Neutrophil Chemiluminescence Defect in Pediatric Patients with Recurrent Pneumonias*

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Recurrent episodes of lower respiratory tract infections are a major problem in pediatric pulmonology. Host defense mechanisms in these patients have not been well-defined and the role of the neutrophil is unresolved. The prompt accumulation of neutrophils in most tissues infected with bacteria is essential for suppression of bacterial growth and eradication of infection. Although isolated observations of increased susceptibility to lower respiratory tract infections have been described in patients with defects of neutrophil movement, decreased neutrophil bactericidal activity, and aberrations of the complement pathway leading to inadequate generation of chemotactic factors, no systematic study of neutrophil function in pediatric patients has been reported.

Due to the lack of existing data, we have studied neutrophil function in pediatric patients with repeated lower respiratory tract infections utilizing two in vitro assays: chemiluminescence, which is a measure of photon emission from neutrophils stimulated by a phagocytizable particle, and chemotaxis which measures cell movement.

Materials and Methods

Patient Population

Twenty-three patients referred to the University of New Mexico Pediatric Pulmonary Center for evaluation of recurrent pneumonias were studied during disease-free periods. The age range of the patient group was four months to 12 years with a mean of 32 months and included 10 females and 13 males. Each patient had at least two episodes of lobar pneumonia documented by chest x-ray examination in the nine months prior to the study with interval clearing noted on x-ray films. These pneumonias were associated with fever and a response to antibiotic therapy. No anatomic or systemic cause for pneumonias could be found and no consistent bacterial or viral etiologic agent was identified. All patients had normal sweat chlorides, immunoglobulins (IgG, IgA, IgM, and IgE), T and B cell numbers, and complement components (C4, C2, C3, and total hemolytic complement). Neutrophil counts, morphology, and nitroblue tetrazolium dye reduction as measured by the semi-quantitative technique of Ochs were normal.

Control Population

The normal population consisted of 11 children (7 boys and 4 girls) with an age range of 12 months to four years.

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Mean age for the control population of patients was 35 months. Other control groups with pulmonary involvement included: a) three pediatric patients with cystic fibrosis who were studied during periods of good control, and b) three pediatric patients with episodic wheezing and atopic histories who were studied during symptom-free periods.

Cell Separation

Polymorphonuclear leukocytes (PMNs) were prepared from heparinized peripheral venous blood (10 units他parin/ml) by Plasmagel (HTI, Buffalo, NY) sedimentation of erythrocytes followed by Ficoll-Hypaque centrifugation to remove mononuclear cells. Cells were washed twice in Hanks balanced salt solution (HBSS) and resuspended in appropriate media. Final cell preparations contained greater than 90 percent neutrophils.

Chemotaxis Assay

Chemotactic assays were performed by the Boyden method as previously described.2 Cells were resuspended in HBSS with 10 percent fetal calf serum and adjusted to a concentration of $5 \times 10^6$ PMN per ml. 0.4 ml of this cell preparation was added to the upper compartment of a nuclerepore chemotaxis chamber. The lower compartment, which was separated from the upper compartment by a 5 μm micropore filter (Sartorius), contained a 10 percent solution of culture supernatant from a 24-hour culture of E coli (strain K12) or purified C5a.4 After a two-hour incubation period at 37°C, filters were removed and stained. The number of neutrophils which responded to chemotactic factor and had migrated to the distal side of the filter were counted in ten high power (400 ×) fields on two duplicate membranes. Results are expressed as percentage of normal simultaneously run adult controls. Chemotactic factors were titrated to give 200 ± 20 neutrophils/high power field in normal donors. Previous studies have shown no differences in chemotactic response of normal adults and children.5

Chemiluminescence Assay

Chemiluminescence was performed as previously described using zymosan particles opsonized with normal serum as a stimulant.6 Light emission was measured in a non-refrigerated Nuclear Chicago beta-scintillation counter used out-of-coincidence with a window setting of 0.10. A mixture of 0.5 ml of PMNs ($5 \times 10^6$) and 0.2 ml of opsonized zymosan (10 mg/ml) were suspended in phosphate-buffered saline solution (pH = 7.4) to a final volume of 1 ml in an 18-hour dark adapted Beckman polyQ scintillation vial. Tubes were counted for 1 minute at 6- to 8-minute time intervals. Chemiluminescence is expressed as peak simulation in counts per minute which occurred between 7 and 9 minutes.

RESULTS

Figure 1 compares peak PMN chemiluminescence values in pediatric patients with recurrent pneumonias to controls. On the far left is the chemiluminescence response of 23 adult controls. The mean chemiluminescence response in adults is 32,908 ± 1,418 (SEM) which did not differ significantly from the mean chemiluminescence in control children, 29,034 ± 1,435. The mean chemiluminescence of the recurrent pneumonia patients was 22,643 ± 2,835 which was significantly depressed as compared to both adult (P < .01) and pediatric (P < .05) control groups. In the patient category, 11 of 23 patients with recurrent pneumonias gave a chemiluminescence response which was greater than two standard deviations below the adult control mean. As shown, the peak chemiluminescence response of neutrophils from the asthma and cystic fibrosis control groups did not differ statistically from control values, 28,883 ± 971 and 25,400 ± 3,858 respectively.

Figure 2 shows the correlation between neutrophil chemotaxis and neutrophil chemiluminescence. In the

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21018/ on 04/08/2017)
function. Alternatively, the defect cases, though shown to be depressed chemotactic activity of 23 pediatric patients with recurrent pneumonias who have defects in neutrophil function as assessed by chemiluminescence and chemotaxis assays. In the lung, the alveolar macrophage is believed to be responsible for the phagocytosis and killing of inhaled particles, while the neutrophil assumes this role for bloodborne bacteria. Since the ability to mobilize neutrophils into the lung appears to be an important lung defense mechanism and since PMN chemiluminescence may be intimately involved in bactericidal activity, it is feasible that defective neutrophil function may be related to the recurrent pneumonias frequently seen in pediatric patients.

**DISCUSSION**

In the normal neutrophil, opsonized particle contact or phagocytosis results in the activation of the hexose monophosphate shunt and increased consumption of oxygen. These increased metabolic activities can result in the generation of the electronically excited molecules including: superoxide anion, singlet oxygen, hydroxyl radicals, and simultaneous photon emission (chemiluminescence). Our study demonstrates that in a group of 23 pediatric patients with recurrent pneumonias, 11 had a depressed neutrophil chemiluminescence of greater than 2 standard deviations below the mean chemiluminescence of normal controls. This depression in chemiluminescence was generally associated with a depressed chemotaxis response indicating that a common defect in the two systems may be present. Only two patients deviated from this pattern showing abnormal chemiluminescence and normal chemotaxis. Although a defect affecting both chemiluminescence and chemotaxis is implied by the data obtained here, some cases exist where these systems diverge, as shown here and in a previous report of a patient with *incontinencia pigmenti* with normal chemiluminescence and abnormal chemotaxis.

Although the nature of the chemiluminescence and chemotactic defect is unknown, it could be either congenital or acquired. Neutrophil chemotaxis has been shown to be depressed following viral infections. Although chemiluminescence has not been studied in these cases, it is possible that our patients could have had a preceding viral infection responsible for neutrophil dysfunction. Alternatively, the defect could represent a maturational delay. Although the age range of our patients was 4 months to 12 years, the mean was 32 months. Neonatal PMNs have been shown to have depressed chemotaxis, phagocytosis, membrane deformability and HMP shunt activation. It is possible that PMN maturation could be delayed in some patients and account for increased lower respiratory tract infections and decreased neutrophil function.

**In summary**, these studies define a group of pediatric patients with recurrent pneumonias who have defects in neutrophil function as assessed by chemiluminescence and chemotaxis assays. In the lung, the alveolar macrophage is believed to be responsible for the phagocytosis and killing of inhaled particles, while the neutrophil assumes this role for bloodborne bacteria. Since the ability to mobilize neutrophils into the lung appears to be an important lung defense mechanism and since PMN chemiluminescence may be intimately involved in bactericidal activity, it is feasible that defective neutrophil function may be related to the recurrent pneumonias frequently seen in pediatric patients.

**REFERENCES**


**DISCUSSION**

*Dr. Murphy:* I might mention as a sideline about 50 percent of our patients are Navajo. In the Navajo children in particular recurrent pneumonias are a tremendous problem, and we're hoping to be able to get at the basic defect in these patients.

*Dr. Oren:* In the patients who had the low values, was there any relationship to their therapy?
Dr. Murphy: The patients were not on any antibiotic therapy for the week prior to study. I haven't made a correlation between the last time they had an infection and their defect.

Dr. Ward: Do you think that this is an acquired defect, secondary to the bacterial infection, or do you think that it's the other way around?

Dr. Murphy: I think in a number of patients it's possibly an acquired defect from a viral infection. We know that respiratory syncytial virus and adenovirus can be really devastating in patients when it's acquired at an early age and certainly young children have an abnormal response to respiratory syncytial virus.

Dr. Rylander: We've seen in our animal experiments throughout the years that following dust exposure animals get an invasion of Gram-negative organisms into the deeper respiratory airways. Since about 50 percent of your population were Navajos, I wonder if exposure to a high indoor fume level could explain some of the effects you see on the neutrophils?

Dr. Murphy: Since 12 of the 23 patients studied had absolutely normal neutrophil function, I think we have to look at another explanation. We haven't seen any particulates on the lavage fluid, but a number of these patients do still live in primitive hogans and they are carried on their mothers' backs. It's possible that the smoke and dust exposure could have an effect. It wouldn't explain the recurrent otitis that we have also seen there, unless you're getting a general depression of the immune system.

Dr. Sanderson: This question is addressed to both Dr. Murphy and Dr. Repine. If one assumes that chemiluminescence and phagocytosis are directly related, wouldn't the integrated light be the best parameter to measure?

Dr. Repine: I agree that both peak and integrated values should be measured. However, the two usually give the same result, especially when we're looking for general patterns such as the pattern between C2 deficient and normal serum.

Dr. Murphy: I would agree with what Dr. Repine has said. We are starting to look at integrated areas and a number of these patients have not only low peaks but very low total curve areas.

Dr. Repine: I'd like to make a comment about the importance of sequential defects in neutrophil function. I have found reversible bactericidal defects in the neutrophil function of 8 patients with untreated bacterial endocarditis. I have also found reversible abnormalities in the simulated locomotion of PMN from untreated patients with blastomycosis. Thus, some neutrophil defects will be acquired due to infection or consequences of the infection. That is why Dr. Murphy's sequential studies will provide the best idea about acquired vs congenital deficiencies of neutrophil function.

Dr. Reynolds: I wish to offer a summary of the host defense session that we have just finished. I think we have been discussing some things of potential importance. You might call them experiments of nature, if you will, in which we're beginning to look at patients who have defects of respiratory cilia, patients who have deficiencies in certain complement factors (C3 or C5) which may impair chemotactant activity which brings effector cells into lung tissue, C2 deficiency which might alter phagocytosis in some cases, selective immunoglobulin deficiencies (IgA), and finally some problems with effector cells themselves which impair the inflammatory response of the polymorphonuclear cell. The point is that many of these patients who have subtle defects in one of these systems do have a propensity for respiratory infections, but rarely are such infections devastating or life-threatening. This emphasizes the flexibility of the host defense mechanism. It's possible to suffer loss in one area but there remain lots of things that seem to compensate for it. If you lose one immunoglobulin, maybe IgM, for example, another one picks up the burden. These are certainly interesting clinical probes for us to look at and to clarify the various components of host defense. We can be thankful that the human being is resilient and rarely does one thing completely determine his fate.