Upper airway cellular pattern in infants with acute bronchiolitis: neutrophils or eosinophils?

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Abstract

Objective: To analyze the cellular pattern of nasopharyngeal secretions in infants with acute bronchiolitis (AB), focusing on the presence or absence of neutrophils and eosinophils.

Method: Hospitalized children with AB admitted to Hospital São Lucas, Porto Alegre, Brazil, between May and July 2002 were recruited. Nasopharyngeal aspirates were collected during the first 48 hours after admission. Slides were stained with May Grunwald and Giemsa. Total cell count and cellular viability were obtained in all samples.

Results: Thirty-eight infants with AB were enrolled. The mean age was 2.2 months (interquartile range: 1.2-3.5), and 21 subjects were male. Neutrophils were the predominant cells in the nasopharyngeal aspirates (median 95%, interquartile: 94-97). No eosinophils were found in the samples studied.

Conclusion: Our results suggest that eosinophils do not play a significant role in the pathophysiogenesis of AB. Infants with AB present a specific inflammatory response to viral infections, which is distinct from the immune response observed in asthma.

Introduction

Acute bronchiolitis (AB) is the most common cause of obstructive disease of the lower respiratory tract during the first two years of life. In developed countries, approximately 1% of patients require hospital admission. Despite mortality being low, the morbidity of this disease is significant among previously healthy infants.1 The factors which control the severity of the acute episode and the recurrence of wheezing are not yet fully understood.
The eosinophilic cationic protein (EPC), a protein which presents eosinophil granuloma and which presents a cytotoxic action in the airways, is increased in the secretions of patients with AB and wheezing during the first two years of life.2-4 These studies suggest the presence of an immunoreponse which, in AB, is predominantly triggered by eosinophils, which are cells that have a central role in the physiopathogenesis of allergic asthma. Nevertheless, studies which have analyzed cytological examinations from patients with AB demonstrate that differential cellularity of nasal secretions and bronchoalveolar lavage presented an absolute predominance of neutrophils.5-8 Therefore, the type of inflammatory response in the airways that characterizes this disease remains to be explained.

A number of different environmental and genetic factors appear to interfere in the development of allergic respiratory disease during the first years of life, with difference in atopic asthma prevalence even existing between developed and developing countries.9,10 There are no reports of studies analyzing the cellular response in the airways of infants with AB in developing countries. Knowledge about this type of cellular response in the respiratory apparatus of these patients is essential to improve the understanding of the physiopathogenesis of AB, and so be able to help in the development of new diagnostic modalities and therapies. Before more specific studies can be developed in order to elucidate this issue, it is necessary to evaluate the cytological examination results of infants with AB from a sample of a population from a developing country. The hypothesis of this study is that the role of the eosinophils in infants with AB is not important. The objective of the study is to analyze the differential cytological examination of nasopharyngeal aspirate from patients with AB, with specific reference to the presence of neutrophils and eosinophils.

Methods

Sample selection

Children younger than two years old were selected, suffering from their first episode of wheezing, with symptoms of acute viral infection (coryza and coughing during the previous 7 days), who required hospitalization at the Hospital São Lucas at the PUCRS because of respiratory difficulties or intolerance of oral route, during the period between May and July of 2002. All cases with previous diagnoses of cystic fibrosis, cardiopathy, congenital immunodeficiencies or chronic pulmonary disease were excluded. Also excluded were all patients who had used a topical or systemic corticoid or whose nasopharyngeal secretion sample was insufficient. Hypoxemia was assumed when saturation (measured by pulse oximetry on admission) was lower than 96%. These patients were indicated oxygen therapy.

Sample collection and processing

The nasopharyngeal aspirate (NPA) was collected with an aspiration probe number 6, introduced into the nasopharynx, connected to an aspiration system and a collecting bottle. All collections were performed during the first 48 hours after admission. The probe, after aspiration, was washed with 1.5 ml of saline solution.

One milliliter of the NPA was separated for the detection of respiratory syncytial virus (RSV), Adenovirus, Influenza and Parainfluenza, by means of direct immunofluorescence.

The remaining material was weighed and centrifuged (2,000 rpm for 2 minutes). The precipitate was suspended in 1 ml of phosphate buffered saline solution (PBS) and 40 µl of this suspension was used for the preparation of slides for differential cytology, in a cytocentrifuge (FANEM, São Paulo, Brazil, Mod. 218), at 500 rpm for 5 minutes. Two slides per patient were fixed with methanol and colored with May Grunwald Giemsa. Cells were analyzed according to their characteristic morphology, always by the same investigator (P.M.C.P.). Cell types, observed through an optical microscope, were expressed in percentages after 200 cells were counted per slide. The slide with the best coloring and cell distribution was chosen for counting. The count level of 200 cells per slide was chosen due to the infrequency of cells in the NPA, similar to that found in sputum or bronchoalveolar lavage.

Total cell count (TCC) and cellular viability were performed for all samples, using the exclusion method with trypan blue, in a Neubauer chamber (Boeco, Germany).

Statistical analysis

For the analysis of variables the t test and the Mann-Whitney tests were used to a significance level of 0.05.

Ethics

This study was approved by the commission for ethics in research at the Hospital São Lucas at the PUCRS. All those responsible for the patients who participated signed an informed consent form.

Results

Forty-five infants hospitalized for AB were recruited. Seven patients were excluded because the sample quantity was insufficient to process the cellular analysis. Thirty-eight infants were included. The demographic and clinical data is summarized in Table 1. Respiratory virus was detected in 29/38 (76%) NPA samples. Respiratory syncytial virus was detected in all samples. Also identified were adenovirus (2/38) and influenza (1/38), all associated with RSV infections. The majority of the patients required oxygen therapy (28/38 cases) and five patients needed mechanical
ventilation. The 10 infants who did not need oxygen therapy were hospitalized due to intolerance of oral route by small infants.

The median of TCC counts of the samples was $1.6 \times 10^6$ cells/ml (interquartile: 0.5-2.6), with a 95% viability (interquartile: 91-97). There was an absolute predominance of neutrophils in the NPA of the patients studied. No eosinophils were detected in the samples. The cytological examination of the NPA revealed a median of 95% neutrophils (interquartile: 94-97), 3% of macrophages (interquartile: 3-4), 1% of lymphocytes and 0% of epithelial cells or eosinophils. The findings from differential cytological examination of the NPA of the patients studied are presented in Figure 1. Neither the TCC nor the differential cytological testing of the NPA demonstrated any significant differences when analyzed in relation to the requirement for oxygen therapy (Table 2). Furthermore, no significant differences were detected when these variables were analyzed in relation to the requirement for mechanical ventilation, smoking family members or positivity for RSV in the NPA.

Discussion

This study found an absolute predominance of neutrophils in the nasopharyngeal aspirate of infants with AB. Furthermore, the presence of eosinophils was not detected in any nasal secretion sample. These results confirm the findings of earlier studies,$^{5,6,8}$ suggesting that neutrophils are the primary cells involved in the acute inflammatory response in the Airways of infants with AB. Additionally, our Results reinforce the questions being asked about the existence of a significant role for the eosinophils in the physiopathogenesis of AB.

No relationship was found between the total cell count or the numbers of neutrophils in the NPA and the severity of the AB episode (requirement for oxygen therapy or mechanical ventilation). Despite the sample of patients being too small for analysis of risk factors for severity, it is probable that a relationship between these variables and the severity of the condition really does not exist. The inclusion of a control group in future studies and also infants with infections of the upper respiratory tract could help to better explain this issue.

In this study nasal secretion samples were used to analyze the cellular response of infants with AB. The NPA samples were correctly collected and processed, taking into consideration the values found with TCC and cellular viability. The NPA may not be representative of the type of inflammatory response found in the lower respiratory tract. Nevertheless, with patients with AB, nasal secretions have been used as means of analysis of the immunoresponse of the lower airways. Nasopharyngeal secretions obtained from the NPA or nasal lavage are quick and easy to collect.

Table 1 - Demographic characteristics of patients studied

<table>
<thead>
<tr>
<th>Patients (n = 38)</th>
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<tbody>
<tr>
<td>Age (months), median</td>
</tr>
<tr>
<td>Sex, male (%)</td>
</tr>
<tr>
<td>Family history of atopy (%)</td>
</tr>
<tr>
<td>Positive RSV (%)</td>
</tr>
<tr>
<td>Oxygen therapy (%)</td>
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<tr>
<td>Mechanic ventilation (%)</td>
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Table 2 - Comparison between total cell count (TCC) and the differential cytological examination of the nasopharyngeal aspirate of infants with acute bronchiolitis regarding the presence of hypoxemia

<table>
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<tr>
<th>Hypoxemia</th>
<th>No hypoxemia</th>
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<tr>
<td>TCC (cells x 10^6/ml)</td>
<td>1.82 (0.86-3.0)</td>
</tr>
<tr>
<td>Percentage of neutrophils (%)</td>
<td>95 (93-97)</td>
</tr>
</tbody>
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*NS: non-significant.
A number of different studies described in literature have used nasal secretions as a method of diagnosis of inflammation of the respiratory apparatus. In wheezing infants, this method has often been used in the study of the immune response. The correlation between findings from the analysis of the inflammatory response of the upper and lower airways is not yet clear. There are, however, certain studies which demonstrate that there is a good correlation between these two locations. Joshi et al., performing simultaneous NPA and tracheal aspirate, and measuring the levels of IL-2 in nine infants with AB, found a significant correlation between the production of this cytokine in the upper and lower airways. Van Schaik et al. also found a high level of correlation between the levels of INF-γ/IL-4 in the NPA and the tracheal aspirate of infants with wheezing caused by viral respiratory infections. Other studies also describe similar results, correlating the levels of IgE, differential cytological test results and lymphocyte subtypes and interleukin levels, in relation to nasopharyngeal secretion and bronchiolar lavage or tracheal aspirate, in this group of patients.

With these results, and because of the ease of obtaining this type of sample, the NPA becomes one of the most often used methods for the analysis of the inflammatory response of patients with AB.

The predominance of neutrophils in the nasopharyngeal secretion in all of the patients studied, suggests that this type of cell must perform an important role in AB. This finding is similar to the results described by other studies which included the analysis of samples from the lower airways. The patients chosen for the current study, selected from a population from a developing country, present the same cellular response characteristics in their airways as patients from developed countries. From these results it can be suggested that the neutrophilic response in the airways of children with AB could perform a central role in inflammation, actively contributing to the processes obstructing the bronchioles. Additionally, this finding could be related to the acute cellular response of airways infected by RSV. The presence of other types of virus in the etiology of AB does not appear to be reflected by a different inflammatory cellular response. In this study, nine patients presented with negative results for respiratory virus in their NPA. Probably these cases were associated with other types of virus which were not tested for. Nevertheless, the NPA samples of these patients presented the same cellular response characteristics as those infected by RSV.

On the other hand, the absence of eosinophils in the nasopharyngeal secretions evaluated, when combined with the evidence found in literature, puts in doubt the hypothesis that AB could be a significant component of the allergic eosinophilic response (Th2). In diseases that are definitively allergic, patients present eosinophils in nasal lavage and in bronchoalveolar lavage. Stevenson et al. demonstrate that children under five years old, with only wheezing and viral respiratory infections, did not
present eosinophils in their bronchoalveolar lavage, in contrast to older children with asthma and atopic disease. Further evidence which also weakens this hypothesis is that the episodes of wheezing in the majority of infants tend to become less frequent and less severe with the passage of time, which does not occur with an atopic condition.20

Together with this information a relevant question comes up in relation to the origin of the elevated levels of EPC production in studies which have analyzed the protein in infants with AB.2,4 In a review article, Gleich claims that samples from lesions which are rich in neutrophils can present elevated levels of EPC.21 This claim originates from a study in which the presence of EPC was found in mature neutrophils, by means of indirect immunofluorescence and radioimmunoassay techniques.22 Therefore the concept that PCE could be a specific marker of inflammation associated with eosinophila, should be re-evaluated. Thus, and this hypothesis is no longer discussed in literature, all of the studies which found elevated levels of EPC in children with AB could simply be reflecting the degree of acute neutrophilic response found in these patients, since no study has documented the minimum presence of eosinophils in the airways of wheezing infants.

No control group of normal infants was included in this study, due to the difficulty of obtaining an NPA sample from children without respiratory infections. Earlier studies that have used nasal lavage from normal infants demonstrated that neutrophils are not the predominant cells at this location. Thus, the findings of this study appear to reflect the altered cellular response in the upper airways of infants with viral respiratory infections.

The results of this study permit the demonstration that a population of infants with AB from a developing country present the same type of cytological response profile found in developed countries. Taking these results into account, the role of the eosinophila in the physiopathogenesis of AB should be further clarified. The allergic response, in part mediated by eosinophils and found in asthma patients, appears not to participate in the physiopathogenesis of AB. Based on the results of this study and of previous studies, analyzing more specific markers for neutrophils and eosinophils by means of the immunocytochemistry of respiratory secretions from infants with AB, could definitley clarify the existence or non-existence of any participation by eosinophils in the physiopathogenesis of AB.

References


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