

Interleukin-1 β and interleukin-1ra levels in nasal lavages during experimental rhinovirus infection in asthmatic and non-asthmatic subjects.

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Summary

Background Exacerbations of asthma are often associated with rhinovirus (RV)-induced common colds. During experimental RV-infection in healthy subjects, increased levels of the pro-inflammatory mediator IL-1 β and the anti-inflammatory IL-1 receptor antagonist (IL-1ra) have been found in nasal lavage.

Objective We postulated that the balance between nasal pro- and anti-inflammatory mediator expression is disturbed in asthma, resulting in more extensive inflammation following RV-exposure in asthma.

Methods We determined IL-1ra, IL-1 β , and IL-8 in nasal lavages (days –2, 3, and 6) of non-asthmatics and asthmatics (with and without pre-treatment with the inhaled steroid budesonide) before and after experimental RV16-infection (days 0 and 1).

Results Following RV16-infection, a significant increase in IL-8 was observed in the placebo- and budesonide-treated asthmatics ($P = 0.033$ and 0.037 , respectively), whereas IL-1 β only increased in the two asthma groups combined ($P = 0.035$). A small, but significant, increase in IL-1ra was only observed in the budesonide-treated asthmatics ($P = 0.047$). At baseline, IL-1ra levels were significantly higher in the non-asthmatics than in the placebo-treated asthmatics ($P = 0.017$).

Conclusion These results demonstrate differences between non-asthmatic and asthmatic subjects in the basal levels of nasal cytokines and their inhibitors, and in the effect of experimental RV-infection on these levels. The results indicate that RV may enhance inflammation more markedly in asthmatics, and suggest that this may in part be explained by lower IL-1ra levels. In addition, the observation that budesonide-treatment may result in higher nasal IL-1ra levels supports the hypothesis that steroids act in part by increasing the endogenous anti-inflammatory screen.

Keywords asthma, IL-1 β , IL-1ra, nasal lavages, RV16

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Introduction

The common cold is a highly prevalent, uncomplicated upper airway disease with a transient nature. Following upper respiratory tract infections, asthmatics suffer from more frequent lower-respiratory-tract (LRT) infections and more severe and longer-lasting LRT symptoms as compared with healthy subjects [1]. Moreover, upper respiratory tract infections, particularly those caused by rhinoviruses (RVs), are frequently associated with asthma exacerbations [2]. These exacerbations are characterized by increased inflammation within the upper and lower airways, a process that is driven by various pro-inflammatory cytokines and mediators [3]. Whereas these results strongly indicate that RV-induced inflammation plays an important role in these exacerbations, it is still unknown whether the effect of RV-infection on

mucosal inflammation differs between healthy subjects and patients with asthma.

Experimental RV infection in humans increases the levels of cytokines in nasal lavages, as demonstrated by e.g. analysis of interleukin (IL)-8 in non-asthmatics and asthmatics [4, 5], and of IL-1 β and IL-1 receptor antagonist (IL-1ra) in non-asthmatics [6, 7]. IL-1ra may modulate IL-1-mediated inflammation, by binding to the IL-1 receptor without inducing an intracellular response [8], and therefore it may act as an endogenous inhibitor of RV-induced airway inflammation. It has been shown that this endogenous anti-inflammatory mechanism may be further enhanced by glucocorticoids. This is demonstrated by studies showing that *in vivo* glucocorticoids selectively inhibit bronchial epithelial expression of IL-1 β , without affecting epithelial IL-1ra in asthmatics [9], and *in vitro* by a steroid-induced increase of IL-1ra by a human airway epithelial cell line [10].

We hypothesized that RV differentially affects pro- and anti-inflammatory mediator production in patients with asthma as compared with healthy subjects. We therefore examined the nasal inflammatory response to a RV infection

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in healthy subjects and patients with asthma. In addition, in the latter subjects we studied the effect of budesonide on RV-induced inflammation. To this end, we determined the levels of IL-1ra, IL-1 β , and IL-8 in nasal lavages of non-asthmatic and asthmatic subjects (with and without steroid treatment) before and after experimental RV16 infection.

Materials and methods

Subjects

Twenty-five atopic asthmatic and 12 non-atopic, non-asthmatic healthy subjects participated in two sequential, previously published studies that used the same study protocol [11, 12]. They had a baseline forced expiratory volume in 1 s (FEV₁) of >70% and >80% predicted, respectively. Airway responsiveness to inhaled histamine (PC₂₀) was <8 mg/mL in the asthmatic group, and >8 mg/mL in the non-asthmatic group. Their non-atopic or atopic status was determined by negative or ≥ 1 positive skin prick tests to 16 common aero-allergen extracts (ALK Abelló, Nieuwegein, the Netherlands), respectively. All subjects had low titres of circulating RV16 neutralizing antibodies ($\leq 1:2$ serum dilution against 20–25 $\times$ 50% tissue culture infective dose – TCID₅₀) [5]. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and the subjects gave their written informed consent.

Study design

Experimental RV16 inoculation was performed on two consecutive days (days 0 and 1). Blood samples were drawn on days –2 and 27, and nasal lavages were carried out on days –2, 3, and 6. In the asthmatics, inhaled budesonide was administered in a randomized, double-blind, placebo-controlled design (budesonide group: $n = 12$, Turbohaler® (AstraZeneca, Zoetermeer, The Netherlands), 800 μ g b.i.d.; placebo group: $n = 13$) for 4 weeks, starting 16 days before RV16 inoculation (day –16) [11, 12].

RV16 inoculation and confirmation

The RV16 inoculum was obtained from the same strain and stock as used in previous experiments in humans *in vivo* [5]. A total dose of $0.6\text{--}24 \times 10^4$ TCID₅₀ diluted in 3 mL Hanks' balanced salt solution (HBSS) with 0.5% (w/v) gelatin was administered to each subject according to a previously described procedure [5]. Confirmation of RV16 infection was established by a fourfold or greater increase in virus-specific neutralizing antibody titre in serum and/or by recovery of the virus from the nasal lavages [5].

Nasal lavages

Nasal lavages were obtained using a modified 'nasal pool device' [5]. This consists of a syringe, connected to a rubber tube, equipped with an inflatable balloon, serving as a nasal adapter. The balloon was inserted into the *vestibulum nasi*, and gently inflated. While the subject was sitting in writing position, 10 mL fluid was instilled by gently compressing the syringe. After 5 min, the fluid was recovered by retracting the syringe. This procedure was carried out in each nostril.

The lavages recovered from the first (Hanks' balanced salt solution with 0.5% gelatin) and second nostril (phosphate-buffered saline) were used for confirmation of RV16 infection and to determine the mediator levels, respectively.

ELISAs

Levels of IL-8 and IL-1 β were determined by ELISA (CLB, Amsterdam, the Netherlands), and levels of IL-1ra were determined by ELISA using commercially available reagents (R&D Systems, Minneapolis, MN, USA). The lower limits of detection were 3, 0.86, and 25 pg/mL, respectively. The levels of samples in which no detectable cytokines were found were set at this lower limit value.

Statistical analysis

The Wilcoxon signed-ranks test and the Mann–Whitney *U*-test were applied to test within- and between-group differences. Relationships between outcome parameters were investigated using Spearman's rank correlation tests. Differences at *P*-values ≤ 0.05 were considered statistically significant.

Results

In the majority of subjects (32 out of 37), RV infection was confirmed. In 5 out of 37 subjects (two out of each asthma group, and one from the non-asthmatic group) RV16 infection was not confirmed, and therefore the results from nasal lavage samples from these subjects obtained after RV16 infection were excluded from the analysis.

IL-8 levels in the nasal lavage fluid obtained from asthmatics were significantly higher after RV16 infection, whereas RV16 infection did not affect these levels in non-asthmatics (Fig. 1a). In the placebo-treated asthmatics, this difference was significant on day 3 as compared with day –2, whereas at day 6 post-infection the level had significantly decreased as compared to day 3. In the budesonide-treated asthmatics, the increase in IL-8 level was significant on day 6 compared with day –2.

In contrast, RV16 did not significantly change IL-1 β levels in either of the three study groups (Fig. 1b). However, when the data from the two asthma groups were analysed together, a significant increase in IL-1 β levels was noted on day 3 as compared with day –2 ($P = 0.035$). RV16 did not affect nasal IL-1ra levels, except for a limited increase in the budesonide-treated asthmatics on day 6 vs. day –2 (Fig. 1c). Interestingly, IL-1ra levels were significantly higher in non-asthmatics as compared with placebo-treated asthmatics on both days –2 and 6 ($P < 0.028$), whereas no such differences were noted between IL-1ra levels in budesonide-treated asthmatics and non-asthmatics. In addition, the IL-1ra/IL-1 β weight ratio was found to be significantly decreased in the placebo-treated asthmatics on day 3 (median [range]: 140 [46–1832] as compared with day –2 (262 [66–2042], $P = 0.01$). This decrease was even more pronounced when we combined the data of the two asthma groups (day –2: 508 [66–2672], day 3: 167 [20–3013], $P = 0.008$).

There were no significant correlations between baseline IL-1ra and the RV16-induced changes in IL-8 or IL-1 β levels. However, the RV16-induced changes in IL-1ra were

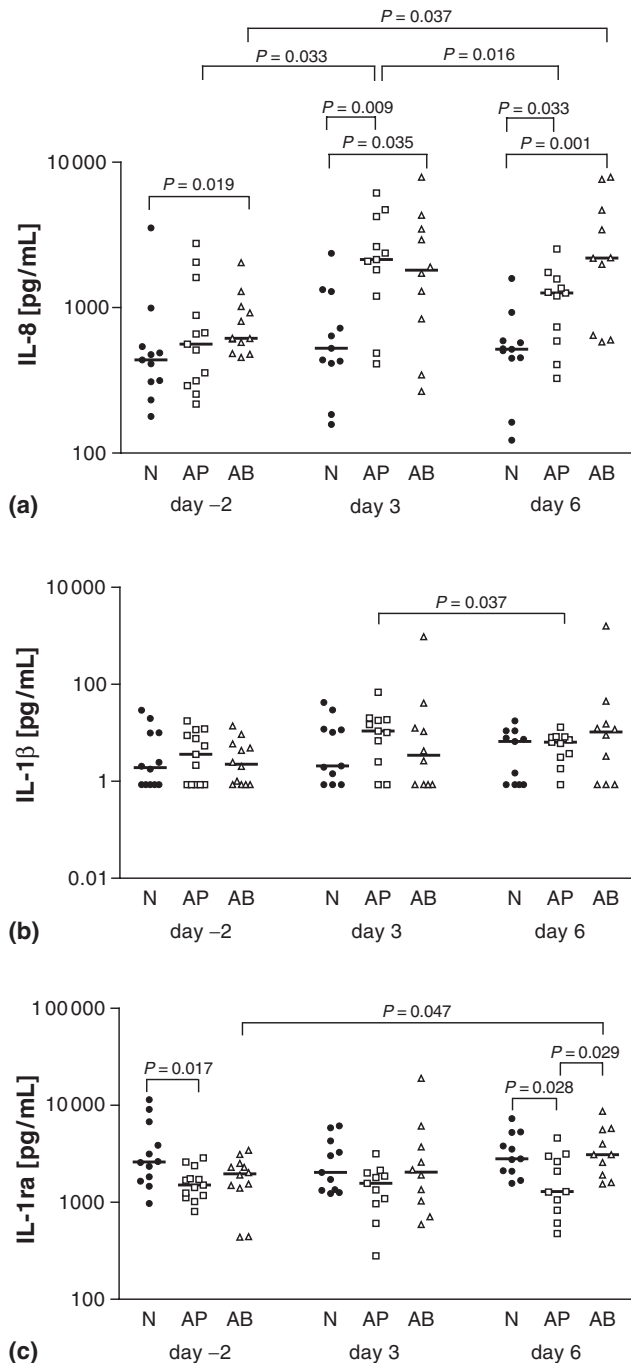


Fig. 1. IL-8, IL-1 β , and IL-1ra levels in nasal lavage fluid. Individual levels of IL-8 (a), IL-1 β (b), and IL-1ra (c) in the non-asthmatic (N: ●), placebo-treated (AP: □), and budesonide-treated (AB: △) asthmatic subjects. Horizontal lines represent the medians. P-values, indicating significant within- and between-group differences, are depicted in the figure.

positively correlated to those in IL-1 β in all three groups studied (Fig. 2; non-asthmatics: $R_s = 0.62$, $P = 0.044$; placebo-treated asthmatics: $R_s = 0.67$, $P = 0.024$; budesonide-treated asthmatics: $R_s = 0.84$, $P = 0.002$).

Discussion

This study shows a difference in the baseline nasal levels of IL-1ra between non-asthmatic and asthmatic subjects. In

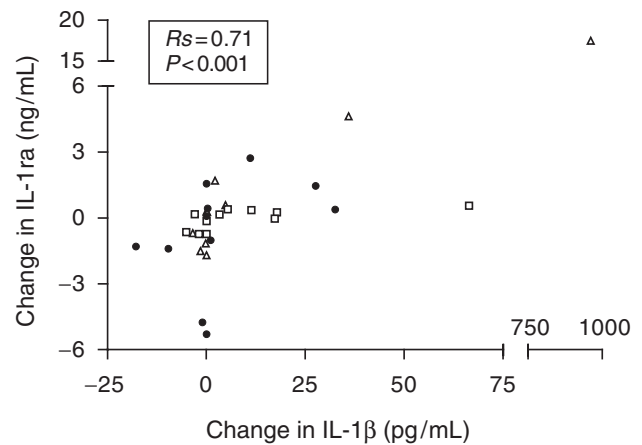


Fig. 2. Relationship between changes in IL-1ra and IL-1 β . Individual levels of changes in IL-1ra and IL-1 β between days -2 and 3, in the non-asthmatic (●), placebo-treated (□), and budesonide-treated (△) asthmatic subjects. There were significant correlations present between the changes in IL-1ra and IL-1 β in all the three study groups (see text). In this figure, the relationship is only shown for all three groups combined.

asthmatic subjects, RV increases nasal lavage IL-8 levels, irrespective of treatment with inhaled steroids, whereas no such increase was observed in normals. During RV infection, an increase in IL-1 β and IL-1ra levels was found in both asthma groups combined and the budesonide-treated asthma group, respectively. Moreover, the changes in IL-1ra levels and the changes in IL-1 β levels showed a significant correlation in all the three study groups. These data indicate that nasal inflammation differs between non-asthmatics and asthmatics, and that the same applies to inflammatory changes following RV infection.

There are no comparative data on the nasal production of IL-1 β and IL-1ra in patients with asthma, with or without RV infection. This study demonstrates for the first time a difference in baseline nasal IL-1ra level between non-asthmatic and asthmatic subjects, with lower levels in the latter group. Moreover, we demonstrated that RV increases IL-1 β in asthma. In contrast, another study revealed increased levels of IL-1ra in bronchoalveolar lavage of patients with status asthmaticus compared with healthy controls, whereas levels in patients with mild asthma did not differ from controls [13]. However, our findings are in line with previous findings showing lower levels of IL-1ra in nasal lavage of patients with perennial allergic rhinitis as compared with controls [14]. The increase in IL-1ra, following RV-infection, in the steroid-treated asthmatics is in line with findings found from *in vitro* studies [10]. However, we did not find an increase in IL-1 β or IL-1ra in the non-asthmatics following infection, which is in contrast to recent findings [6, 7].

Although the nasal lavages were obtained from two sequential studies, identical protocols [11, 12] and the same virus batch were used [5], and all samples were analysed at the same time. Whereas other studies have reported an increase in nasal IL-1 β levels following RV exposure [6, 7], in the present study such an increase was only detected when both asthma groups were combined in the analysis, and was not found in the separate groups. This may be explained by the fact that the IL-1 β levels in part (30%) of the lavages were below the detection limit of the assay used, and therefore were

arbitrarily set at the lower limit. This may have limited the possibility to detect differences. Another explanation could be the time-point at which the nasal lavages were collected. In the two studies that did report an increase, levels of IL-1 β peaked on day 2 after RV inoculation [6, 7], whereas we performed nasal lavages on day 3. Finally, the procedure used to obtain nasal lavage may have affected the results, because the nasal pool device used in the present study may result in a different recovery of cytokines from the mucosal surface when compared to the methods used in the other studies.

How do we interpret these data? The pro-inflammatory cytokine (e.g. IL-1 β) network is part of the normal innate immune response, which is inhibited by anti-inflammatory mechanisms involving mediators such as IL-1ra. This study shows that nasal IL-1 β , which could contribute to the pathogenesis of a common cold, is increased following experimental RV infection in asthmatics. IL-1ra expression is induced by a number of cytokines (e.g. IL-4), viral products, and acute phase proteins [8], and may play a role in the resolution of symptoms [7]. The IL-1ra/IL-1 β ratio is proposed to be more important in (defence against) RV-induced inflammatory changes than the absolute levels of IL-1ra *per se* [8]. Moreover, it has been reported that this ratio is significantly higher in patients with status asthmaticus as compared with patients with stable asthma and healthy controls [13]. In the present study, this ratio was found to be significantly decreased in the asthmatics on day 3 as compared with day -2. One-third of the weight ratio values were lower than 100, and it has been previously suggested that in this case the levels of IL-1ra may not suffice to inhibit IL-1 β [7, 8]. This would indicate that RV16 alters the balance between pro- (IL-1 β) and anti-inflammatory (IL-1ra) mediators, thus promoting inflammation.

The balance between IL-1ra and IL-1 β may also be affected by steroids that have been shown to decrease IL-1 β *in vivo* [9] and increase IL-1ra *in vitro* [10] in epithelial cells. Our observation that nasal production of IL-1ra is increased in budesonide-treated asthmatics, even in the presence of a RV infection, is in line with these *in vitro* findings. The effect of inhaled steroids on measures of nasal inflammation may appear to be unexpected. Indeed, this study was not designed to investigate the steroid-induced effects on nasal inflammation, but was designed to explore the effects of steroids on lower airway inflammation and function [11]. It is likely that intranasal steroids would have exerted a larger effect than observed in the present study using inhaled steroids. Nevertheless, there is reason to assume that even orally inhaled steroids display anti-inflammatory effects in the nose [15]. Therefore, we conclude that our results contribute to our insight into the effects of steroids on baseline and RV-induced airway inflammation in asthma.

In conclusion, the results from the present study reveal differences in the effect of experimental RV infection on cytokine levels in nasal lavage between non-asthmatics and asthmatic subjects. Increased levels of especially IL-8 suggest a higher degree of inflammation in asthmatics following RV16 infection as compared with non-asthmatics. The lower nasal IL-1ra levels in asthmatics at baseline point towards an impaired endogenous protection against inflammation in this disease. Our findings suggest that this may be restored by glucocorticosteroid therapy.

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