
Discrepancy between cytokine production from peripheral blood mononuclear cells and nasal secretions among infants with acute bronchiolitis

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Background: Many studies have measured cytokine production derived from peripheral blood mononuclear cells (PBMCs) to evaluate the immune response in acute bronchiolitis (AB), but no previous reports have examined the association between PBMC release of cytokines and concomitant airway immune response.

Objective: To determine whether interferon- γ (IFN- γ), interleukin 4 (IL-4), and IL-10 levels from PBMCs are associated with concurrent cytokine release in the airways of infants with AB.

Methods: Infants with acute viral-associated first episode of wheezing who required hospitalization between May and September 2002 were recruited. Nasopharyngeal aspirates (NPAs) and PBMC samples were collected simultaneously. The concentrations of IFN- γ , IL-4, and IL-10 in NPA and PBMC supernatants were determined by enzyme-linked immunosorbent assay.

Results: Twenty infants with AB were enrolled in the study of whom 17 (85%) had positive NPA immunofluorescence results for viral detection and respiratory syncytial virus. Median total cell count and viability from NPA samples were 2.2×10^6 cells/mL (SD, 1.7 cells/mL) and 92% (SD, 6.0%), respectively. There was a significant correlation between IL-4 levels from NPA and PBMC samples ($r = 0.5$, $P = .02$); however, we did not find an association between IFN- γ and IL-10 levels.

Conclusions: Cytokines produced by in vitro PBMCs may not necessarily reflect the concurrent cytokine pattern production at the mucosal surface in the respiratory tract of infants with AB. Further studies are required to determine whether peripheral blood is a reliable sample for airway inflammation evaluation and to explain the discrepancies of cytokine productions found in this study.

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INTRODUCTION

Acute bronchiolitis (AB) is the most common lower airway tract infection in infancy, resulting in high morbidity worldwide.¹ The immune response to this illness, often caused by respiratory syncytial virus (RSV), has been widely studied during the last 2 decades.² However, the role of immune response (T_H1/T_H2 lymphocyte cytokine production, neutrophil and eosinophil responses, and inflammatory mediators) in respiratory viral infections and the association with predisposition to AB and severity of disease are not yet well understood.^{3–8} Furthermore, T_H1/T_H2 immune responses in AB have shown controversial results in previous reports.^{3,4,7,9,10} The different sites from where cytokine levels are analyzed and the methods of evaluation of the immune response used in these studies may contribute to these contradictory findings.

Many studies have measured cytokine production derived from peripheral blood mononuclear cells (PBMCs) to evalu-

ate the immune response in AB.^{3,9,11,12} To our knowledge, there are no previous reports of the association between PBMC release of cytokines and the concomitant airway immune response. It has been a matter of speculation whether the PBMC cytokine production reflects the immune response in the airways of infants with AB. The aim of this study was to determine whether interferon- γ (IFN- γ), interleukin 4 (IL-4), and IL-10 levels from PBMCs are associated with concurrent cytokine release in the airways of infants with AB.

METHODS

Patients

Infants with acute viral-associated first episode of wheezing who required hospitalization between May and September 2002 were recruited. AB was defined as coryza, cough, and respiratory distress associated with the first episode of wheezing. Patients with a history of more than 7 days of respiratory symptoms, cardiopulmonary diseases, immunodeficiencies, and history of prematurity or previous wheezing episodes were excluded. Patients who had required steroid treatment were also excluded.

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Nasal Secretion Samples

Nasopharyngeal aspirates (NPAs) were collected in the first 48 hours of admission, using the routine hospital procedure, by placing a polyethylene suction catheter into the nasopharynx and applying gentle suction with the patient in a sitting position. Secretions were collected in a sterile mucous trap. Additional secretions remaining in the catheter were recovered by flushing with 1 mL of phosphate-buffered saline solution. The samples were then centrifuged at 2,000 rpm for 2 minutes and the supernatant removed and stored at -80°C for cytokine analysis. Samples were tested for the presence of RSV, parainfluenza types 1, 2, and 3, influenza groups A and B, and adenovirus by direct immunofluorescence. Total cell count and viability were evaluated with a hemocytometer (Boeco, Hamburg, Germany) with the trypan blue exclusion method.

Collection of Peripheral Blood and Isolation of Mononuclear Cells

Two milliliters of peripheral blood was collected by venipuncture in the first 24 hours of admission and samples stored in lithium-heparin tubes before analyses. The PBMCs were isolated by centrifugation over a Ficoll-Hypaque (Sigma Chemical Co, St. Louis, MO) gradient (900g, 30 minutes). Cells were counted by means of microscopy (100 $\times$) and viability always exceeded 95%, as judged from their ability to exclude trypan blue (Sigma). The NPAs and peripheral blood samples were collected simultaneously.

Cell Cultures

The PBMCs (1.5×10^6 cells/mL) were stimulated with 1% phytohemagglutinin (Gibco/Invitrogen, Carlsbad, CA) in complete medium (RPMI-1640, supplemented with 0.5% gentamicin, 1% glutamine, 1% HEPES, and 10% fetal calf serum; all from Sigma) for 48 hours at 37°C in 5% carbon dioxide. After the incubation, supernatants were transferred to Eppendorf tubes, centrifuged (900g, 15 minutes), and stored at -80°C before analysis by enzyme-linked immunosorbent assay (ELISA).

Cytokine Analysis

The concentrations of IFN- γ , IL-4, and IL-10 in NPA and PBMC supernatants were determined by ELISA (Pharmin-gen, San Diego, CA), with a detection limit of 7.8 pg/mL. When concentration of the cytokine was undetectable, values of half of the lowest detectable concentration were considered for statistical analysis. The Pearson or Spearman correlation test was used to evaluate the correlation between cytokine levels from NPA and PBMC samples, according to variable distribution. Statistical significance was defined as $P < .05$. The study was approved by the São Lucas Hospital Ethics Committee. All parents gave written informed consent.

RESULTS

Twenty infants with AB were enrolled in the study. Their mean age was 3.5 months (SD, 2.6 months), and 12 (60%) were male. The NPA immunofluorescence result was positive

for viral detection in 17 patients (85%). All 17 of these patients were positive for RSV. Adenovirus associated with RSV infection was detected in 1 patient.

All NPA and PBMC samples were processed. The median total cell count and viability from NPA samples were 2.2×10^6 cells/mL (SD, 1.7 cells/mL) and 92% (SD, 6.0%), respectively. The IL-10 measurements could not be obtained in 1 NPA sample due to small volume.

The correlation of cytokine production between NPAs and PBMCs is shown in Figure 1. There was a significant correlation between IL-4 levels from NPA and PBMC samples ($r = 0.5$, $P = .02$); however, we did not find an association between IFN- γ and IL-10 levels from both sites. Correlations did not differ significantly when RSV-positive subjects were analyzed separately.

DISCUSSION

This study has demonstrated that cytokine production from PBMCs may not reflect the cytokine release occurring simultaneously in the airways. The inflammatory response in AB has been evaluated by many methods and from different tissues.^{3-6,13} It can be assumed that airways inflammation is better evaluated by examination of respiratory secretions than by other tissues, such as peripheral blood. However, PBMCs could be useful as a method for assessing markers (cytokines) of severity or prognosis rather than for understanding the pathogenesis of AB. To our knowledge, this is the first study to compare the cytokine production between peripheral blood with production in the airways of infants with AB.

We have found a significant correlation between IL-4 levels from peripheral blood and nasal secretions. However, IFN- γ and IL-10 did not show any significant association between these 2 sites. The NPA total cell counts and viability results indicate that these samples were properly collected, and consequently the cytokine concentrations may be considered reliable. Furthermore, even though PBMC cytokine production was stimulated with phytohemagglutinin, we speculated that both methods used in the present study for cytokine production analysis between the airways and peripheral blood (supernatant of aspirate vs supernatant of PBMC culture) should demonstrate a correlation if the immune response in these tissues were similar.

Our results may be influenced by many factors. Type of virus, stage of the disease, and source of cytokine production may affect cytokine production in different tissues. Different cytokine productions between peripheral blood and respiratory secretions may be found in viral infections that are restricted to the airways (as in RSV infection). In the present study, most cases were RSV positive, and our results did not differ when only RSV-infected patients were analyzed separately.

The fact that samples were collected in the acute phase of AB and also that cytokines are largely produced by different cell types in different tissues could explain the lack of association observed with IL-10 and IFN- γ responses. Bont and colleagues¹³ have demonstrated that PBMC IL-10 response in

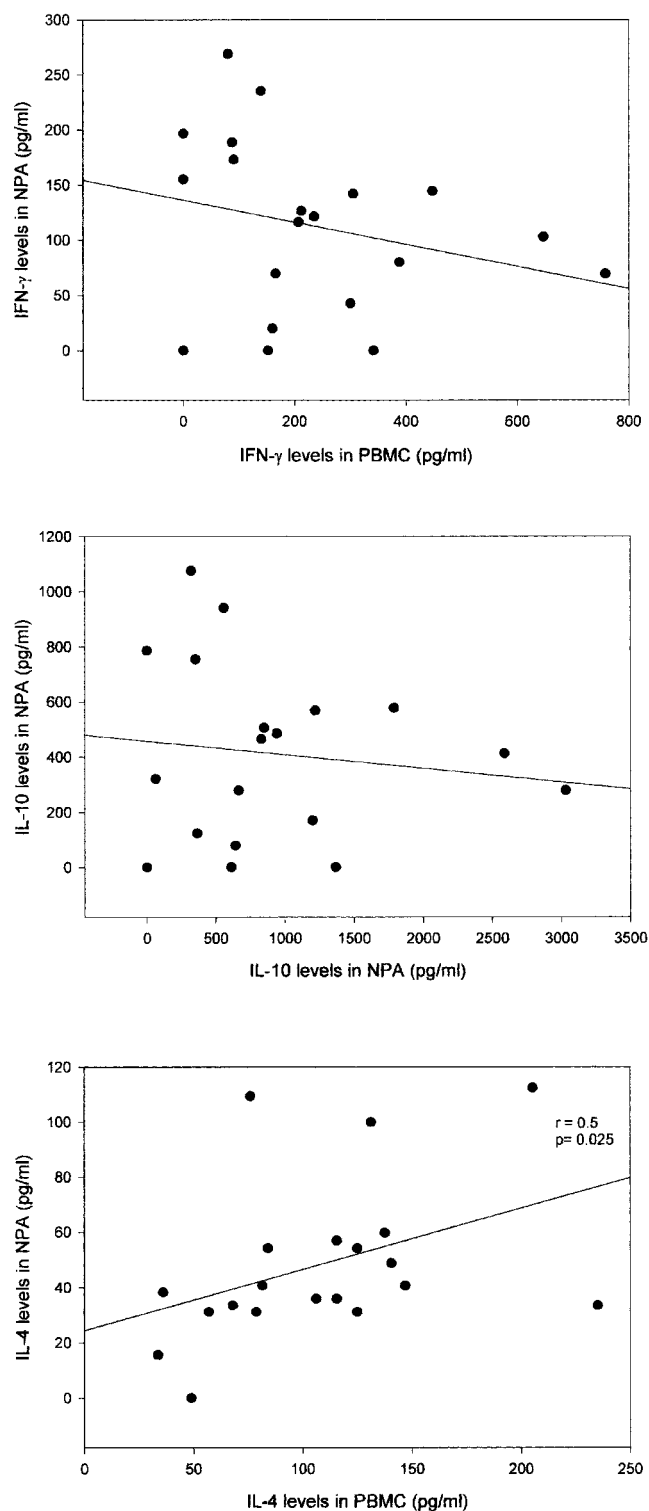


Figure 1. Correlation of cytokine production (interferon- γ [IFN- γ], interleukin 10 [IL-10], and IL-4) between nasopharyngeal aspirates (NPAs) and phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs).

AB may be only associated with recurrent wheezing at the convalescent phase of AB. Furthermore, IL-10, like other cytokines, is produced by respiratory epithelial cells,¹⁴ which could increase local release of this cytokine in the viral-infected airways. In our opinion, this evidence could partially explain the different levels of cytokine production between these 2 sites.

The association of IL-4 released by PBMCs and airway cells may not have a clear explanation. There is significant controversy regarding the role of either T_H1 or T_H2 immune responses.^{3,4,7,9,10} However, unlike the source of production of IL-10 that includes respiratory epithelial cells, IL-4 is secreted predominantly by lymphocytes. This could explain the IL-4 results (lymphocytes from the mucosa of the respiratory tract and lymphocytes from peripheral blood).

Previous reports have demonstrated that nasal secretions in AB may reflect inflammation in the lower airways.^{4,5,15} Therefore, during a lower respiratory tract viral infection, as in AB, the upper and the lower airways seem to be similarly affected. However, a difference between the inflammatory response from peripheral blood and the mucosal surface of the respiratory tract was demonstrated by one previous report. Ingram et al⁶ have shown that the level of airway eosinophilic cationic protein from many infants and children with wheezing is greater than 20-fold higher than parallel measurements of this mediator in the blood. This finding suggests that the evaluation of the inflammatory response in peripheral blood may underestimate the specific response in the airways. Even though the methods of cytokine production used in our study in NPAs and PBMCs were different, we would expect a significant association between these 2 sites if the immune response would be naturally similar.

In summary, our findings suggest that cytokines produced by in vitro PBMCs may not necessarily reflect the concurrent cytokine pattern production at the mucosal surface in the respiratory tract in infants with AB and that immune response is organ dependent. The present data indicate that studies that assess airway inflammation by PBMC cytokine production should be interpreted with caution. Further studies are required to determine whether peripheral blood is a reliable sample for airway inflammation evaluation and also to explain the discrepancies of cytokine productions found in the present study.

REFERENCES

1. Dubois DB, Ray CG. Viral infections of the lower respiratory tract. In: Taussig LM, Landau LI, editors. *Pediatric Respiratory Medicine*. St. Louis, MO: Mosby; 1999:572–579.
2. van Schaik SM, Welliver RC, Kimpen JL. Novel pathways in the pathogenesis of respiratory syncytial virus disease. *Pediatr Pulmonol*. 2000;30:131–138.
3. Román M, Calhoun WJ, Hinton KL, et al. Respiratory syncytial virus infection in infants is associated with predominant T_H2 -like response. *Am J Respir Crit Care Med*. 1997;156:190–195.
4. van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC 2nd, Welliver RC. Increased production of INF- γ and

-
- cysteinyl leukotrienes in virus-induced wheezing. *J Allergy Clin Immunol.* 1999;103:630–636.
5. Everard ML, Swarbrick A, Wraitham M, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. *Arch Dis Child.* 1994;71:428–432.
 6. Ingram JM, Rakes GP, Hoover GE, Platts-Mills TA, Heymann PW. Eosinophil cationic protein in serum and nasal washes from wheezing infants and children. *J Pediatr.* 1995;127:558–564.
 7. Brandenburg AH, Kleinjan A, van het Land B, et al. Type 1-like immune response is found in children with respiratory syncytial virus infection regardless of clinical severity. *J Med Virol.* 2000;62:267–277.
 8. Mobbs KJ, Smyth RL, O’Hea U, Ashby D, Ritson P, Hart CA. Cytokines in severe respiratory syncytial virus bronchiolitis. *Pediatr Pulmonol.* 2002;33:449–452.
 9. Tripp RA, Moore D, Barskey A IV, et al. Peripheral blood mononuclear cells from infants hospitalized because of respiratory syncytial virus infection express T helper-1 and T helper-2 cytokines and CC chemokine messenger RNA. *J Infect Dis.* 2002;185:1388–1394.
 10. Welliver RC, Garofalo RP, Ogra PL. β -chemokines, but neither T helper type 1 nor T helper type 2 cytokines, correlate with severity of illness during respiratory syncytial virus infection. *Pediatr Infect Dis J.* 2002;21:457–461.
 11. Bont L, Heijnen CJ, Kavelaars A, et al. Peripheral blood cytokine responses and disease severity in respiratory syncytial virus bronchiolitis. *Eur Respir J.* 1999;14:144–149.
 12. Díaz PV, Calhoun WJ, Hinton KL, et al. Differential effects of respiratory syncytial virus and adenovirus on mononuclear cell cytokine responses. *Am J Respir Crit Care Med.* 1999;160:1157–1164.
 13. Bont L, Heijnen CJ, Kavelaars A, et al. Monocyte IL-10 production during respiratory syncytial virus bronchiolitis is associated with recurrent wheezing in a one-year follow-up study. *Am J Respir Crit Care Med.* 2000;161:1518–1523.
 14. Bonfield TL, Konstan MW, Burfeind P, Panuska JR, Hilliard JB, Berger M. Normal bronchial epithelial cells constitutively produce the anti-inflammatory cytokine interleukin-10, which is downregulated in cystic fibrosis. *Am J Respir Cell Mol Biol.* 1995;13:257–261.
 15. Joshi P, Kakakios A, Jayasekera J, Issacs D. A comparison of IL-2 levels in nasopharyngeal and endotracheal aspirates of babies with respiratory syncytial viral bronchiolitis. *J Allergy Clin Immunol.* 1998;102:618–620.
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