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0031-3998/85/1903-0297\$02.00/0

PEDIATRIC RESEARCH

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Vol. 19, No. 3, 1985

Printed in U.S.A.

Newborn Sepsis following Antepartum Group B Streptococcal Maternal Infection in Rats

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ABSTRACT. Group B streptococcus is an important pathogen in man and infection due to this bacteria is responsible for significant mortality and morbidity in neonates. An animal model of neonatal infection caused by group B streptococcus that results from vertical transmission is described. Nine pregnant Sprague-Dawley rats received intraperitoneal inoculation of 10^2 – 10^{10} colony forming units of group B streptococcus on day 20 or 21 of gestation. Four of nine rats died following inoculation. A total of 51 pups was born to the surviving five mothers. Pups were sacrificed at 4- to 8-h intervals and cultures of blood, brain, liver, and spleen were obtained. Nineteen of 51 pups (37%) had group B streptococcus isolated from blood or tissues within the first 48 h of life. Results suggest that antepartum systemic infection in rats can result in vertical transmission of disease. This animal model can be used to further study the mechanisms of transmission of group B streptococcus and the pathogenesis and treatment of neonatal sepsis caused by this pathogen. (*Pediatr Res* 19: 297–299, 1985)

Abbreviations

GBS, group B streptococcus
ip, intraperitoneal
CFU, colony forming units

Group B streptococcal infection occurs in two to three newborns per 1000 live births in the United States (1). Prophylactic intrapartum antibiotic regimens directed at interrupting transmission of GBS from mother to neonate has resulted in reduction of newborn colonization and infection (2–4). Difficulties in identifying women at risk for delivering infants who may develop group B streptococcal infections, however, have made indications for these regimens unclear.

Although an animal model for newborn group B streptococcal

sepsis in the rat has been established (5) and GBS surface colonization of pups born to mothers colonized with GBS has been demonstrated (6), a model in the rat that involves newborn sepsis resulting from maternal colonization of infection has not been described. The purpose of this study was to develop an animal model of vertical transmission of newborn sepsis following group B streptococcal challenge of mothers. A better understanding of the mechanisms of transmission of GBS and the pathogenesis of early-onset neonatal sepsis may help in our approach to new prophylactic and therapeutic regimens.

MATERIALS AND METHODS

Preparation of organisms. A human isolate of GBS type III further characterized as resistant to opsonization (7) was incubated in Todd-Hewitt broth (Difco Labs) at 37° C for 18 h and adjusted to 10^8 – 10^9 CFU/ml in normal saline.

Animals. Pregnant outbred Sprague-Dawley rats (Charles River Labs) of 15–18 days gestation were obtained and housed in our animal quarters. On day 20 or 21 of gestation animals were given 2 ml of GBS inoculum (10^9 – 10^{10} CFU) ip through a 25-gauge needle in the right upper quadrant of the abdomen. Care was taken not to inoculate viscera or uterus. Blood cultures obtained by incising the distal tail were taken at 2, 24, 48, and 72 h following inoculation. Animals were closely observed and time of delivery was recorded. Soon after birth, and at regular intervals during the first 48 h of life, pups were sacrificed and cultures were obtained.

Isolation of GBS. Cultures were obtained from all dead adult females, stillborns, and sacrificed pups.

Blood for culture was obtained by aseptic intracardiac puncture with a 25-gauge needle attached to a tuberculin syringe and inoculated onto blood agar plates which were incubated overnight at 37° C.

Brain specimens were obtained by aseptic puncture of the cranium and aspiration with a 21-gauge needle attached to a tuberculin syringe. Brain tissue was inoculated onto blood agar plates and incubated.

Liver and spleen were aseptically removed from each animal upon opening the abdominal cavity. Care was taken not to incise the bowel or stomach and minimal bleeding was appreciated

Received August 24, 1984; accepted October 29, 1984.

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This research was supported in part by a grant from the Thrasher Foundation.

during this procedure. Tissue was homogenized in sterile Petri dishes and inoculated onto blood agar plates and incubated.

All colonies on blood agar plates consistent with GBS were subcultured and were considered to be GBS after demonstrating accentuation of hemolysis on blood agar when incubated with *Staphylococcus aureus* (CAMP phenomena).

RESULTS

Mothers. Nine pregnant dams were challenged with GBS (Table 1). Three received an inoculum of 1.8×10^9 CFU (group 1) and 6 received 1×10^{10} CFU (group 2). All three animals in group 1 survived. Four animals in group 2 died within 78 h of inoculation without giving birth. These four animals were less active than surviving mothers and were noted to be tachypneic 24–48 h prior to their death. GBS was isolated from blood, brain, liver, and spleen of these four animals postmortem. Surviving mothers appeared active, fed well, and delivered litters within 26 to 54 h after inoculation with GBS. Surviving mothers appeared to care for their pups appropriately.

All mothers in group 1 were bacteremic at 2, 24, 48, and 72 h following challenge and thus were documented to be bacteremic with GBS prior to and after labor. In group 2, bacteremia was documented at 24 h following inoculation (26 h prior to delivery) in one surviving mother. The other surviving mother in group 2 had no organisms isolated from blood cultures taken 2, 48, and 72 h following inoculation. Blood culture was not obtained in this animal 24 h following inoculation because she was in labor.

Newborns. A total of 51 pups was born. Four were stillborn (three in group 1). Stillborn pups were not malformed or runted. Sixteen of 47 liveborn pups, and three of four stillborn pups had GBS isolated from blood or tissues. Four unborn pups from each

Table 1. Mortality and frequency of bacteremia following inoculation of pregnant rats with group B streptococcus

	Group 1 (n = 3)	Group 2 (n = 6)
Size of inoculum (CFU)	1.8×10^9	1×10^{10}
Mortality	0% (0/3)	67% (4/6)
Bacteremia	100% (3/3)	83% (5/6)*

* Four animals had group B streptococcus isolated from postmortem blood culture.

Table 2. Frequency of isolation of GBS from blood and tissues in born pups

	Group	
	1 (n = 36)	2 (n = 15)
Blood	17% (6)	13% (2)
Brain	8% (3)	20% (3)
Liver	19% (7)	33% (5)
Spleen	19% (7)	33% (5)
One or more source	31% (11)	53% (8)

Table 3. Frequency of isolation of GBS from born pups at time of sacrifice or death

Time (h)	Group		Total
	1	2	
0–4	4/10 (40%)	2/4 (50%)	6/14 (43%)
4–12	2/9 (22%)	1/2 (50%)	3/11 (27%)
12–24	1/10 (10%)	3/6 (50%)	4/16 (25%)
24–36	4/5 (80%)	1/1 (100%)	5/6 (83%)
36–48	0/2 (0%)	1/2 (50%)	1/4 (25%)

of the females that died prior to delivery had blood, brain, liver, and spleen cultures which were positive for GBS.

Table 2 summarizes the frequency of GBS isolation from blood and tissues in live-born and stillborn pups. GBS was most frequently isolated from liver and spleen homogenates. Two pups had GBS isolated from their spleen in the absence of other positive blood or tissue cultures. Four pups had GBS isolated from their liver in the absence of positive blood or tissue cultures.

Table 3 summarizes the frequency of GBS-positive cultures from pups with regard to hour of sacrifice or death. Of pups from whom GBS was isolated, six of 19 (32%) had bacteria isolated from cultures obtained prior to 4 h of life. Five of these six pups had GBS isolated from their liver or spleen in the absence of positive blood or brain cultures. The other pup had GBS isolated from both liver and spleen in the absence of positive cultures of the blood or brain. Nine of 13 pups from whom GBS was isolated from cultures obtained after 4 h of life, had bacteria isolated from more than one source. All six positive blood cultures in newborn were obtained from pups sacrificed after 4 h of life.

Three pups died at 12–16 h of age before sacrifice (one in group 1). GBS was isolated from blood, brain, liver, and spleen of one of these animals (group 2). One pup died at 4 h of age (group 2); GBS was isolated from its spleen. GBS was not isolated from the other two pups.

DISCUSSION

Group B streptococcal infection in neonatal rats followed antepartum ip bacterial inoculation of mothers. It was apparent in our experiments that sublethal ip inoculation could establish maternal infection without affecting delivery or postpartum maternal-newborn interactions. Documentation of bacteremia in the majority of mothers within 2 h and persisting as long as 72 h following inoculation, suggests that maternal infection can be quickly and effectively produced after GBS ip challenge.

Thirty-seven percent (19/51) of pups born to GBS challenged mothers had GBS isolated from their blood or tissues. The frequency of newborn infection appeared to increase with the size of inoculum that mother received. It has been our experience that the isolation of bacteria from the blood, brain, liver, and spleen of newborn rats occurs following ip inoculation of their mothers and that isolation of GBS from blood and tissues is more sensitive than mortality in determining infection in the newborn rat. The greater frequency of isolation from liver and spleen when compared to blood (Table 2) suggests that isolation of GBS from liver and spleen does not solely reflect bacteremia and that bacteria may localize in these organs.

The frequency of isolation of GBS from pups at the time of sacrifice appears to be constant over the first 48 h. Isolation of GBS from pups immediately after birth and from stillborns suggests that antepartum infection of newborns occurs in this model. Slightly higher frequencies of GBS isolation from pups greater than 24 h of age were noted (Table 3) and suggest that evolving infection may occur in the 1st day of life following vertical transmission.

The mechanism of vertical transmission of GBS in this model is not clear. Mothers did not appear to have abnormal or prolonged deliveries and it seems unlikely that ascending infection occurred in these animals. It may be that aspiration or swallowing of infected maternal blood during birth was a mode of transmission in some pups, however, this mechanism is not consistent with the isolation of GBS from pups immediately after birth or in stillborns. It is probable that antepartum infection occurred in pups in the absence of ascending infection which suggests that transplacental hematogenous transmission is important in this animal model. The pathogenesis of early-onset infection in human neonates born to colonized mothers with intact or recently ruptured membranes could be similar to that occurring in this animal model. Hematogenous transmission of GBS from mother

to infant has been suspected as occurring, particularly in the presence of intact membranes (8). The importance of this mode of transmission of infection, however, is not well understood. It may be that antepartum maternal bacteremia increases the risk to the newborn for acquiring early-onset disease and that preventing newborn early-onset sepsis is dependent on effectively treating or preventing antepartum GBS maternal bacteremia.

Previously described animal models for GBS sepsis have included inoculation of bacteria into amniotic fluid (9) and directly into newborns (5, 10, 11) and have not included maternal infection. This animal model of vertical transmission can be used to study the mechanisms of transmission of GBS from mother to infant and to investigate regimens aimed at preventing early-onset group B streptococcal infection.

Acknowledgments. The authors thank Harry R. Hill, M.D. for reviewing this manuscript and Doris Gutierrez for her technical assistance.

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0031-3998/85/1903-0299\$02.00/0

PEDIATRIC RESEARCH

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Vol. 19, No. 3, 1985

Printed in U.S.A.

Humoral and Cell-Mediated Immunity in Growth Hormone-Deficient Children: Effect of Therapy with Human Growth Hormone

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ABSTRACT. To delineate the role of growth hormone (GH) in the development and function of the immune system, immunological parameters including quantitative immunoglobulins, T and B lymphocytes, phytohemagglutinin lymphoproliferative response and delayed hypersensitivity skin tests were studied in nine GH-deficient children prior to GH therapy and at 2 months and 9 to 12 months following therapy. The phytohemagglutinin response (74.1 ± 37.6 , mean \pm SD), and the T rosette ($58.3\% \pm 9.4$), B rosette ($21.1\% \pm 6.1$), IgG (810 ± 241 mg/dl), (IgA 140 ± 85), and IgM (176 ± 70) levels in GH-deficient children were comparable to age adjusted values in normal children. Following GH therapy the phytohemagglutinin response increased significantly at 9 to 12 months post-therapy, 123.2 ± 51.9 versus 74.1 ± 37.6 , $p < 0.05$. T and B rosettes, immunoglobulin concentrations, and hypersensitivity skin tests were not affected by GH therapy. Al-

though an effect of GH was not demonstrable by these studies, a positive role of GH cannot be entirely excluded since total GH deficiency did not exist in all children. (*Pediatr Res* 19: 299-301, 1985)

Abbreviations

GH, growth hormone
PHA, phytohemagglutinin

GH plays a significant role in the normal development of thymic-dependent immune function in lower mammals (3, 4, 11, 14). Although immune deficiency states in man are frequently associated with impaired growth, abnormalities of GH secretion has not been implicated in such conditions. Moreover, the precise role of GH in the development and maturation of the thymic-dependent and thymic-independent immune functions in humans is as yet unknown. The purpose of the study

Received June 15, 1984; accepted October 31, 1984.

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