

A comparison of IL-2 levels in nasopharyngeal and endotracheal aspirates of babies with respiratory syncytial viral bronchiolitis

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Background: Cytokines such as IL-2 are thought to be important in the pathogenesis of respiratory tract inflammation. Cytokine levels in nasopharyngeal aspirates (NPAs) have been used as a measure of respiratory inflammation in children with viral infections, but it is unclear whether they reflect levels in the lower respiratory tract.

Objective: We sought to assess the correlation between IL-2 levels in the nasopharyngeal and endotracheal secretions of children intubated with respiratory syncytial virus (RSV)-positive bronchiolitis.

Methods: NPA and endotracheal aspirates were collected concurrently from intubated infants with RSV-positive bronchiolitis. IL-2 levels were assayed by ELISA, and the results were compared according to collection site.

Results: Nine paired specimens were collected. IL-2 levels ranged from 31 pg/mL to 8040 pg/mL. No significant difference was found in the geometric mean IL-2 values from the 2 collection sites. The intraclass correlation coefficient between NPA IL-2 levels and endotracheal aspirate IL-2 levels was 0.83.

Conclusion: IL-2 levels in NPAs are comparable with those in the lower respiratory tracts of infants with RSV-positive bronchiolitis. NPA cytokine levels provide a simple and useful means of assessing respiratory tract inflammation. (*J Allergy Clin Immunol* 1998;102:618-20.)

Key words: Cytokine, nasopharynx, respiratory syncytial virus, IL-2

There is ongoing interest in the role of viral respiratory tract infections, particularly those caused by respiratory syncytial virus (RSV), in modifying the immune response early in life. Cytokines are thought to be important in the pathogenesis of inflammation in the respiratory tract. The proinflammatory cytokine IL-2 is produced during viral infections by lymphocytes in culture.^{1,2} There are little human data which document the *in vivo* nasal production of IL-2 during viral infections. Lower respiratory tract secretions for cytokine measurements are usually obtained by bronchoalveolar lavage, which is an invasive technique

Abbreviations used

NPA: Nasopharyngeal aspirate

RSV: Respiratory syncytial virus

and would require the use of an anaesthetic if used in young children. For this reason, NPAs are often used to measure the cytokine response in children.

The aim of this study was to determine whether the cytokine levels in the nasopharyngeal aspirates (NPAs) of children with a specific respiratory disease reflect the cytokine levels in the lower respiratory tract.

METHODS

Patients

Fifteen infants were intubated with RSV-positive bronchiolitis in the intensive care unit of the Royal Alexandra Hospital for Children, Sydney, between June 1 and July 15, 1997. The diagnosis of bronchiolitis was based on the clinical criteria of tachypnea, coryzal symptoms, and respiratory distress with or without apnea. RSV was detected by using indirect immunofluorescence on NPAs. Babies were included in the study if no other pathogens were detected in their secretions.

Specimen collection

Paired NPA and endotracheal specimens were collected by suctioning the nasopharynx and the endotracheal tube, respectively. In each collection the child was placed in the supine position. A sterile feeding tube with an attached mucus trap (Indoplas) was passed into the nasopharynx. Gentle suction was applied by means of the wall suction unit. No saline was instilled before suction. The feeding tube and mucus trap were changed, and the same procedure was used to suction the endotracheal tube. Collection was timed such that suctioning of the nasopharyngeal and endotracheal sites occurred within 5 minutes of each other. The specimens were refrigerated immediately and were frozen within 15 minutes of collection at -80°C for later testing.

Specimen processing

Each specimen was made up to 2 mL with PBS. The specimen was centrifuged at 375g for 5 minutes, and the supernatant was tested for IL-2 by means of ELISA. The ELISA protocol was a variation of the standard technique³ optimized for local reagents. Briefly, Maxisorp plates (Nunc) were coated with monoclonal rat anti-IL-2 capture antibody (Pharmingen). The plates were incubated overnight after which nonattached sites were blocked with 1% BSA in PBS. The IL-2 level was then measured by using polyclonal biotinylated detection antibodies (Pharmingen) with streptavidin-peroxidase. Development was carried out with ortho phenylene diamine dihydrochloride, and the color was measured at 490 nm

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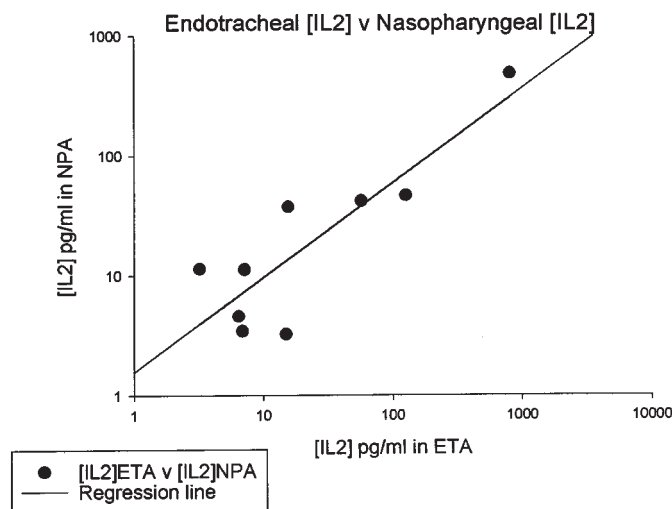
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ETA=endotracheal aspirate. NPA=nasopharyngeal aspirate.

FIG 1. The concentration of IL-2 in endotracheal and nasopharyngeal aspirates.

immediately after stopping the reaction with H₂SO₄. The standards were measured by using cytokines supplied by Pharmingen. A range of 62.5 to 4000 pg/mL was used in our assays. Any IL-2 level that fell below 62.5 pg/mL but above 0 was expressed as 31 pg/mL, which was equivalent to half of the lowest standard dilution. The sensitivity of this assay was 15 pg/mL.

Statistics

Because the data were not normally distributed, log transformation was used, and paired *t* tests were used to compare the geometric means. The intraclass correlation coefficient, a measure of repeatability, was calculated.⁴

RESULTS

The infants were aged between 3 weeks to 6 months, with a mean age of 8.5 weeks. Twelve paired specimens were collected from 15 intubated babies. Of the 3 not included, 1 baby grew multiple pathogens from the endotracheal aspirate, 1 baby was clinically unstable and could not tolerate suctioning of both sites within the required time, and 1 baby was extubated before the investigators were notified of the admission to intensive care. Nine samples were included in the study. Three samples were excluded because the secretions were not refrigerated after collection or they were not collected within 5 minutes from both sites. Each specimen was 0.5 mL in volume.

The geometric mean of the endotracheal aspirate IL-2 levels was 224 pg/mL and that of the NPA levels was 183 pg/mL. The mean difference between endotracheal aspirate IL-2 levels and NPA levels in log₁₀ units was 0.09 (95% CI: -0.21, 0.39), which was not significantly different from 0 (*P* = .52).

Fig 1 summarizes the range of IL-2 levels found in the specimens and demonstrates the correlation between the 2 sets of values. The intraclass correlation coefficient was 0.83.

DISCUSSION

Investigation of the cytokine response to respiratory viral infections in children has been hampered by difficulties in obtaining appropriate samples for analysis. Adult studies frequently use bronchoalveolar lavage,⁵⁻⁷ a technique that can not be easily applied to children. On the other hand, NPAs are often used for diagnosis of viral respiratory tract infections in children. Investigators have measured cytokine levels in nasal washings and nasal mucosal biopsies as a potential model for the investigation of lower airway inflammation.⁸⁻¹⁰ One of the concerns about using NPA sampling is that the cytokine levels of the upper respiratory tract may not reflect those of the lower respiratory tract. To our knowledge, the correlation between cytokines in NPAs and the lower respiratory tract has not previously been studied.

An intraclass correlation coefficient of 0.83 suggests the IL-2 levels from nasopharyngeal and endotracheal aspirates have a high level of agreement. This study shows the level of at least 1 proinflammatory cytokine, IL-2, in NPAs is comparable to that in the lower respiratory tract of infants with RSV-positive bronchiolitis. The study also demonstrates that IL-2 is produced in the nasopharyngeal and endotracheal aspirates during severe RSV-positive bronchiolitis in babies. To our knowledge, this finding has not been previously reported.

Although these results provide evidence for the use of NPAs as an alternative to endotracheal aspirates in the measurement of cytokine responses in RSV-positive bronchiolitis, they may not apply to cytokine responses seen in other conditions. A further study to examine children with other types of viral respiratory tract infections is planned. The results of these studies will contribute to a larger study, which looks at the cytokine response in the

respiratory tract of infants with viral infections and the subsequent development of asthma.

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