Effect of C2-Deficiency on the Bactericidal Activity and Chemiluminescence Responses of Human Neutrophils in vitro*

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Effective ingestion and killing of invading bacteria by neutrophilic polymorphonuclear leukocytes (PMN) is an important aspect of pulmonary host defense. Opsonins play a key role in the process by facilitating uptake of bacteria by PMN. Opsonic substances in serum include heat-stable components such as specific antibody, which usually develops later in the course of infection, and heat-labile factors, like those produced rapidly by activation of the complement cascade. Following engulfment of opsonized bacteria, PMN consume oxygen, undergo a series of metabolic reactions, and produce highly reactive oxygen intermediates. These oxygen byproducts are necessary for optimal killing of ingested bacteria by PMN. In association with production of these oxygen intermediates, PMN generate chemiluminescence. This light can be measured in a scintillation counter and used to accurately quantitate the metabolic reactions of PMN to engulfment of bacteria. The nature of the requirements for the complex processes by which PMN pick up and kill bacteria and produce chemiluminescence remain poorly defined.

In the present investigation, we evaluated the effect of a selective absence of the second component of complement (C2) on the uptake of bacteria by PMN, using both a standard assay of PMN bactericidal activity and an assay of the quantitative chemiluminescence response of PMN stimulated by bacteria. The patient we studied had a complete selective absence of C2 by radial immunodiffusion or immunoelectrophoresis analysis and frequent infections with a propensity for respiratory infections. PMN and sera were collected by venipuncture from the patient or uninfected, drug-free control subjects and prepared as previously described. PMN were separated by Ficoll-Hypaque technique and triply-washed. Serum was allowed to clot once, recovered by centrifugation, frozen once, and stored at -70°C for less than two months. Bactericidal activity was measured for various combinations of patient or control PMN with patient or control serum. Chemiluminescence responses were measured at physiologic temperatures (37°C) in a liquid scintillation counter using methods reported in a prior publication.

The results show that C2 is necessary for optimal killing by PMN of *S. aureus* (Fig 1, left) but not of *E. coli* (Fig 1, right). In the absence of serum replacement, triply-washed control PMN killed only 5 ± 2 percent (mean ± SE) of the initial inoculum of *S. aureus*. Addition of increasing amounts of serum improved killing of *S. aureus* by control PMN to a peak bactericidal activity of 81 ± 7 percent at 4 percent serum. In contrast, in 4 percent C2-deficient serum, control PMN killed only 20 ± 6 percent of the *S. aureus*. Increasing the concentration of C2-deficient serum added from 4 percent to 16 percent did not increase the percentage of *S. aureus* killed by triply-washed control PMN. The specificity of the need for C2 for optimal bactericidal activity of control PMN against *S. aureus* was confirmed by showing that addition of purified C2 (Cordis Corp, 2,500 units/ml serum) completely corrected the bactericidal defect. In marked contrast to the findings for *S. aureus*, the bactericidal activity of control PMN against *E. coli* was the same in 4 percent control or C2-deficient serum.

Similar findings were obtained when chemiluminescence responses produced by control PMN stimulated by *S. aureus* (Fig 2, top) or by *E. coli* (Fig 2, bottom) were compared. The results clearly show that C2 is also needed for optimal chemiluminescence by control PMN stimulated by *S. aureus* but not by *E. coli*.

Parallel studies using PMN from the patient corroborated the above findings and documented normal bac-

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**Figure 1. Bactericidal activity after 60 minutes for *S. aureus* (left), 502A of triply-washed control PMN (4×10⁶) with no added serum (○), 4% control serum (●), 4% C2-deficient serum (△), or 4% C2-deficient serum reconstituted with purified C2 (▲). The test ratio was 1.25 bacteria/PMN. The bacterial activity of control PMN in C2-deficient serum was significantly (P<.001) from control serum. Right: Similar studies using *E. coli* as the test organism.**

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tericidal activity and chemiluminescence responses of patient PMN in the presence of control serum. Only when patient PMNs were studied in the absence of serum replacement or in C2-deficient serum, with S aureus as the test organism, was any abnormality found in the bactericidal activity or chemiluminescence production of patient PMN.

These studies suggest that opsonization of S aureus, 502A has a major dependence on C2 and probably the classic pathway of complement activation. In contrast, interaction of PMN with E coli ON2 appears to be independent of C2. The magnitude and the kinetics of the chemiluminescence response by control PMN in the presence of E coli is the same with C2-deficient serum as with control serum. At least at these concentrations and incubation times, the findings suggest that an intact classic pathway is not an absolute requirement for optimal activation of the alternative pathway and opsonization of E coli.

The demonstrable necessity for C2 for optimal bactericidal activity against S aureus, and potentially other organisms which may depend on complement activation by the classic pathway, may be a factor contributing to the recurrent infections of patients with complete C2-deficiency or other defects of classical pathway components. The association of C2-deficiency and recurrent infections does not establish a causal relationship. However, the present study suggests a possible mechanism for this clinical observation. Further clarification will require detailed prospective study of the clinical features of individual patients, as well as laboratory study of specific infecting organisms in the presence of serum and cells from these patients. The present results do suggest that assay of chemiluminescence may be a useful way to evaluate the opsonic requirements for those organisms where standard bactericidal assay is unsatisfactory due to difficulty in obtaining reproducible growth and colony counts of the microorganisms.

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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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