

Peripheral blood eosinophil counts and eosinophil cationic protein content of respiratory secretions in bronchiolitis: relationship to severity of disease

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Infants and young children with acute viral respiratory illness were studied to determine the association of peripheral blood eosinophil counts and concentrations of eosinophil cationic protein (ECP) in nasopharyngeal secretions with the development and severity of bronchiolitis. Subjects included those with upper respiratory illness (URI) alone, pneumonia or bronchiolitis. Controls consisted of healthy infants, and those hospitalized with non-respiratory illnesses. While peripheral blood eosinophil counts were suppressed in all infected infants greater than two months of age, eosinophil counts in patients with bronchiolitis were significantly greater than in those with URI alone. ECP concentrations were significantly greater among individuals with bronchiolitis than other infected infants. For bronchiolitis cases with detectable peripheral blood eosinophils, eosinophil counts correlated weakly and inversely with oxygen saturations. In contrast, ECP concentrations were strongly inversely correlated with initial oxygen saturation. ECP concentrations were also significantly correlated with peripheral blood eosinophil counts. Viral infections suppress peripheral blood eosinophil counts in infants greater than two months of age, although the effect is somewhat overcome in patients with bronchiolitis. The form and severity of bronchiolitis is much more strongly related to degranulation of eosinophils in the respiratory tract than to peripheral blood eosinophil counts.

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Individuals with asthma are known to have increased peripheral blood eosinophil counts (1–3) and increased concentrations of eosinophil cationic protein (ECP) in their respiratory secretions (4), particularly after allergen challenge (5). Peripheral blood eosinophil counts correlate with the severity of asthma and with reductions in pulmonary function (1, 4) and with the degree of airway hyperreactivity (2, 3). Concentrations of ECP in respiratory secretions also correlate with severity of illness (4) and increase at the time of late-phase allergic reactions in the airway (5).

While eosinophils appear to be important in the pathogenesis of both extrinsic (allergen-induced) and intrinsic (frequently viral-induced) asthma (6) (7), there has been comparatively little investigation of the potential role of eosinophils in bronchiolitis, a

wheezing-associated disease of infants and young children precipitated by viruses. Concentrations of ECP in respiratory secretions of infants with respiratory syncytial virus (RSV) bronchiolitis are significantly greater than those of infants with other forms of illness due to RSV (8), suggesting a role for eosinophils in the pathogenesis of bronchiolitis. We therefore wondered if peripheral blood eosinophil counts were abnormal in infantile bronchiolitis. Previous attempts to determine eosinophil counts in bronchiolitis have been inconclusive (9, 10), and there has been no attempt to relate these to severity of illness. The present study was therefore undertaken to determine peripheral blood eosinophil counts in infants with various forms of viral respiratory illness, and the relationship of these to the form and severity of illness. Results were compared to the

Table 1. Demographic features of study subjects

	Form of illness:				
	URI	Pneumonia	Bronchiolitis	Non-respiratory	Healthy
Number enrolled	34	17	67	24	38
Age, months (Mean $\pm$ S.D.)	4.3 $\pm$ 5	4.7 $\pm$ 5	4.3 $\pm$ 4	4.5 $\pm$ 5	11.1 $\pm$ 3
Caucasian	20 (59)	15 (88)	44 (66)	15 (63)	24 (63)
Males	20 (59)	9 (53)	28 (42)	14 (58)	22 (58)

ECP content of nasopharyngeal secretions (NPS) obtained from these same patients.

Patients and methods

Study subjects – This study was approved by the Institutional Review Board of Children's Hospital of Buffalo, and parents of each subject gave written informed consent. The study population consisted of 120 infants and young children from birth to 18 months of age who were hospitalized because of acute viral respiratory infection. Individuals referred to as RSV-positive had RSV infection documented by identification of viral antigen in nasopharyngeal secretions using indirect immunofluorescence (11) and had RSV recovered in cell culture. Individuals referred to as RSV-negative were recruited before the onset of the RSV season, and were negative for RSV by both indirect immunofluorescence and cell culture infectivity. Various features of the study participants are shown in Table 1. Patients with upper respiratory illness alone had no abnormal findings on auscultation of the chest, negative chest radiographs as determined by a radiologist, and oxygen saturations of $\geq 96\%$ (see below). Patients diagnosed as having pneumonia had crackles but not wheezes on auscultation of the chest, and had infiltrates on chest radiographs. Patients diagnosed as having bronchiolitis either had wheezing on auscultation of the chest or, in a few cases, had neither crackles nor wheezing on auscultation of the chest, had chest radiographs interpreted as showing hyperinflation without infiltrates and had oxygen saturations of $< 96\%$. Of 64 patients defined as having bronchiolitis, 9 (14.8%) had a history of previous wheezing. These patients with recurrent wheezing did not differ in any of the demographic factors listed in Table 1, in peripheral blood eosinophil counts or in ECP content of nasopharyngeal secretions from those individuals with bronchiolitis who had not had previous wheezing episodes. Therefore all of these patients were included in the study population.

All patients with bronchiolitis received 1 to 3 treatments with aerosolized beta-adrenergic agents at the time that blood samples were obtained for eosinophil counts, and before secretions were obtained for ECP determinations. However, patients

who had been treated with corticosteroids or oral beta-adrenergic agents before the time of presentation to the Emergency Room were not recruited into the study because of the reported effects of these compounds on peripheral blood eosinophil counts or ECP content of secretions (12–14).

Because previous studies have suggested that eosinophil counts in nasal mucosa (15–17) and peripheral blood (16) (17) may be greater in the newborn period than at 2 to 3 months of age, peripheral blood eosinophil counts and ECP content of NPS in infants less than 2 months of age were considered separately. These infants included 9 with URI alone, 4 with pneumonia, and 10 with bronchiolitis. Of these 7 URI, all 4 pneumonias, and 9 bronchiolitis cases were due to RSV infection.

Controls consisted of healthy infants ($n = 38$) who had peripheral blood obtained at the time of normal newborn examinations at 9 to 12 months of age, as well as "ill" infants ($n = 24$) 3 to 18 months of age who had peripheral blood obtained at the time of hospitalization for non-respiratory illnesses (mostly idiopathic seizures, trauma and gastroesophageal reflux).

Sample collection – Blood samples for determination of peripheral blood eosinophil counts were obtained at the time of evaluation of study subjects in the emergency room before admission. Eosinophil counts were determined by multiplying the percentage of eosinophils in a 100-cell differential by the total white blood cell count. Samples of nasopharyngeal secretions for ECP determination were obtained by passing a size 8 polyethylene catheter into the nasopharynx via the nares and applying gentle suction. Samples were rinsed into a collection vial using 0.5 ml of PBS. Samples were kept frozen at -70°C . ECP was measured using a radioimmunoassay (Pharmacia, Uppsala, Sweden). No attempt was made to correct ECP concentrations to any substance present in secretions because of the lack of documentation that this enhances accuracy of measurements (18). Treatment of NPS samples with detergent or acid did not enhance the measured ECP content.

Severity of illness – Severity of illness was defined

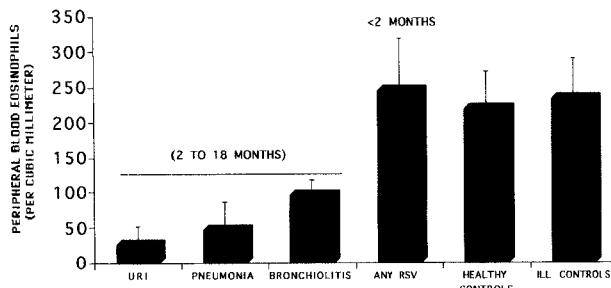


Fig. 1. Peripheral blood eosinophil counts in infants with viral infections, healthy infants and ill controls. The first three bars indicate all infants 2 to 18 months of age with different forms of viral respiratory infection. The fourth bar refers to RSV-infected infants less than two months of age, with any form of respiratory illness. The fifth bar indicates healthy controls. The sixth bar indicates infants with non-respiratory illnesses. Numbers of subjects: I = 20, II = 12, III = 54, IV = 23, V = 24, VI = 38.

by the oxygen saturation while breathing room air obtained at the time of the initial Emergency Room evaluation, using the Biox 3740 pulse oximeter (Ohmeda, Louisville, CO). Final readings were those obtained when the oximeter revealed a normal signal, and when the heart rate on the monitor matched that of the patient.

Statistical analysis – The BMDP statistical software program (University of California: Berkeley) was used for all data analysis. Differences in eosinophil counts and differences in ECP concentrations between patient groups were performed using the Mann-Whitney statistic. repeated measures paired comparisons were performed. Correlations of eosinophil counts and ECP concentrations with measures of severity of illness were performed using Spearman correlation coefficients with adjustments for multiple comparison testing. All mean values are expressed as mean ± standard error.

Results

Peripheral blood eosinophil counts in controls

Eosinophil counts in healthy controls ($n = 38$) were 221.6 ± 36 , and eosinophil counts in individuals hospitalized with non-respiratory illnesses ($n = 24$) were 233.4 ± 46 (Fig. 1). These values are well within the reported normal range for infants and children in this age group (16, 17, 19). The difference between these two groups was not statistically significant. Median values for eosinophil counts were 58 in healthy controls and 176 in those with non-respiratory illnesses.

Peripheral blood eosinophil counts in infants <2 months of age

The mean ($\pm$ S.E.) peripheral blood eosinophil counts for infants <2 months of age at the time of

respiratory infection was 244 ± 57 (Fig. 1). The median value was 157. For infants in this age group, peripheral blood eosinophil counts did not differ by form of illness. Thus, for subjects with URI alone, the mean peripheral blood eosinophil count was 226 ± 97 , while for patients with pneumonia it was 259 ± 164 , and for patients with bronchiolitis it was 229 ± 71 . None of these values differed in a statistically significant fashion from eosinophil counts in healthy controls or the control group with non-respiratory illnesses (Fig. 1).

Peripheral blood eosinophil counts in infants 2 to 18 months of age with respiratory illness

Peripheral blood eosinophil counts in older infants with various forms of respiratory illness are analyzed by form of illness in Fig. 1 and Table 2. Fig. 1 includes data on all infants, while Table 2 separates the study groups into those with RSV infection and those with infection due to other agents. Peripheral blood eosinophil counts appeared to be markedly suppressed in individuals with RSV infection or infants with RSV-negative infection in this age group. Individuals with RSV-positive URI had 25 ± 25 cells/mm² and patients with RSV-positive pneumonia had eosinophil counts of 46 ± 34 cells/mm². Peripheral blood eosinophil counts in patients with RSV bronchiolitis appeared to be intermediate between those with non-wheezing RSV illness and control groups. Eosinophil counts in patients with RSV bronchiolitis were 95 ± 23 cells/mm². Median values were zero in each illness group. Eosinophil counts in infants with RSV-negative respiratory illnesses exhibited approximately the same pattern. Statistically significant differences were found when comparing peripheral blood eosinophil counts of all 54 patients age 2 to 18 months with bronchiolitis (93.5 ± 21.6 cells) versus all 19 patients age 2 to 18 months with URI alone (31.5 ± 16.2 cells/mm², $p < .05$, data from combined groups

Table 2. Peripheral blood eosinophil counts in infants 2 to 18 months of age with respiratory infections

Study group	Eosinophils/mm ³ (means $\pm$ S.E.)
RSV-positive	
URI (9)	25 $\pm$ 25
pneumonia (7)	46 $\pm$ 34
bronchiolitis (49)	95 $\pm$ 23
RSV-negative	
URI (11)	37 $\pm$ 22
pneumonia (5)	25 $\pm$ 21
bronchiolitis (5)	76 $\pm$ 76

Degree of significance:

RSV bronchiolitis vs. RSV URI: $p = 0.09$

All bronchiolitis vs. all URI: $p < 0.05$

Table 3. Peripheral blood eosinophil counts in healthy males and females and in those 2 to 18 months of age with URI or bronchiolitis

Subject group	Peripheral blood eosinophil counts per mm ³		
	Males	Females	Probability
All bronchiolitis	173.5 ± 48	46.4 ± 18	<0.02
All URI	16.2 ± 12	47.8 ± 31	<.25
Healthy controls	223 ± 72	218 ± 105	<.25

not shown in table). The difference in peripheral blood eosinophil counts between patients with RSV bronchiolitis and those with URI due to RSV (95 ± 23 vs. 25 ± 25) was not statistically significant ($p = .09$).

Because eosinophil counts were greater in infants less than 2 months of age regardless of the form of illness, all subsequent analyses were carried out in 2- to 18-month-old children only, except when noted. Further analysis of the bronchiolitis group revealed that peripheral blood eosinophil counts were higher in males with bronchiolitis (173.5 ± 48 cells) than in females with bronchiolitis (46.4 ± 18 cells, $p < .02$, Table 3). This difference in peripheral blood eosinophil counts between males and females was not observed in patients with URI alone or in healthy controls (Table 3).

Eosinophil counts were similar in females with bronchiolitis or URI, but were significantly higher in males with bronchiolitis (173.5 ± 48) than in males with URI (16.2 ± 12 , $p < .005$).

ECP concentrations analyzed by form of illness

Table 4 shows the ECP content of nasopharyngeal secretions of all infants with acute respiratory illness. In contrast to the results of peripheral blood eosinophil analyses, the ECP content of nasopharyngeal secretions of infants <2 months of age with different forms of respiratory illness was similar to that of older infants with the same form of respiratory illness. For each age group shown in the table, the concentration of ECP in subjects with bronchiolitis was greater than the ECP content of the corresponding group with URI alone (each $p < .05$). The concentration of ECP among patients with bronchiolitis is also greater than the concentration in subjects with pneumonia for each group except the RSV-positive group 2 to 18 months of age (middle column).

ECP concentrations were quite similar in males (235 ± 24) and females (241 ± 24) with bronchiolitis.

Peripheral blood eosinophils, ECP and severity of illness

Correlations of peripheral blood eosinophil counts and the ECP content of nasopharyngeal secretions

Table 4. ECP content of nasopharyngeal secretions of infants from birth to 18 months

Form of illness	ECP Content (ng/ml of fluid) for Subjects:		
	< 2 Months of Age	2 to 18 Months; RSV-Positive	2 to 18 Months; RSV-Negative
URI alone	88 ± 33 (8)	51 ± 18 (4)	59 ± 30 (12)
Pneumonia	67 ± 25 (4)	149 ± 90 (4)	27 ± 10 (6)
Bronchiolitis	220 ± 44 (10)	254 ± 19 (38)	144 ± 49 (5)

Values represent mean ± S.E., with number tested in parenthesis.

For each age group, ECP content in patients with bronchiolitis is greater (each $p < .05$) than patients with URI alone.

For each age group, ECP content in patients with bronchiolitis is greater (each $p < .05$) than patients with pneumonia, except for RSV-positive cases (center column).

with initial oxygen saturation values were completed in patients with bronchiolitis. For patients (both sexes included) with RSV-positive or RSV-negative bronchiolitis (aged 2 to 18 months), there was no statistically significant correlation of peripheral blood eosinophil counts with oxygen saturation ($r = -.24$, $p = .10$). When only male infants were evaluated, a negative correlation which attained statistical significance was observed ($r = -.49$, $p < .05$).

In contrast, the ECP content of nasopharyngeal secretions was much more strongly correlated with oxygen saturation. For infants with RSV-positive or RSV-negative bronchiolitis, the correlation coefficient was $-.55$, $p < .001$ (Fig. 2). All correlations were strengthened somewhat by including subjects with upper respiratory illness alone or pneumonia, since the vast majority of these individuals had low eosinophil counts, low ECP values and high oxygen saturations.

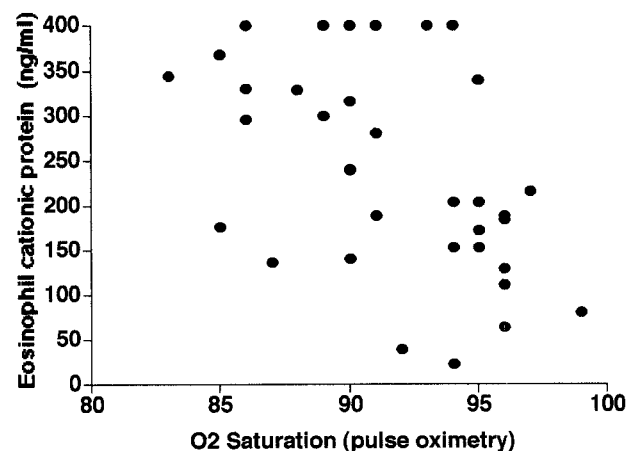


Fig. 2. The relationship of eosinophil cationic protein concentrations in nasopharyngeal secretions to oxygen saturation. Infants 2 to 18 months of age with bronchiolitis are included in the figure. The correlation coefficient is $-.55$, $p < .001$.

Relationship of peripheral blood eosinophil counts to ECP content of secretions

A statistically significant correlation ($r = .33$, $p < .025$) between peripheral blood eosinophil counts in infants with bronchiolitis ($n = 41$) and the ECP content of nasopharyngeal secretions was observed. The coefficient of correlation was somewhat stronger for males with bronchiolitis, although not statistically significant ($r = .48$, $p = .06$), probably because of a smaller sample size ($n = 15$).

Discussion

This study was undertaken to determine whether peripheral blood eosinophil counts are increased in bronchiolitis, as they are in asthma (1–3). While a limited trend was observed (which was particularly evident in males), the present study also identified other factors which influence peripheral blood eosinophil counts in subjects with bronchiolitis. These include age and the degree of reduction of peripheral blood eosinophilia related to infection itself. Infected infants less than two months of age were studied separately because infants of this age have been reported to have higher circulating eosinophil counts than older infants (16, 17). In the present study, infected infants less than 2 months of age had higher eosinophil counts than older infected infants. Also, eosinophil counts in infected subjects older than 2 months were less than those of healthy controls, or those with non-respiratory illnesses. In the 2- to 18-month age group, eosinophil counts in subjects with bronchiolitis were slightly higher than among those with URI alone, an effect essentially observed only in males. This suggests that infection reduced eosinophil counts in this age group, and that this suppression could be partially overcome by some factor which is active in males with bronchiolitis. Studying only male individuals, a weak negative correlation (-0.49 , $p < .05$) of peripheral blood eosinophil counts with oxygen saturation was noted. This further suggests that, once the suppressive factor is overcome, eosinophils may then participate in pathogenetic mechanisms.

This study also attempted to determine if eosinophils are activated during infection in the respiratory tract. As in a previous study of a separate group of individuals with RSV infection (8), current study subjects with bronchiolitis had increased concentrations of ECP in nasopharyngeal secretions. The present study extends the previous findings by demonstrating that the association of ECP concentrations in secretions with both the form and severity of illness was much stronger than that of eosinophil counts in peripheral blood. The ECP content of secretions of infants with bronchiolitis was greater than that of

individuals with other forms of respiratory illness, regardless of age or gender. In addition the correlation of ECP content to severity of illness was much stronger than that of peripheral blood eosinophil counts to severity of illness, and was highly statistically significant ($p < .001$). It has been suggested (20) that 100 times as many eosinophils reside in human tissues, particularly mucosal tissues, as circulate in the peripheral blood. Therefore the activation of respiratory tract eosinophils would be expected to correlate more closely with illness than events which influence eosinophils in peripheral blood. The fact that males and females with bronchiolitis had equal ECP concentrations in secretions, while males had higher peripheral blood eosinophil counts, also suggests that local factors cause eosinophil degranulation in the respiratory mucosa. These local factors appear to be independent of the factors which suppress peripheral blood eosinophil counts. ECP may contribute to the pathogenesis of bronchiolitis by causing sloughing of respiratory epithelium (21) or increasing airway mucus production (22).

Previous studies of peripheral blood eosinophil counts in bronchiolitis have been inconclusive. Simon & Jordan (9) studied children less than five years of age with first episodes or multiple episodes of wheezing. Patients with RSV-positive first episodes of wheezing all had eosinophil counts of less than 247/ml of peripheral blood, while 38% of 48 RSV-negative cases had this degree of eosinophilia. Eighteen percent of children with asthmatic bronchitis had similar degrees of eosinophilia. Unfortunately, all cases of bronchiolitis were seen during the time that RSV would have been present in the community and cell culture alone was used to detect RSV infection. The use of cell culture alone may miss a significant percentage of individuals with RSV infection (11). Therefore, many "RSV-negative" subjects may have had RSV infection. More importantly, eosinophil counts in patients with bronchiolitis were not compared to individuals with URI alone, or to normal controls. Therefore it was impossible to determine if blood eosinophilia was less suppressed in bronchiolitis patients than in infected controls. Laing and colleagues (10) found no difference in peripheral blood eosinophil counts between bronchiolitis patients and controls without respiratory illness. No control group with respiratory illness was included, so, again, it was not possible to determine whether eosinophil counts were greater in patients with bronchiolitis than in those with URI alone. Two studies (10) (23) have come to different conclusions as to whether eosinophils are present in nasopharyngeal secretions of patients with acute bronchiolitis. All of the above data are consistent with the concept that peripheral blood eosinophil counts are, at most, only slightly different in bron-

chiolitis patients than in controls. In the present study, the presence of detectable eosinophils in peripheral blood was weakly associated with the development of bronchiolitis, and with severity of illness. The associations were evident in male infants in particular. The lack of a stronger correlation is probably due in part to the multiple factors (age, gender and suppression by infection) which can also affect eosinophil counts.

In the present study, eosinophil counts among infants with RSV or other viral infections were markedly lower than in controls. A variety of bacterial and viral illnesses are known to suppress eosinophil counts in peripheral blood (24). The mechanism is unclear, but it does not appear to be simply due to increased corticosteroid levels as a result of stress. Eosinophils were not suppressed in infants hospitalized with non-respiratory illnesses in the present study in whom a stress response also could be presumed. In animal models the degree of eosinophil suppression is not correlated with serum corticosteroid concentrations and, in fact, the suppression occurs even in adrenalectomized animals (25, 26).

This study has the potential limitation of using eosinophil counts, calculated by multiplying percentages of eosinophils in 100-cell differentials times total white blood cell counts, rather than determining absolute eosinophil counts. Determination of absolute eosinophil counts is presumably more accurate, but would have required a second venepuncture. Any error involved appears to have been minimal, since the eosinophil counts obtained in both control groups were easily within the range of reported normal values (200–300 cells/mm³) in this age group (16, 17, 19). In addition, any error induced by use of cell differentials should have been constant across all subject groups.

In the present study, had the ECP content of serum been measured, it may have correlated better with the ECP content of nasopharyngeal secretions and severity of illness than did the peripheral blood eosinophil counts. Nevertheless the goal of the study was to determine if eosinophil counts, a simple and reproducible measure, were associated with the development of bronchiolitis or predicted its severity. Future studies may determine whether the ECP content of serum is increased in bronchiolitis.

Finally studies of infants dying with bronchiolitis have not revealed eosinophils in the airway walls (27). It is possible that most, if not all, available eosinophils had already degranulated, and the suppression of peripheral blood eosinophilia had prevented migration of new eosinophils into the airway. Also in animal models of bronchiolitis (28) guinea pigs infected with parainfluenza virus do not show eosinophils in the airway walls, but do have in-

creased numbers of eosinophils recoverable in bronchoalveolar lavages in comparison to uninfected animals. Lavage fluids have not been evaluated for eosinophil content in human bronchiolitis. Infants who died of RSV infection following immunization with a formalin-inactivated RSV vaccine had increased numbers of eosinophils present in the airway at autopsy (29), as well as in peripheral blood (30). These infants had a clinical picture typical of bronchiolitis, with the exception of increased severity.

In summary eosinophils may contribute to the pathogenesis of viral bronchiolitis, although eosinophils in the respiratory tract may have a more direct role than circulating eosinophils. The findings may be interpreted as providing a basis for trials of medications which stabilize eosinophils in preventing or treating bronchiolitis.

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