Efficacy of Orally Administered Immune Serum Globulin against Type III Group B Streptococcal Colonization and Systemic Disease in an Infant Rat Model

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ABSTRACT. We established an experimental animal model of the gastrointestinal colonization and systemic disease following oral challenge of type III group B streptococcal strain in 3-day-old newborn rats. Two type III group B streptococcal strains isolated from the cerebrospinal fluid of septic newborn infants produced colonization in 57–87% of the challenged animals and 13–31% of these colonized animals developed systemic disease. Using this new animal model, we evaluated the effect of orally administered human immune serum globulin on the colonization and systemic disease. This antiserum contained 21 µg/ml of type III group B streptococcal antibody of human IgG class. Animals fed with immune serum globulin developed significantly lower rates of colonization and systemic disease than those of control (albumin or saline) (23 versus 71%, $p < 0.001$ for colonization; 7 versus 31%, $p < 0.05$ for systemic disease). However, rates of the development of systemic disease among the colonized animals were not significantly different between the two groups (33 versus 43%, $p > 0.1$). These findings suggest that orally administered immune serum globulin is beneficial in the prevention of colonization and systemic disease in this rat model and that this protective effect of oral immune serum globulin occurs primarily at the mucosal level. (*Pediatr Res* 18: 1329–1331, 1984)

Abbreviations

GBS, group B streptococcus
ISG, immune serum globulin
CFU, colony-forming units
CSF, cerebrospinal fluid
ELISA, enzyme-linked immunosorbent assay

The importance of GBS as a major cause of neonatal bacterial infection has been well documented. Several lines of evidence suggests that type-specific antibody plays a critical role in protec-

tion against GBS disease (4, 13). Recently, with the advent of ISG modified for intravenous use, passive administration of GBS antibody has been feasible. Studies from several laboratories have shown that this modified preparation contains opsonic activity against type III GBS and is also effective in providing significant protection against systemically challenged type III GBS in suckling rats (10, 18). Moreover, a recent study has demonstrated that modified ISG preparations retain opsonic activity after passage through the gastrointestinal tract of low birth weight infants (6).

The purpose of the present study was to examine the effect of orally administered modified ISG preparation on the colonization and systemic disease by type III GBS in a newly established experimental animal model.

MATERIALS AND METHODS

Organisms. Logarithmic phase cultures of two type III GBS strains (K75 and K79) in Mueller-Hinton broth (Difco Laboratories, Detroit, MI) were stored at -70°C in aliquots until used in this study (14). These strains had been isolated from the CSF of septic newborn infants.

Immune serum. A lyophilized preparation of ISG for oral administration (lot 2850) was obtained from Cutter Laboratories, Berkeley, CA. It is free of mercury-containing thimerosal preservative and prepared by treatment of ISG at pH 3.8–4.2 (10). When reconstituted in 10 ml of sterile water, the solution contains 5% dextrose and 10% protein, of which at least 90% is γ -globulin. The content of type III GBS antibody of human IgG class was 21 µg/ml as determined by the ELISA (1).

Animal studies. Outbred, pathogen-free Sprague-Dawley pregnant rats with timed conception were purchased from Charles River Breeding Laboratories, Wilmington, MA and gave birth in our vivarium 5–7 days after arrival. Each mother and her pups were housed in solid polypropylene opaque cages with filter hoods to minimize cross-infection. All experiments were begun when the pups were 3 days old.

The first studies were performed to develop a model for GBS gastrointestinal colonization. Three litters (29 pups) were used for two type III GBS strains. GBS grown to late logarithmic-phase (10^8 CFU/ml) (14) were concentrated 10-fold in sterile water and 20 µl of concentrated GBS (approximately 10^7 CFU) were fed twice (morning and evening) to 3-day-old newborn rats. This volume was delivered in two 10-µl doses using the microliter pipette (Oxford). The pups were permitted to drink the inoculum from the pipette tip to avoid aspiration or traumatizing the mucosa. Stool cultures were done 1, 2, and 3 days after inocula-

Received April 9, 1984; accepted June 13, 1984.

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This study was supported in part by Research Grants HD-09800, AI-15332, and AI-19454 from the National Institutes of Health.

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tion and gastrointestinal colonization was identified by positive stool cultures. Stool samples were obtained by gentle pressure of the suprapubic area or by gentle irritation of the rectum with polyethylene tubing (0.024-inch outer diameter, Clay Adams, Parsippany, NJ), thoroughly mixed in 1 ml of selective broth medium (Todd-Hewitt broth containing 10 µg/ml of colistin sulfate and 15 µg/ml of nalidixic acid), incubated overnight at 37° C, and spread onto selective broth agar (tryptose blood agar base with 6.6% sheep blood, 10 µg/ml of colistin sulfate, and 15 µg/ml of nalidixic acid). GBS was tentatively identified by the CAMP test (7) and grouped and typed by the methods of Lancefield (17) and Wilkinson *et al.* (20). Cultures of the blood and CSF were done only in moribund animals by external jugular venipuncture and cisternal taps, as previously described (15).

We next evaluated the effect of orally administered ISG on the gastrointestinal colonization and systemic disease by GBS in infant rats. A total of 78 newborn rats from eight litters were used to minimize the effect of interlitter variability. At 2 days of age, all members of each litter were removed from the mother 1 h before the administration of serum to allow some emptying of the stomach. Each litter was divided into three groups and each group was fed twice daily (0800 and 1600) with ISG (lot 2850), 10% human serum albumin (Sigma Chemical Co., St. Louis, MO), or saline in a dose of 50 µl/10 g of body weight for 8 days. Approximately 10⁷ CFU of K79 strain was fed twice daily (0900 and 1700, 1 h after ISG) for 2 days beginning at 3 days of age. Cultures of stool and blood were obtained at 5, 7, and 9 days of age. The efficacy of ISG was determined by comparing rates of colonization and systemic disease among the three groups.

Statistical methods. The χ^2 test with Yates' correction was used (8). *p* values ≤ 0.05 were considered significant.

RESULTS

Cumulative rates of gastrointestinal colonization following oral administration of two type III GBS strains of 3-day-old newborn rats were 57% (8 of 14) for K75 strain and 87% (13 of 15) for K79 strain. It was of note that one animal colonized with K75 (1 of 8 or 13%) and four with K79 (4 of 13 or 31%) were found to be moribund in 2 days after inoculation. Cultures of the blood and CSF were all positive for type III GBS in these animals.

Tables 1 and 2 summarize rates of colonization and bacteremia at 1, 3, and 5 days after oral administration of the K79 strain among animals receiving ISG, albumin, or saline. K79 strain was used because this strain appeared to produce a higher incidence of colonization and systemic disease following oral chal-

lenge. Animals fed with albumin or saline developed colonization in 71% of the animals *versus* 23% of the animals receiving ISG developed colonization ($p < 0.001$, Table 1). Of note, most colonization (28 of 43 or 65%) occurred early, *i.e.* at 1 day after bacterial challenge. Among these animals colonized early, colonization was persistent in approximately 50% of the animals receiving albumin or saline, whereas colonization disappeared in all animals receiving ISG.

A total of 18 animals developed bacteremia following oral administration of the K79 strain (Table 2): two of the ISG group (2 of 26 or 7%) and 16 of the control group (16 of 52 or 31%, $p < 0.05$). Three of the bacteremic animals in the control group died between 2 and 4 days after bacterial challenge. It was interesting to note that stool cultures were also positive in all bacteremic animals. Thus, once animals became colonized, rates of the development of bacteremia were not significantly different between the ISG and control group (2 of 6 or 33% for ISG *versus* 16 of 37 or 56% for control, $p > 0.1$).

DISCUSSION

The infant rat has been used previously to reproduce bacteremia and meningitis with GBS in studies of the pathogenesis, prevention, and therapy of neonatal GBS infections (9, 10, 16, 18). In these studies, GBS were inoculated either intraperitoneally, subcutaneously, or intranasally. In contrast, it is widely believed that in the early onset form of neonatal GBS disease, human newborn infants acquire GBS from the maternal genital tract via either swallowing or aspiration. In addition, the gastrointestinal tract has been suggested as a primary site of GBS colonization in humans with secondary transfer to their birth canal (2, 3).

In this study, we established an animal model of GBS colonization and systemic disease following oral challenge, which may be analogous to the natural acquisition of GBS by the human newborn infants. GBS fed to 3-day-old newborn rats resulted in the gastrointestinal colonization in most and in systemic disease in some. The present findings of GBS colonization and systemic disease following oral challenge were different from those of Ferrieri *et al.* (9) who reported no systemic disease following oral challenge of 10⁶–10⁸ type III GBS. However, the methods employed were different: age of animals was 5–7 *versus* 3 days for our study and frequency of GBS feeding was once *versus* two to four times for our study.

With the availability of this animal model, we evaluated the effect of oral ISG on the gastrointestinal colonization and sys-

Table 1. Rates of gastrointestinal colonization at 1, 3, and 5 days after oral administration of a type III group B streptococcal strain (K79) among animals receiving ISG, albumin, or saline*

Groups	Colonization (%) at days after challenge			Cumulative
	1	3	5	
ISG (<i>n</i> = 26)	4/26 (15%)	0/26	2/26 (8%)	6/26 (23%)
Control (<i>n</i> = 52)	24/52 (46%)	20/50 (40%)	17/49 (34%)	37/52 (71%)
Albumin (<i>n</i> = 25)	13/25 (52%)	10/24 (42%)	9/23 (39%)	20/25 (80%)
Saline (<i>n</i> = 27)	11/27 (41%)	10/26 (38%)	8/26 (31%)	17/27 (62%)

* *p* values are by the χ^2 test with Yates' correction.

Table 2. Rates of bacteremia at 1, 3, and 5 days after oral administration of a type III group B streptococcal strain (K79) among animals receiving ISG, albumin, or saline

Groups	Bacteremia (%) at days after challenge			Cumulative*
	1	3	5	
ISG (<i>n</i> = 26)	2/26 (7%)	0/26	0/26	2/26 (7%)
Control (<i>n</i> = 52)	15/52 (29%)	7/50 (14%)	7/49 (14%)	16/52 (31%)
Albumin (<i>n</i> = 25)	9/25 (36%)	4/24 (17%)	3/23 (13%)	9/25 (36%)
Saline (<i>n</i> = 27)	6/27 (22%)	3/26 (12%)	4/26 (15%)	7/27 (25%)

* *p* value is by the χ^2 test with Yates' correction.

temic disease by GBS. This study was performed based on the following information: 1) orally administered human ISG retains opsonic activity against GBS after passage through the gastrointestinal tract of low birth weight infants (6); 2) oral ISG administration to human newborn infants and lambs protects them from diarrhea caused by rotavirus (5, 19); and 3) preliminary data from our laboratory have shown that oral administration of ISG (10%) 5 ml/kg to 5-day-old newborn rats is not accompanied by demonstrable serum levels of specific antibody (<50 ng of IgG antibody/ml by the ELISA), suggesting no significant absorption of orally administered human immunoglobulin by the gut of the young rat. This finding corroborates the observation of Halliday (11, 12) who found that the absorption of orally administered immunoglobulin in the young rat was selective; immune sera prepared in cows or in humans did not appear to be readily absorbed by the gut of the young rat. Our results suggest that oral immune serum globulin prevents the gastrointestinal colonization and systemic disease by GBS in this newborn rat model. However, rates of the development of systemic disease among the colonized animals were not significantly different between the ISG and control groups, suggesting that the protection exerted by oral ISG is mainly by the effect of local antibody at the mucosal level. Whether this effect is due to the opsonic activity of immunoglobulin leading to phagocytosis and killing or the inhibition of microbial adherence and invasion by specific antibody remains to be determined.

Acknowledgment. The authors thank Bascom F. Anthony for providing the ELISA for measurement of type III group B streptococcal antibody.

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