THE IMPORTANCE OF NEUTROPHILS IN RESISTANCE TO PNEUMOCOCCAL PNEUMONIA IN ADULT AND NEONATAL MICE

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Abstract—Neonatal mice succumbed to intranasally-inoculated Streptococcus pneumoniae doses which were as much as 250 times less than the doses that adult mice were resistant to. Neutrophil migration into lungs of neonates was similar in kinetics and intensity to that in adults in response to lethal doses of S. pneumoniae. Interestingly, neutrophil infiltration into the lung alveoli of neonates occurred at lower doses of bacteria than that required for similar responses in adults. Furthermore, depletion of neutrophils in adult and neonatal mice inoculated with low doses of bacteria resulted in significantly higher lung burdens of bacteria in neonatal mice as compared to adults. These data indicate that increased susceptibility of neonates to S. pneumoniae is not the result of incompletely developed neutrophil function and infact, indicate that neutrophils contribute more to resistance to low doses of S. pneumoniae in neonates than they do in adult mice.

INTRODUCTION

Acute respiratory diseases are a leading cause of illness and death in infants and young children in developing countries. Resident alveolar macrophages and elicited neutrophils are known to be important in defense against pathogens in adult lungs. However, little is known about the role phagocytes play in fighting bacterial infections in neonatal lungs. Even so, it has been suggested that neonatal susceptibility to lung infection may be the result of a deficiency in phagocyte function (1–6).

Human neonatal cord blood neutrophils have been reported to be deficient chemotactically compared to adult peripheral blood neutrophils (7, 8). This deficiency may be a result of decreased expression of adhesion molecules such as CR3 (9, 10) and L-selectin (11). Additionally, neutrophils of neonates appear to have cytoskeletal differences which may contribute to diminished ability to
change shape which is necessary for their movement into sites of infection (12). In vivo studies indicate that the magnitude of neutrophil migration into the peritoneum of neonatal rats in response to FMLP, *Escherichia coli*, or Group B *Streptococcus* was significantly less than in adults (13). Neutrophil storage pools have also been reported to be more quickly depleted upon peritoneal challenge in neonatal rats as opposed to adult rats (3).

Although neutrophils from neonates appear to have inferior capabilities in vitro as compared to neutrophils from adults, it is not clear whether such differences affect host resistance to acute bacterial lung infections. This is an important issue since the natural route of infection of many pathogens of neonates is the lungs. This study examined whether increased susceptibility of neonatal mice to *S. pneumoniae* is the result of a not yet fully developed and functional neutrophil response in these hosts.

**MATERIAL AND METHODS**

*Materials.* Hybridoma clone RB6-8C5 which produces a rat IgG2b MAb against GR-1 was a gift from Dr. R. Coffman, DNAX Research Institute (Palo Alto, California). Clone TIB210, anti-CD8, was used as an isotype-matched control MAb and was obtained from American Type Tissue Culture (Rockville, Maryland). MAbs were partially purified from ascites using ammonium sulfate precipitation.

*Bacteria.* *S. pneumoniae* type 4 was obtained from American Type Tissue Culture (Rockville, Maryland) and kept frozen in Todd-Hewitt broth with 5% glycerol in 1 ml aliquots. For inoculation into mice, a vial of *S. pneumoniae* was grown in Todd-Hewitt broth for 3–6 hours and pelleted at 3000 xg for 15 minutes. Bacteria were resuspended in PBS and the approximate concentration determined by O.D. at 630 nm. Multiple dilutions of the inoculum were plated onto blood agar and incubated at 35°C overnight for determination of the actual CFU inoculated into mice.

*Mice and Experimental Design.* BALB/c mice were bred in the animal breeding facility at the Trudeau Institute following NIH guidelines for housing and treatment. Adult male BALB/c mice were routinely 8–10 weeks of age when used. Neonatal mice (and dams) were obtained from the animal breeding facility at 24 ± 12 hours of age and were either inoculated with *S. pneumoniae* immediately or the next morning. Adults and neonates received equal CFU per gram body weight. Neonatal and adult mice were placed under light halothane anaesthesia and inoculated intranasally (*i.n.*) with 10 μl and 50 μl, respectively, of *S. pneumoniae* suspensions. Mice were sacrificed at various times post-inoculation. In some experiments, mice were depleted of mature neutrophils 24 hours before infection by intraperitoneal (*i.p.*) injections of 50 μg (neonates) or 200 μg (adults) of a MAb against GR-1, a surface molecule on mature murine granulocytes. This antibody has been shown to significantly depress neutrophils and eosinophils in the blood, spleen, liver, and peritoneal cavity of adult mice within 2 days of administration in vivo (14, 15). As an isotype control, some mice received injections of the MAb against CD8. To determine if neutrophils were able to respond to a secondary site of infection, experiments were performed in which mice were inoculated *i.p.* with *S. pneumoniae* 24 hours after an initial *i.n.* inoculation with the bacteria. Mice were sacrificed 6 hours post-*i.p.* inoculation.

*Comparison of Neutrophil Infiltration with Level of Infection.* Neonatal and adult mice were