135% compared with the production of leukotriene in the absence of the cytokine. Pre-incubation of eosinophils with IL-5 (1 000 U/ml) for 10 min and subsequent stimulation with STZ or A23187 showed a similar potentiation of leukotriene synthesis as measured without pre-incubation (Table 2). In pilot experiments, longer pre-incubation times of eosinophils with IL-5 (up to 60 min) did not show a further enhancement of leukotriene synthesis. Leukotriene synthesis by eosinophils decreased after pre-incubation above 15 min. This was observed both in the absence or presence of IL-5. There were large donor-to-donor variations in eosinophil-leukotriene production and its IL-5 enhancement.

![Graph A](image1)  
![Graph B](image2)

**Figure 1.** The effect of different concentrations of IL-5 on human eosinophil leukotriene C_4/D_4/E_4 synthesis induced with (A) STZ (n = 11) or (B) calcium ionophore A23187 (n = 4). Results are presented as means ± S.E.M. IL-5 and the stimulating agents were added simultaneously. *p < 0.05 and **p < 0.01 as determined with the paired Student's t-test and compared with the leukotriene synthesis in the absence of IL-5.

**Table 1.** The direct effect of different concentrations of IL-5 on human eosinophil leukotriene C_4/D_4/E_4 synthesis (n = 3)

| IL-5 (U/ml) | LT synthesis (pg/10^6 cells) |
|------------|-----------------------------|
| 0          | 36.7 ± 3.6                  |
| 10         | 37.5 ± 3.2                  |
| 100        | 42.3 ± 4.0                  |
| 1000       | 47.9 ± 2.7                  |

Results are presented as means ± S.E.M.

**Table 2.** The effect of pre-incubation time on human eosinophil with IL-5 on STZ- or A23187-induced leukotriene C_4/D_4/E_4 synthesis

| Pre-incubation time (min) | STZ      | A23187    |
|---------------------------|----------|-----------|
|                           | −IL-5    | + IL-5    |
| 0                         | 8.6 ± 4.0| 13.1 ± 5.7* |
|                           | 16.8 ± 6.4| 56.5 ± 14.8* |
| 10                        | 9.2 ± 4.2| 14.5 ± 5.2**|
|                           | 12.4 ± 6.2| 62.9 ± 6.8**|

*p < 0.05 and **p < 0.01 as determined with the paired Student’s t-test and compared with the control. Results (ng/10^6 cells) are expressed as mean ± S.E.M. (n = 6 for STZ, n = 3 for A23187). IL-5 was used as 1 000 U/ml.
with the present findings, it can be speculated that IL-5 may play an important role in the pathogenesis of asthma.

References

1. Gleich GJ. The eosinophil and bronchial asthma: current understanding. J Allergy Clin Immunol 1990; 86: 422-436.
2. Dijkstra R, Roche WR, Wilton JW, Beasley CRW, Twempean OP, Howarth PH, Holgate ST. Mucosal inflammation in asthma. Am Rev Respir Dis 1990; 142: 434-457.
3. Van Oosterhout AJM, Nijkamp FP. Minireview: lymphocytes and bronchial hyperresponsiveness. Life Sci 1990; 46: 1255-1266.
4. Frigos E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. Lab Invest 1980; 42: 35-43.
5. Motojima S, Frigos E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. Am Rev Respir Dis 1989; 139: 801-805.
6. Gudend RH, Lerts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. J Clin Invest 1991; 87: 1470-1473.
7. Uchida DA, Ackerman SJ, Coley AJ, Larsen GL, Weller PF, Freed J, Irvin CG. The effect of human eosinophil granule major basic protein on airway responsiveness in the rat in vivo. Am Rev Respir Dis 1993; 147: 982-988.
8. Arm JP, Spur BW, Lee TH. The effects of inhaled leukotriene E4 on the airway responsiveness to histamine in subjects with asthma and normal subjects. J Allergy Clin Immunol 1988; 82: 654-660.
9. O'Hickey SP, Hawkesworth RJ, Fong CY, Arm JP, Spur BW, Lee TH. Leukotrienes C4, D4, and E4 enhance histamine responsiveness in asthmatic subjects. Am Rev Respir Dis 1991; 144: 1035-1057.
10. Jacques CAJ, Spur BW, Johnson M, Lee TH. The mechanism of LTBE-induced histamine hyperresponsiveness in guinea-pig tracheal and human bronchial smooth muscle. In vitro. Br J Pharmacol 1991; 104: 859-866.
11. Patterson R, Harris KE, Bentzmire PB, Krell RD. Aerosolized leukotriene D4 converts monkeys that are negative aerosolized Ascaris responders to positive airway responders. Life Sci 1986; 38: 1179-1184.
12. Frew AJ, Kay AB. Eosinophils and T-lymphocytes in late-phase allergic reactions. J Allergy Clin Immunol 1990; 85: 533-539.
13. Lopez AF, Sanderson CJ, Gamble JR, Campbell HR, Young IG, Vadas MA. Recombinant human interleukin-5 is a selective activator of human eosinophilic function. J Exp Med 1988; 167: 219-224.
14. Silberstein DS, David JR. The regulation of human eosinophil function by cytokines. Immunol Today 1987; 8: 380-385.
15. Silberstein DS, Owen WF, Gasson JC et al. Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (re-combinant) granulocyte-macrophage colony-stimulating factor. J Immunol 1986; 137: 3290-3294.
16. Fukushima T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. J Immunol 1990; 144: 642-646.
17. Walsh GM, Hartnell A, Warnall AJ, Kuribara K, Sanderson CJ, Kay AB. IL-5 enhances the in vitro adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD11b/CD18)-dependent manner. Immunology 1993; 71: 258-265.
18. Wang JM, Rambaldi A, Bianchi D, Chen ZG, Sanderson CJ, Mantovani A. Recombinant human interleukin-5 is a selective eosinophil chemotactant. J Exp Med 1989; 169: 791-795.
19. Koenderman L, Kok PTM, Hametink ML, Verheuven JAJ, Bruijinses PLB. An improved method for the isolation of eosinophilic granulocytes from peripheral blood of normal individuals. J Leukocyte Biol 1988; 44: 79-86.
20. Bischoff SC, Brunner T, Deweck AL, Dabendien CA. Interleukin-5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. J Exp Med 1990; 172: 1577-1582.
21. Hirai K, Yamaguchi M, Misaki Y, et al. Enhancement of human basophil histamine release by interleukin-5. J Exp Med 1990; 172: 1525-1528.
22. Hamid Q, Assman W, Ying S, et al. Expression of messenger RNA for interleukin-5 in mucosal bronchial biopsies from asthmatics. J Clin Invest 1991; 87: 1541-1546.
23. Robinson DS, Hamid Q, Ying S, et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in asthmatic New Engl J Med 1992; 326: 298-304.
24. Van Oosterhout AJM, Ladensius ARC, Savelkoul HJF, Van Ark I, Deelman KC, Nijkamp FP. Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. Am Rev Respir Dis 1993; 147: 548-552.
25. Van Oosterhout AJM, Van Ark I, Hofman G, Savelkoul HJF, Nijkamp FP. Recombinant interleukin-5 induces in vivo airway hyperresponsiveness to histamine in guinea pigs. Eur J Pharmacol 1993; 236: 379-383.

ACKNOWLEDGEMENT. This study was supported by a research grant of the Dutch Asthma Foundation (87.29). The authors thank R. Ladensius for carefully reading this manuscript.

Received 2 September 1993; accepted in revised form 24 November 1993