Functional Leukocyte Administration in Protection against Experimental Neonatal Infection

JOSÉ IGNACIO SANTOS,⁽²²⁾ ANN O. SHIGEOKA, AND HARRY R. HILL

Howard Hughes Medical Institute Laboratory, Division of Clinical Immunology and Allergy, Department of Pediatrics, and the Department of Pathology, University of Utah College of Medicine, Salt Lake City, Utah, USA

Summary

Studies have shown that human neonates who develop group B streptococcal sepsis usually lack opsonic antibody (Ab) to their infecting strain and that these neonates may also have impaired polymorphonuclear leukocyte (PMN) function. The present study was designed to determine the efficacy of administration of PMNs or opsonic Ab-containing serum in protecting against group B streptococcal infection in a newborn rat model. After intraperitoneal (IP) injection of $\approx 5 \times 10^6$ streptococci, animals received separate IP injections of saline, serum lacking opsonic antibody (Ab negative), Ab positive serum or washed adult human PMNs (2×10^6). The mortality rate in 55 neonatal rats infected with group B streptococci who received saline or Ab negative serum was 91%. In contrast, 40 animals who received adult human PMNs at the time of inoculation had a survival rate of 50% ($P < 0.001$). Human serum containing opsonic antibody also provided significant protection against mortality in this model (survival rate 51%, $P < 0.001$). Leukocytes from normal term neonates, stressed neonates, or ones pretreated with cytochalasin B offered less protection than did functional adult human cell ($P < 0.001$).

Speculation

This study provides evidence for polymorphonuclear leukocyte (PMN) dysfunction as another risk factor in group B streptococcal disease and suggests that fully functional PMNs are required for protection. Furthermore, this model involving the administration of human PMNs to an animal appears to have the potential for the *in vivo* evaluation of PMN function in neonates and other compromised hosts.

The mechanisms of host resistance to streptococci of Lancefield's group B have not been precisely defined. Mouse protection studies by Lancefield *et al.* (14, 15) and Fischer *et al.* (9) have shown the protective effect of antibodies against group B streptococci. Hemming *et al.* (10), Shigeoka *et al.* (17), and Anthony (2) have demonstrated the absolute requirement of specific antibody for significant opsonization of group B streptococci. In previous studies, we have shown that human neonates who develop group B streptococcal sepsis usually lack opsonic antibody to their infecting strains (10) and that these neonates may also have impaired polymorphonuclear leukocyte function (19). Inasmuch as opsonization and phagocytosis represent the most important host defense mechanisms in clearing the body of these bacteria, the present study was designed to determine the effects of passively supplying functional human polymorphonuclear leukocytes (PMNs) or serum containing opsonic antibody in protecting against group B streptococcal infection in a newborn rat model.

METHODS

PREPARATION OF ORGANISMS

Group B streptococci type III were isolated from the blood and cerebrospinal fluid of infected infants. The strains were cultured

at 37°C in Todd-Hewitt broth (Difco Laboratories, Detroit, MI), washed and concentrated in phosphate-buffered saline by centrifugation to contain 5.0×10^8 to 1.0×10^9 colony-forming units per ml, as previously described (10, 17).

NEONATAL RAT INFECTIONS

Sprague-Dawley outbred pregnant female rats (Holtzman) were obtained one wk before delivery. When tested, maternal rat serum did not appear to contain significant opsonic activity against most of the strains we used. The neonatal progeny less than 24 hr-old were randomly assigned to groups and entered into the study. After intraperitoneal (IP) injection of 5.0×10^6 streptococci with a Hamilton syringe, the animals received separate IP equal volume (10 μ l) injections of saline, Ab negative serum, Ab positive serum, washed adult human PMNs (2×10^6) or PMNs pretreated with cytochalasin B and washed thoroughly. The rat litters were observed at 6-hr intervals for mortality. Those neonatal rats who died were aseptically dissected, and in all instances in which cultures were taken, group B streptococci were cultured from one or several of the following organs: brain, lungs, kidney, liver, and spleen.

PREPARATION OF PMN

Heparinized blood (10 units/ml) was collected from healthy adult volunteers, gravity sedimented to remove erythrocytes, and washed twice with PBS. PMNs were then counted and adjusted to a concentration of 1.0×10^7 /cc representing 50 to 70% of the cells in the suspension. In a similar fashion, leukocytes were collected and prepared from cord blood of noninfected term human neonates and from neonates whose delivery was stressed by maternal preeclampsia or premature rupture of membranes.

ASSESSMENT OF NEUTROPHIL VIABILITY AFTER IP INJECTION

Latex particles, 0.81 μ (Difco Laboratories) were washed once in 10 ml PBS, and the pellet was resuspended in PBS to a concentration of 1×10^8 in a sterile plastic tube. Group B streptococci type III were prepared as previously described. Sprague-Dawley outbred adult and neonatal rats were injected IP with equal volume injections (10 μ l) of either latex particles or group B streptococci before receiving washed adult human PMNs (2×10^6). Thirty min after injection, animals underwent peritoneal washout with 10 ml (adults) or 2 ml (neonates) sterile PBS. The exudates were cytocentrifuged onto slides and visual evidence of phagocytic uptake of either latex particles or group B streptococci was evaluated by examination of Giemsa-stained smears (10).

SERUM TREATMENT

Fresh human serum or frozen serum (at -70°C thawed only on use) was utilized. Opsonic antibody titers were determined by a chemiluminescence procedure developed in our laboratory (10) and confirmed in most instances by a radio-labeled bacterial uptake technique (1).

RESULTS

The survival rate in 55 neonatal rats infected with group B streptococci, type III, who received saline or opsonic antibody negative serum was only 9% (Table 1). In contrast, animals who received human serum containing opsonic antibody to the infecting strain had a significantly improved survival rate (50%). Similarly, a marked increase in survival was also observed in animals who received group B streptococci and a separate intraperitoneal injection of 2×10^6 adult human PMNs. Thus, both human serum containing opsonic antibody to the infecting strain as well as functional adult human leukocytes significantly improve survival in infected neonatal rats. Saline and opsonic antibody negative serum did not decrease mortality. All 30 uninfected animals who received human leukocytes alone survived.

Giemsa-stained smears of peritoneal exudates from both adult and neonatal rats revealed that human PMNs (evidenced by relative size and nuclear configuration) had phagocytized both latex particles and group B streptococci equally well. Thus, the human cells remained viable and functionally active within the rat peritoneal cavity.

In addition to investigating adult humoral and cellular factors in the immune response to group B streptococci, we also evaluated PMNs from stressed human neonates because we and others have previously detected metabolic, chemotactic, and bactericidal defects in these cells (13, 16, 19). The data in Figure 1 is a comparison between the protection provided by adult and neonatal PMNs in newborn rats infected with a different strain of type III group B streptococci. As shown, adult PMNs provided significant protection against the infecting strain resulting in an overall survival rate of 69% versus 0% in the saline-treated animals. In contrast, the same number of PMNs from stressed neonates showed much less protection with an overall survival of only 28%. The difference in protection supplied by the functional adult PMNs and the stressed neonatal PMNs is significant at $P < 0.001$. In a similar fashion, we next compared the protective effect of adult PMNs with the

Fig. 2. Effect of cytochalasin treatment of leukocytes on neonatal rat protection in type III group B streptococcus (GBS) infection.

same number of PMNs from uninfected, healthy, term neonates. The survival rate in 28 infected animals who received adult PMNs was 50% (14 of 28) compared to 25% (8 of 32) in animals receiving PMNs from healthy, term neonates.

To further confirm that the administered leukocytes must be functional to improve survival in the neonatal rat model, adult human leukocytes were incubated with cytochalasin B (Aldrich Chemical Co., Milwaukee, WI) in concentrations (10 $\mu\text{g}/\text{ml}$) known to have an effect on both chemotaxis and phagocytosis *in vitro* (5, 7). The protective effect of these cells was compared with that of untreated leukocytes. The survival rate in 27 neonatal rats infected with group B streptococci who received adult human PMNs pretreated with cytochalasin B was only 26%, whereas animals receiving untreated PMNs had a survival rate of 71% (Fig. 2; $P < 0.001$). Pretreatment of leukocytes with cytochalasin appears, therefore, to inhibit PMN antibacterial function when tested *in vivo* in this animal model.

DISCUSSION

Group B streptococci have emerged over the last decade as a major cause of morbidity and mortality in neonates. Group B disease is known to occur in other compromised hosts including diabetics (4, 8), patients with liver disease, and ones with renal failure (4). Moreover, we have demonstrated that acutely stressed neonates with group B streptococcal sepsis or meningitis often develop neutropenia (6) and have PMN chemotactic and bactericidal abnormalities (19). Clinical studies relating neonatal and maternal antibody levels to infection have previously suggested the importance of antibody in host defense against these strains (3, 10). Similarly, limited observations (18) have shown that infants infected with group B streptococci who received transfusions of whole blood containing opsonic antibody to the infecting strain had improved survival when compared to infants receiving blood lacking antibody to their infecting organism.

Studies in animals have also suggested the importance of antibody in the protection against group B disease (9, 14, 15). Our data indicate that human PMNs are both viable and capable of phagocytosis after being introduced into the peritoneal cavity of both adult and neonatal rats. For these reasons, we have utilized a neonatal rat model of group B disease and have sought to determine the effects of opsonic antibody and leukocyte administration on the survival of these experimentally infected animals.

Our data indicate that functional human leukocyte administration affords protection in this animal model. Caution should be exercised, however, in extrapolating the clinical applicability of our results to the human neonate. We are not recommending leukocyte transfusion in such patients. It is conceivable, however, that for limited conditions such as overwhelming group B streptococcal sepsis the combination of opsonic antibody and func-

Table 1. Effect of administering functional leukocytes or opsonic antibody on the survival of group B infected rats

Group	n	% survival at 96 hr	
		n	P^1
GBS ² $\pm$ saline or AB ⁻ serum	55	5/55 (9) ³	
GBS + AB ⁺ serum	40	20/40 (50)	<0.001
GBS + PMNs	56	29/56 (51)	<0.001

¹ P = Fisher exact test.

² Δ GBS, Group B streptococci.

³ Numbers in parentheses, percent.

Fig. 1. Effect of leukocyte administration from human stressed neonates and adult controls on survival of neonatal rats infected with type III group B streptococci (GBS).

tional cellular elements (PMNs) may prove to be efficacious. Considerable additional work in animal models such as the one described here needs to be carried out before clinical trials can even be considered. Then the problem of possible graft *versus* host disease or transmission of infection via blood and blood products will have to be considered. Still, such avenues should be investigated in attempts to improve survival in this type of overwhelming infection with mortality rates in the 25 to 75% range. The study also provides evidence for PMN dysfunction being another risk factor in group B disease because neonatal animals given antibody to their infecting strain often still had a mortality rate of 40 to 50%. We were also able to show that human neonatal PMNs were less effective in providing protection in this animal model.

PMNs from healthy term neonates behave very similar to those from normal adult controls in assays of chemiluminescence generation or bactericidal activity when tested *in vitro* (19). In contrast, PMNs from stressed neonates have a subtle bactericidal defect when challenged with a high bacteria to PMN ratio. The present observations suggest that PMNs from both healthy term and stressed neonates are deficient in their ability to protect newborn rats from group B streptococcal infection. Giemsa-stained smears of peritoneal exudates from neonatal rats revealed that human PMNs from adult controls as well as healthy term and stressed neonates are capable of phagocytizing both latex particles and group B streptococci equally well. Inasmuch as healthy and stressed neonatal PMNs are both deficient in their ability to protect neonatal rats, it is unlikely that the subtle bactericidal defect observed *in vitro* in stressed PMNs contributes to this phenomenon. Both healthy and term neonatal PMNs have defective chemotactic activity (13, 16) which would partially explain the abnormality observed. Of interest is the fact that diabetic individuals who have an increased incidence of group B streptococcal infections (4, 8) also have defects in PMN chemotactic activity (11, 12).

Additional studies with cytochalasin B tended to support the conclusions that functional PMNs are required for protection in this model. Cytochalasin B has been reported to inhibit spontaneous motility and chemotactic responses of rabbit and human leukocytes (5). Davis *et al.* (7) also reported the inhibition of bacterial phagocytosis by human polymorphonuclear leukocytes in the presence of cytochalasin B. Our results support these observations and aid in delineating the importance of PMN chemotaxis and phagocytosis in the host defense mechanism against group B streptococci. The data from these experiments also confirm that significant protection in this model results only when functional PMNs are utilized. Furthermore, this model involving the administration of human PMNs to an animal appears to have the potential for the *in vivo* evaluation of PMNs from neonates and other compromised hosts.

REFERENCES AND NOTES

1. Allred, C. D., Shigeoka, A. O., and Hill, H. R.: Evaluation of group B streptococcal opsonins by radiolabeled bacterial uptake. *J. Immunol. Methods*, 26: 355 (1979).
2. Anthony, B. F.: Immunity to the group B streptococci: Interaction of serum and macrophages with types Ia, Ib, and Ic. *J. Exp. Med.*, 143: 1186 (1976).
3. Baker, C. J., and Kasper, D. L.: Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. *N. Engl. J. Med.*, 294: 753 (1976).
4. Bayer, A. S., Chow, A. W., Anthony, B. F., and Guze, L. B.: Serious infections in adults due to group B streptococci. *Am. J. Med.*, 61: 498 (1976).
5. Becker, E. L., Davis, A. T., Estensen, R. D., and Quie, P. G.: Cytochalasin B. IV. Inhibition and stimulation of chemotaxis of rabbit and human polymorphonuclear leukocytes. *J. Immunol.*, 108: 396 (1972).
6. Christensen, R. D., Shigeoka, A. O., Hill, H. R., and Rothstein, G.: Neutropenia and bone marrow exhaustion in human and experimental neonatal sepsis. *Pediatr. Res.*, 14: 806 (1980).
7. Davis, A. T., Estensen, R., and Quie, P. G.: Cytochalasin B. III. Inhibition of human polymorphonuclear leukocyte phagocytosis. *Proc. Soc. Exp. Biol. Med.*, 137: 161 (1971).
8. Eickhoff, T. C., Klein, J. O., Daly, A. K., Ingall, D., and Finland, M.: Neonatal sepsis and other infections due to group B beta-hemolytic streptococci. *N. Engl. J. Med.*, 271: 1221 (1964).
9. Fischer, G. W., Lowell, G. H., Crumrine, M. H., and Bass, J. W.: Demonstration of opsonic activity and *in vivo* protection against group B streptococci type III by *Streptococcus pneumoniae* type 14 antiserum. *J. Exp. Med.*, 148: 776 (1978).
10. Hemming, V. G., Hall, R. T., Rhodes, P. G., Shigeoka, A. O., and Hill, H. R.: Assessment of group B streptococcal opsonins in human and rabbit serum by neutrophil chemiluminescence. *J. Clin. Invest.*, 58: 1379 (1976).
11. Hill, H. R., Sauls, H. S., Dettloff, J. L., and Quie, P. G.: Impaired leukotactic responsiveness in patients with juvenile diabetes mellitus. *Clin. Immunol. Immunopathol.*, 3: 395 (1974).
12. Hogan, N. A., Rallison, M. L., Santos, J. I., and Hill, H. R.: Abnormal monocyte chemotactic responsiveness in diabetes mellitus. *Clin. Res.*, 28: 108A (1980).
13. Klein, R. B., Fischer, T. J., Gard, S. E., Biberstein, M., Rich, K. C., and Stiehm, E. R.: Decreased mononuclear and polymorphonuclear chemotaxis in human newborns, infants, and young children. *Pediatrics*, 60: 467 (1977).
14. Lancefield, R. C., and Freimer, E. H.: Type specific polysaccharide antigens of group B streptococci. *J. Hyg. (Cambridge)*, 64: 191 (1966).
15. Lancefield, R. C., McCarty, M., and Everly, W. N.: Multiple mouse-protective antibodies directed against group B streptococci. *J. Exp. Med.*, 142: 165 (1975).
16. Miller, M. E.: Chemotactic function in the human neonate. Humoral and cellular aspects. *Pediatr. Res.*, 5: 487 (1971).
17. Shigeoka, A. O., Hall, R. T., Hemming, V. G., Allred, C. D., and Hill, H. R.: Role of antibody and complement in opsonization of group B streptococci. *Infect. Immun.*, 21: 34 (1978).
18. Shigeoka, A. O., Hall, R. T., and Hill, H. R.: Blood-transfusion in group B streptococcal sepsis. *Lancet*, 1: 636 (1978).
19. Shigeoka, A. O., Santos, J. I., and Hill, H. R.: Functional analysis of neutrophil granulocytes from healthy, infected and stressed neonates. *J. Pediatr.*, 95: 454 (1979).
20. Presented, in part, at the meeting of the Society for Pediatric Research, Atlanta, May 4, 1979.
21. The authors thank Val Callanan for secretarial assistance.
22. Requests for reprints should be addressed to: José Ignacio Santos, M.D., Maxwell Finland Laboratory for Infectious Diseases, 774 Albany St., Boston City Hospital, Boston, MA, 02118 (USA).
23. This research was supported in part by United States Public Health Service Grants AI13150 and AM21140 and a grant from the Thrasher Foundation.
24. Received for publication February 1, 1980.
25. Accepted for publication May 19, 1980.