Association Between Interleukin-8 Concentration in Nasal Secretions and Severity of Symptoms of Experimental Rhinovirus Colds

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The purpose of this study was to examine the association between experimental rhinovirus infection and the elaboration of interleukin-8 (IL-8) into nasal secretions of volunteers and to determine the effect of pentoxifylline on IL-8 elaboration and rhinovirus-associated common cold symptoms. Fifty-four subjects with experimental rhinovirus infections and 20 sham-inoculated subjects were studied. Pentoxifylline had no effect on rhinovirus-induced symptoms or nasal-secretion IL-8 concentrations. IL-8 concentrations were significantly greater in nasal secretions from infected symptomatic subjects than in those from infected asymptomatic or sham-challenged subjects on days 2–4 after virus challenge. In infected subjects, there was significant rank correlation between nasal obstruction severity, rhinorrhea severity, and nasal-wash albumin concentrations and the change in IL-8 concentration from baseline on days 2–4 after virus challenge.

The rhinoviruses are the predominant pathogens responsible for common cold symptoms. Although the association between infection and symptoms is clear, the mechanism by which the virus infection of the nasal epithelium results in the symptom complex of the cold is not known. Histopathologic studies found little evidence of epithelial damage during rhinovirus colds and led to the suggestion that the host inflammatory response may play a role in the pathogenesis of rhinovirus-induced common cold symptoms [1–3]. The subsequent observation of an influx of polymorphonuclear leukocytes (PMNs) into nasal mucosa and nasal secretions that was not seen in asymptomatic infections or uninfected subjects provided further evidence of a role for host factors in production of symptoms [4–6].

IL-8, a potent chemoattractant for PMNs, has been found in media supernatants from rhinovirus-infected cell cultures and in nasal secretions from rhinovirus-infected volunteers, and intranasal administration of IL-8 to normal volunteers produces some symptoms of common-cold illness [7–9]. These observations suggest that inhibition of rhinovirus-induced IL-8 might have a beneficial effect on rhinovirus-associated common-cold symptoms in volunteers.

See the editorial comment by Hendley on pages 847–8.

Pentoxifylline, a methylxanthine that has been shown to inhibit the production and effects of some inflammatory cytokines, has also been shown to inhibit rhinovirus-induced IL-8 elaboration in vitro [10–14]. Our objectives were to determine the effect of experimental rhinovirus infection on the elaboration of IL-8 into nasal secretions of volunteers in a prospective, controlled study and to determine the effect of pentoxifylline on rhinovirus-associated common-cold symptoms.

Materials and Methods

Subjects. Healthy subjects 18–60 years of age were recruited from the Medical University of South Carolina community. Subjects with underlying conditions that might interfere with symptom assessment or that might place the subject at increased risk from the rhinovirus infection or administration of pentoxifylline were excluded from the study. All subjects were required to have a serum titer of $\leq 1:4$ of neutralizing antibody to rhinovirus type 23.

Five hundred twenty-eight volunteers were screened for participation in the study. Two-hundred nine subjects met the antibody criteria for participation; of these, 85 declined participation in the hotel-isolation phase of the study, 30 were not contacted to participate, and 11 failed other inclusion or exclu-
sion criteria. The first 80 subjects who were eligible and agreed to participate were included in the hotel-isolation phase of the study, and the remaining three subjects participated in the study as alternates until the time of the virus challenge. This study was reviewed and approved by the Investigative Review Board for Human Subjects of the Medical University of South Carolina. All subjects gave written informed consent for participation.

**Study medication.** Pentoxifylline was purchased commercially as Trental 400-mg controlled-release tablets (Hoechst-Roussel Pharmaceuticals, Somerville, NJ) and encapsulated into opaque gelatin capsules. Identical placebo capsules contained cellulose. Subjects received either active treatment or placebo treatment, according to random assignment of the treatments to subject numbers. Subject numbers were then assigned sequentially as subjects were enrolled in the trial.

Each subject took one study capsule (of pentoxifylline or placebo, hereafter termed study medication) at approximately 8 A.M., 4 P.M., and midnight on each treatment day. Capsules for the duration of the study were packaged in a blister pack for each study subject. Compliance with the doses of study medication given before isolation in the hotel was assessed by asking the subjects to return the empty blister packs for these doses. One volunteer missed a single dose of study medication.

**Nasal wash.** Nasal-wash specimens were collected by instillation of drops into the nose. Two inocula of 250 μL per nostril were given ~15 minutes apart while the subjects were supine. Sham-challenged subjects were inoculated in an identical manner with balanced salt solution. The challenge virus used in this study had been safety-tested according to consensus guidelines [15].

**Nasal lavage.** Nasal-wash specimens were collected by instillation of 5 mL of sterile 0.9% saline into each nostril. This wash was then expelled into a waxed paper cup and kept chilled until processed for viral cultures and for determination of IL-8 and albumin concentrations.

**Measures of infection.** Nasal-wash specimens were centrifuged at 1,500 g (Beckman GS, Beckman Instruments, Palo Alto, CA) for 15 minutes and then mixed 3:1 with 4× concentrated virus-collecting broth. Each specimen was inoculated into two tubes (0.2 mL/tube) of human embryonic lung fibroblast cells (MRC-5, Biowhittaker, Walkersville, MD, and Viromed, Minneapolis), incubated in a roller drum at 33°C, and examined every 60 minutes for the development of typical rhinoviral cytopathic effect. Virus-neutralizing antibody titers in sera collected immediately before virus challenge and 21 days after challenge were determined by a standard microtiter assay. Subjects who were viral culture-positive on any of the postchallenge study days or had at least a fourfold rise in titer of antibody to the challenge virus were considered infected.

**IL-8 assay.** Concentrations of IL-8 in nasal lavage were determined with a commercially available ELISA (R&D Systems, Minneapolis). The lower limit of detection for the assay was 31.25 pg/mL. The precision of the assay for measurement of IL-8 in nasal wash was determined prior to the study with pools of nasal wash spiked with known concentrations of IL-8. Intra-assay variability was found to range from 3.8% to 5.7%, and interassay variability was found to range from 2.7% to 11% for nasal wash containing high and low concentrations of IL-8, respectively. The recovery of IL-8 in nasal wash ranged from 88% to 136% of predicted concentrations. Specimens that had IL-8 concentrations greater than the operating range of the assay were diluted 1:10 and 1:100 and re-assayed.

**Symptom scoring.** Prior to taking each dose of study medication (three times each day), subjects were to judge the severity of the symptoms of sneezing, rhinorrhea, nasal obstruction, sore throat, cough, headache, malaise, and chilliness over the previous 8 hours. Each symptom was assigned a severity score of 0–4, corresponding to a reported symptom severity of absent, mild, moderate, severe, or very severe. The three symptom scores recorded for each symptom on a given study day were averaged to provide a daily symptom score for that day.

The total symptom score for each study day was the sum of the daily symptom scores for the individual symptoms. Subjects with a total symptom score of >6 over the course of the study were defined as having symptomatic colds.

**Nasal mucus weights.** The weight of expelled nasal secretions was determined by providing all subjects with packets of preweighed nasal tissues. Used tissues were stored in an airtight (zipper-closing) plastic bag until the following morning, when they were collected, weighed, and counted. Values reported are nasal mucus weights per 24-hour period.

**Anterior rhinomanometry.** Nasal airflow was measured daily by single-nostril, active anterior rhinomanometry [16]. Airflow was recorded during inspiration of four separate breaths at a transnasal pressure of −1.5 cm H2O. Values reported are the total airflow (through both nostrils) measured on each study day. Prior to the airflow measurement, each subject was asked to rate the severity of their nasal obstruction at the time of the measurement, with use of the scale described above.

**Conduct of the study.** Eighty volunteers began treatment with either pentoxifylline or placebo 3 days prior to virus challenge (study day −3). The subjects were confined in individual hotel rooms from 24 hours before until 5 days after virus challenge. After 11 doses of study medication (study day 0), 60 subjects (29 treated with active drug and 31 treated with placebo) were challenged with rhinovirus type 23, while 20 subjects (10 treated with active drug and 10 treated with placebo) were sham-challenged. Nasal-wash specimens for viral culture and for determination of IL-8 and albumin concentrations and serum specimens for viral serology were collected immediately prior to virus challenge.

On study days 1–4, subjects continued treatment with study medication, and on each of these days a nasal wash specimen was obtained for viral culture and determination of IL-8 con-
centration and albumin concentration. Symptom severity was monitored by assessment of symptom scores and measurement of nasal airflow and nasal secretion weights. On study day 21 a convalescent serum specimen was obtained for viral serology.

**Statistical analysis.** Initial analysis of the data revealed no effect of pentoxifylline treatment on symptom scores or IL-8 concentrations. On the basis of this result, the two treatment groups were combined for the analysis of the correlation of IL-8 concentrations with symptoms and albumin concentrations. The IL-8 concentrations were not normally distributed, and all analyses were done by nonparametric methods. The changes in IL-8 concentrations from baseline in the virus-infected and sham-challenged groups were compared with use of the Mann-Whitney U test. Correlations between IL-8 concentrations and symptom scores or albumin concentrations were determined with the Spearman rank-correlation test.

**Results**

Eighty subjects were confined in the hotel for the virus-challenge phase of the study. Of the 60 subjects challenged with rhinovirus, 54 (90%) completed the study and met the study definition for rhinovirus infection. Six subjects were excluded from the data analysis. One of these subjects withdrew from the study before the virus challenge because of an adverse event, and one had a positive virus culture on study day 0, before the virus challenge. Four subjects did not become infected with the challenge virus. All 20 of the sham-challenged volunteers completed the study as planned and were included in the data analysis. The volunteers included in the data analysis from the virus- and sham-challenged groups were comparable prior to challenge.

Of the 54 evaluable virus-infected subjects, 26 were treated with pentoxifylline and 28 were treated with placebo. Treatment with pentoxifylline did not affect rhinovirus infection or viral shedding. Of the virus-challenged subjects, 26 (96%) of 27 in the pentoxifylline-treated group and 28 (93%) of 31 in the placebo group were infected. Rhinovirus was isolated from 81 (62%) of 130 culture specimens from pentoxifylline-treated subjects and from 90 (64%) of 140 culture specimens from placebo-treated subjects. Nausea was the only side effect reported more frequently by the pentoxifylline-treated subjects (13%) than by controls.

Pentoxifylline had no effect on nasal-lavage IL-8 concentrations in this study. Median IL-8 concentrations were 60.7, 168.2, 306.2, and 265.1 pg/mL in the pentoxifylline-treated subjects and 83.5, 198.4, 218.4, and 272.5 pg/mL in the placebo-treated subjects on study days 1–4, respectively. There was also no significant difference in rhinorrhea or nasal-obstruction severity scores between the pentoxifylline and placebo treatment groups. The mean (±SE) areas under the severity-time curve were 44.3 (±7.9) and 34.6 (±8.5) for rhinorrhea and 75.2 (±9.4) and 69.3 (±9.4) for nasal obstruction in the treated and placebo groups, respectively.

The effects of pentoxifylline treatment on nasal-wash IL-8 concentrations and symptom severity were analyzed by multivariate repeated-measures ANOVA with treatment, inoculation group (virus vs. sham), and treatment by inoculation as model terms. No effect of pentoxifylline was detected in this analysis, and the pentoxifylline and placebo groups were combined for the subsequent analyses.

**Association of IL-8 with virus infection and common cold symptoms.** The concentrations of IL-8 and the changes in concentration from baseline were significantly higher in the rhinovirus-infected subjects than in sham-challenged subjects on study days 2 ($P < .03$), 3 ($P < .006$), and 4 ($P < .006$). There were 37 rhinovirus-infected subjects whose total symptom scores for the study were $>6$ (symptomatic) and 17 whose scores were $\leq6$ (asymptomatic). IL-8 concentrations in nasal lavage specimens were significantly greater for those subjects with symptomatic infections than for those with asymptomatic infections on study days 2 ($P = .008$), 3 ($P = .004$), and 4 ($P = .003$) (figure 1). The IL-8 concentrations in subjects with asymptomatic infections were not significantly different from those in the sham-challenged subjects.

**Correlation between IL-8 concentration and symptom severity.** The rhinorrhea severity score (figure 2), nasal-obstruction severity score (figure 3), and total daily symptom-severity score correlated with the change in nasal-wash IL-8 concentration from baseline. The correlation between rhinorrhea severity and change in IL-8 concentration was statistically significant on day 2 ($r_s \text{ [rank correlation coefficient]} = .378, P = .005$), day 3 ($r_s = .298, P = .033$), and day 4 ($r_s = .320, P = .021$). The
Figure 2. Correlation between the change in nasal-lavage-specimen interleukin-8 (IL-8) concentration (pg/mL) from baseline and rhinorrhea severity scores. The Spearman rank-correlation between rhinorrhea severity and change in IL-8 concentration was statistically significant on day 2 ($r_s = .378, P = .005$), day 3 ($r_s = .298, P = .033$), and day 4 ($r_s = .320, P = .021$).

The correlation between nasal-obstruction severity and change in IL-8 concentration was statistically significant on day 2 ($r_s = .359, P = .008$), day 3 ($r_s = .277, P = .046$) and day 4 ($r_s = .329, P = .017$). The correlation between total symptom score and change in IL-8 concentration (data not shown) was statistically significant on day 2 ($r_s = .349, P = .011$) and day 4 ($r_s = .339, P = .014$).

The correlation between the change in IL-8 concentration and objective measures of symptom severity was also determined. The change in nasal-wash IL-8 concentration from baseline was correlated with the concentration of albumin in the nasal wash (figure 4). The correlation between change in IL-8 and albumin concentration was statistically significant on day 2 ($r_s = .560, P < .001$), day 3 ($r_s = .573, P < .001$), and day 4 ($r_s = .513, P < .001$). There was no correlation between the daily nasal mucus weights or nasal airflow and change in IL-8 concentration from baseline on any study day.

Figure 3. Correlation between the change in nasal-lavage-specimen interleukin-8 (IL-8) concentration (pg/mL) from baseline and nasal obstruction severity scores. The Spearman rank-correlation between nasal obstruction severity and change in IL-8 concentration was statistically significant on day 2 ($r_s = .359, P = .008$), day 3 ($r_s = .277, P = .046$), and day 4 ($r_s = .329, P = .017$).
Discussion

Several recent studies have explored the relationship between IL-8 and viral respiratory pathogens. IL-8 is elaborated by cell cultures challenged with rhinoviruses, respiratory syncytial virus, influenza virus, and adenovirus [7, 17–21]. IL-8 has also been found in increased concentrations in the nasal secretions of individuals with natural colds [22]. Two recent studies have also found increased concentrations of IL-8 in the nasal secretions of asthmatic subjects with either naturally acquired viral infection or experimental rhinovirus colds [23, 24].

The rise in IL-8 concentration in asthmatic subjects with experimental rhinovirus colds was directly correlated with the severity of common-cold symptoms and inversely correlated with airway hyperresponsiveness. Our study demonstrates that similar IL-8 responses are seen in normal subjects and that the magnitude of the rise in IL-8 is directly correlated with the severity of rhinovirus-associated symptoms. Although other inflammatory mediators have been found to be associated with rhinovirus infection, only IL-6 has been shown to have a direct correlation with symptom severity [6, 25, 26].

We have assumed in this study that the concentration of IL-8 in nasal lavage fluid accurately reflects the concentration of IL-8 in nasal secretions. This assumption may be affected by either dilution or matrix effects. No attempt was made to adjust the IL-8 concentrations for the dilutional effects of the lavage procedure; however, the volume of nasal secretions recovered is generally relatively small in comparison to the amount of saline instilled, and the variation in dilution from sample to sample is unlikely to explain the findings of this study.

The lack of correlation between daily nasal mucus weights and IL-8 concentrations provides indirect evidence that a relative increase in the volume of mucus recovered was not the source of the increase in IL-8 concentration. Although the problem of potential dilutional effects has been raised in other studies, there is no consensus about whether or how an adjustment for these effects should be made [27].

A second variable that may affect the accurate measurement of cytokines in nasal lavage fluid is the change in the composition of the nasal secretions that occurs over the course of the cold [28]. There is evidence that a low protein content in the nasal lavage fluid can adversely affect the recovery of cytokines (personal communication, M. W. Seibel and T. M. Engel, The Procter & Gamble Co.). The impact of this or other potential matrix effects on the results of our study is unknown.

The prestudy evaluation of the IL-8 assay we used involved assessment of the recovery of IL-8 from nasal lavage fluid. The nasal lavage specimens were obtained from asymptomatic volunteers and would be expected to contain lower concentrations of protein than those obtained from subjects with symptomatic colds. Under these conditions, the recovery of IL-8 in nasal lavage fluid ranged from 88% to 136% of predicted concentrations and was within the expected performance capabilities of the assay.

This study was not designed to establish a cause-and-effect relationship between the elaboration of IL-8 in nasal secretions and the symptoms of the common cold. The observations that a chemoattractant for PMNs (IL-8) is elaborated during colds and that both this chemoattractant and PMNs are associated with symptomatic infection are consistent with the hypothesis that IL-8-mediated PMN migration plays a role in the pathogen-
esis of rhinovirus colds. However, there are alternative explanations for the increased concentration of IL-8 in nasal secretions during colds.

The concentration of IL-8 increases in the plasma of patients with viral respiratory disease [29, 30]. It is conceivable that the IL-8 present in nasal secretions is a result of the transudation of serum proteins into the nasal cavity during the cold [28]. A second possibility is that the IL-8 is produced in part by rhinovirus stimulation of the PMNs that infiltrate the nasal mucosa during rhinovirus infections. Respiratory syncytial virus has been shown to stimulate IL-8 elaboration by PMNs [31]. Previous studies of natural colds have demonstrated increased cytokine mRNA in nasal epithelial cells, suggesting that at least a portion of the cytokines in the nasal secretions are a result of local production [22].

One of the objectives of this study was to use pentoxifylline as an inhibitor of rhinovirus-induced IL-8 elaboration and to determine the effect of pentoxifylline on symptom severity. In spite of using maximum approved doses of pentoxifylline by the oral route, no effect was seen on either outcome measure. Pentoxifylline has been shown to be an effective inhibitor of tumor necrosis factor (TNF) and IL-1 elaboration and activity [10–13]. In vitro studies of human embryonic lung fibroblast cells revealed that pentoxifylline at a concentration of 100 μM/mL reduced the elaboration of rhinovirus-induced IL-8 by ~50% [14]. This effect was not dependent on inhibition of TNF or IL-1 and was not associated with any antiviral or cytotoxic effect.

The reason for the failure of pentoxifylline to inhibit rhinovirus-induced IL-8 elaboration in vivo is not clear. The mechanism of IL-8 elaboration appears to vary in response to different stimuli and in different cell types. It is possible that the inhibitory effect of pentoxifylline on virus-induced IL-8 elaboration is limited to the fibroblast cell line. It is also likely that the pentoxifylline concentrations in the nasal mucosa in this study were substantially less than the effective concentrations in the studies in vitro.

The results of this study provide evidence that rhinovirus infection is associated with an increased concentration of IL-8 in nasal secretions and that the severity of symptoms in rhinovirus colds is correlated with the magnitude of the rise in nasal IL-8 concentrations. These observations are consistent with the hypothesis that the host response in general and IL-8 in particular play a role in the pathogenesis of rhinovirus colds. Further evaluation of the role of IL-8 in rhinovirus infection will depend upon identification of agents that can specifically inhibit the production of rhinovirus-induced IL-8 in vivo.

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References


