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Experimental Vaginal Colonization and Mother-Infant Transmission of Group B Streptococci in Rats

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An animal model for group B streptococcal vaginal colonization and neonatal acquisition was developed with albino rats. Intravaginal inoculation of genital isolates of group B streptococci of serotypes Ia, II, and III either once or on 3 successive days resulted in carriage of the organisms for 7 days or longer in 26% of the virgin animals and 43% of the pregnant animals. Throat and perianal cultures of the offspring of pregnant rats revealed that 51% of the rat pups acquired the organisms at some time. Litter exchange studies were done to explore the contributions of environmental and intralitter spread. Significantly more infants born to mothers with positive vaginal cultures acquired the organisms than infants of culture-negative mothers who were suckled by positive adoptive mothers. However, 13% of the offspring of vaginal-culture-negative rats who were suckled by animals with positive genital cultures acquired group B streptococci. This model may be valuable in understanding the dynamics of vaginal carriage and mother-infant transmission of group B streptococci.

Since the emergence of streptococci of Lancefield group B as major pathogens in the neonatal period, there has been much interest in the epidemiology and pathogenesis of disease caused by these organisms (2, 3, 8, 17, 21). The most information is available for the early-onset type of disease, which is a septic, fulminant form associated with a high mortality where the source of the organism is ordinarily the mother's genital tract (2, 3, 21). This form occurs in 2 to 6 per 1,000 live births or approximately 1 to 3 per 100 colonized infants (3, 16, 21). Although this disease is common, the factors which promote vaginal carriage and neonatal colonization have not been well defined. We have developed an animal model for group B streptococcal vaginal colonization and neonatal acquisition of group B streptococci which may help further our understanding of the dynamics of carriage and transmission of these organisms.

MATERIALS AND METHODS

Rats. Albino Sprague-Dawley rats, 80 to 90 days old and obtained from Bio-Lab Corp. (White Bear Lake, Minn.), were used in the virgin rat carriage experiments. The pregnant rat colonization experiments were done with outbred Sprague-Dawley rats, obtained from either Bio-Lab Corp. or Holtzman Co. (Madison, Wis.), or with virgin rats mated in our own colony. Litter exchange studies were done with rats with known, synchronous matings (Holtzman Co.). Adult rats and rat pups with their mothers were housed separately in solid, opaque cages with filter hoods made from spun-bonded polyester (Lab Products, Inc., Garfield, N.J.) under standard conditions (25°C; relative humidity, 40%) with a 7 a.m.-to-7 p.m. light schedule. Purina rat chow plus water were available ad libitum.

Bacteria. The group B streptococci used were recent human genital isolates. Identification of their serological groups was by the hot acid extraction-capillary precipitin method of Lancefield (11), and typing was done by immunodiffusion in agar (18), using antisera prepared in our laboratory (grouping) and antisera provided by the Center for Disease Control, Atlanta, Ga. (typing). Cultures of the original isolate were either lyophilized or frozen at −20°C in Todd-Hewitt blood broth and had not been passaged in the laboratory.

The streptococcal strains were grown up to late log phase and frozen in small aliquots at −70°C. For each inoculation, the organisms were incubated overnight, transferred to fresh Todd-Hewitt broth (THB), and incubated at 37°C for 3 h. This culture was then washed one to three times in THB and resuspended in 5 ml of THB. Quantitation was done with standard curves of optical density on a Coleman Jr. II spectrophotometer (Coleman Instruments Div., Oak Brook, Ill.), with pour plates done periodically to confirm inoculum size. Supernatants of these cultures were tested for pH on a Corning pH meter (Corning Scientific Instruments, Medfield, Mass.) for the vaginal carriage experiments in virgin animals. Representative streptococcal cultures recovered from the animals were grouped and typed to confirm their identification.

Vaginal carriage in virgin and pregnant rats. Pregnant or 80- to 90-day-old virgin rats were inoculated with 0.1 ml of a bacterial suspension containing 10^7 to 10^9 colony-forming units of a type Ia, II, or III isolate by inserting an automatic pipette (Pipetman; Rainin Instrument Co. Inc., Brighton, Mass.) atrau-

matically into the vagina. Virgin animals were staged in estrous cycle by observation of external genitalia according to the method of Long and Evans (14). This method divides the estrous cycle into five stages spanning 4 days, with diestrus (stage V) occupying approximately one-half of the total cycle. Animals were inoculated either once or on 3 successive days. Most of the animals inoculated once were in diestrus, whereas those inoculated on 3 successive days were in at least two stages of their cycle. Vaginal cultures were done by rotating a cotton swab, moistened with THB, in the vaginal orifice. Pre-inoculation cultures were streaked on plates of tryptose-blood agar base (Difco Laboratories, Detroit, Mich.) containing 6% sheep blood. All other cultures were streaked on plates of Columbia blood agar base containing 10 μg of colistin sulfate per ml and 15 μg of nalidixic acid (Columbia CNA blood agar base; Difco Laboratories) per ml. Plates were read on a semiquantitative basis—1+ (1 to 10 colonies), 2+ (11 to 50 colonies), 3+ (>50 colonies), and 4+ (pure growth). Vaginal cultures were obtained before each inoculation and on 1, 3, 5, 7, 10, and 14 days post-inoculation. Animals with positive cultures on day 14 were cultured approximately weekly thereafter. Rectal cultures were done consistently on eight of the animals; these were processed in the manner described. Vaginal cultures were obtained from all of the adult rats before beginning any experiments, and the cultures were all negative for group B streptococci.

In eight of the virgin animals, all aerobic organisms were isolated and tested for inhibition of group B streptococci by using a spotting technique (5) on a lawn of group B streptococci.

Studies of streptococcal acquisition in newborn rats. All newborn rats born to mothers who had positive or negative group B streptococcal vaginal cultures at the time of delivery were cultured. Cultures of the throat and perianal area were obtained with a cotton swab moistered in THB. These were directly plated on either blood agar or, if previously colonized with a swarming *Proteus* species, Columbia CNA blood agar base plates. Except for the first three litters of pups, swabs were also inoculated into THB containing 5% defribrinated sheep's blood, 8 μg of gentamicin (Schering Laboratories, Bloomfield, N. J.) per ml, and 15 μg of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) per ml (4). The inhibitory broth cultures were subcultured on blood agar plates at 24 to 48 h and read as positive or negative for group B streptococci. Blood cultures were obtained from 32 pups by removing 5 μl of blood from the tail vein on each of the first 3 days of life.

Litter exchanges. In separate experiments from the above, litters from pregnant rats with vaginal cultures positive for group B streptococci of serotype II were exchanged with the litters of uninoculated, culture-negative rats. Litters were born within 18 h of one another and were exchanged by 24 h of age. The throats and perianal areas of all rat pups were cultured on blood agar plates and in inhibitory broth before exchange (day 1) and again on days 3, 5, 7, 10, and 14. The mother rats were cultured vaginally on the same schedule, and rectal cultures were obtained on days 5 and 7.

Statistical analysis. The chi-square test was used to determine the significance of the data. When numbers were small in any of the subgroups, the Yates correction was used.

RESULTS

Vaginal colonization in virgin and pregnant rats. The overall vaginal colonization rate after intravaginal inoculation of serotypes Ia, II, and III for 1 week or longer for virgin animals was 26% (11/42) (Table 1). There was no significant difference in frequency of successful colonization among the three serotypes tested. The mean duration of carriage in those animals carrying the organism for at least 7 days was 17.3 days.

The carriage rate for 7 days or longer in virgin rats inoculated on a single occasion was 27% (7/19) versus 22% (5/23) for those inoculated on 3 successive days. Since the animals inoculated on a single occasion were predominately in the diestrus (stage V) phase of their cycle and the others were inoculated in at least two stages of their cycle, it appeared that the stage of estrous cycle was not a major determinant of streptococcal carriage in this model.

Of the 40 pregnant rats inoculated on 3 successive days, 17 (43%) carried the organism for 7 or more days (Table 1). The mean duration of carriage for these animals was 10.2 days. The numbers were too small for valid statistical analysis of serotypic differences in achieving colonization in these animals.

There was no significant difference between pregnant and virgin rats in the overall rates of vaginal colonization with group B streptococci on days 1, 3, 5, and 7 after inoculation (Table 2). Nearly all animals converted to positive by 1 day after the last inoculation, and the carriage rate declined over the first 7 days to 26% in virgin animals and to 43% in pregnant rats.

Since the normal gestation of the rat is approximately 21 days, we attempted to examine

TABLE 1. *Comparison of vaginal colonization with group B streptococcal serotypes in virgin and pregnant rats*

Serotype	Virgin animals			Pregnant animals		
	Total[a]	With positive cultures for ≥7 days		Total[a]	With positive cultures for ≥7 days	
		No.	%		No.	%
Ia	6	1	17	8	4	50
II	22	8	36	26	8	31
III	14	2	14	6	5	83
All	42	11	26	40	17	43

[a] Number of animals inoculated intravaginally with group B streptococci.

TABLE 2. *Group B streptococcal carriage rates in virgin and pregnant rats at intervals after inoculation*

Days post-inoculation	Positive cultures in:			
	Virgin rats (N = 42[a])		Pregnant rats (N = 40[a])	
	No.	%	No.	%
1	39	93	39	98
3	28	67	26	65
5	17	40[b]	21	53[b]
7	11	26[c]	17	43[c]

[a] All animals were cultured at the specified times.
[b] $P > 0.10$.
[c] $P > 0.10$.

the influence of stage of pregnancy on vaginal carriage of group B streptococci by separating animals into those inoculated either more than or less than 7 days before birth of their litters. Twenty-five of the pregnant rats completed the three vaginal inoculations within 7 days before delivery, and 12 (48%) became carriers of the organism for 7 days or longer. Fifteen animals completed the inoculations more than 7 days before delivery, and five (33%) were carriers of the organism for 7 days or longer; this difference was not statistically significant, $P > 0.50$.

Other aerobic organisms recovered from the vaginas of animals before inoculation were tested for bacteriocin-like activity (5), but no inhibition of group B streptococci was found. There appeared to be little qualitative change in the vaginal flora, consisting predominately of gram-positive cocci, after the introduction or disappearance of group B streptococci. The effect of the pH of the bacterial inoculum on success of colonization with group B streptococci was studied. There was no significant difference in the rate of streptococcal carriage in animals who received an inoculum with a lower (5.7 to 5.9) versus a higher (6.9 to 7.1) pH.

Streptococcal acquisition by newborn rats. Rat pups born to inoculated mothers who had converted to negative streptococcal cultures by time of delivery had a zero acquisition rate of group B streptococci. The cumulative group B streptococcal acquisition rate in rat pups born to mother rats with streptococcal vaginal colonization was 51% (72/142). The daily prevalence rates ranged from 27 to 12% with the lowest carriage rates on day 7 (Fig. 1). There were no significant differences in recovery rates in animals acquiring type II versus type III group B streptococci. The cumulative acquisition rate for infant rats for type II was 54% (43/80) versus 47% (29/62) for type III.

Positive cultures were evenly divided between the two sites cultured. The swabs of the throats yielded group B streptococci in 11% of the cultures, whereas 10% of all perianal cultures were positive.

The mean duration of carriage of group B streptococci (day of first positive culture to day of positive culture after which organism was no longer recoverable) was 3.5 days. Some animals were only positive on 1 day at a single site, whereas the longest duration of carriage was 21 days.

Of the 72 pups who acquired group B streptococci, 61 became culture positive while the mothers were still positive, and 10 of these pups were positive at both sites sampled. The other 11 pups became culture positive for the first time after their mothers' vaginal cultures converted from positive to negative, and none of them had two positive sites. These latter findings could be interpreted as intralitter or environmental transmission or both. Maternal rectal and nipple cultures were done and none was positive.

None of the blood cultures obtained from the 32 rat pups was positive.

Litter exchange studies. To further evaluate the role of spread from a maternal source versus intralitter or environmental spread or both, litter exchange experiments were carried out with mother rats and litters distinct from those used in the previous studies. The mother rats accepted the exchanged litters without difficulty, and there were no episodes of litter rejection.

Before transfer to a culture-negative adoptive mother, 15% of animals born to culture-positive mothers harbored streptococci (Table 3). The overall group B streptococcal acquisition rate for animals born to mothers with positive vaginal cultures and suckled by culture-negative mothers was 32% (17/53), whereas the rate for pups born to culture-negative mothers but suc-

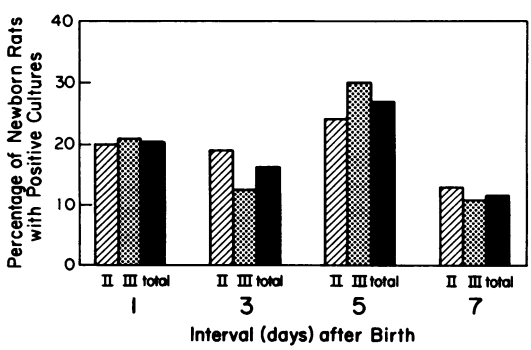

FIG. 1. *Prevalence of positive throat or perianal cultures or both for group B streptococci in suckling rats born to mothers with experimentally produced vaginal colonization with serotypes II and III.*

TABLE 3. *Group B streptococcal acquisition in newborn rats of litter exchange studies*

Culture status of:		Culture results of newborn rats					
				Positive after transfer			
Biological mother	Adoptive mother	Positive before transfer[a]		Before elimination of 15 pups from erratic litter		After elimination of 15 pups from erratic litter[b]	
		No.	%	No.	%	No.	%
+	−	8/53	15	9/41[c]	22	9/26	35
−	+	0/49	0	6/45[d]	13	6/45	13

[a] Cultures obtained within 24 h of birth.
[b] See text.
[c] Denominator excludes four animals lost and eight animals excluded because of previous positive culture.
[d] Denominator excludes four animals lost.

kled by culture-positive mothers was 13% (6/45), $P < 0.05$. All positive mothers had similar semiquantitative culture results at the time of delivery. Of the 41 rat pups who were born to culture-positive mothers but had negative cultures at the time of exchange, 9 (22%) subsequently developed positive cultures for group B streptococci. In comparison, 6 of 45 (13%) pups born to culture-negative mothers but suckled by culture-positive rats developed positive cultures, $P > 0.10$. If we exclude the single litter of 15 pups born to a culture-positive mother in which no pup was colonized before exchange, the secondary acquisition rate in the group born to culture-positive mothers but suckled by culture-negative animals increased from 22% to 35%. When this latter rate is compared with 13%, the difference is still not significant, $P > 0.05$.

There was no significant difference in the density of streptococci recovered from the infant rats with early versus that from rats with later acquisition of streptococci. However, there was a slight trend for both sites to be culture positive and for persistence of positive cultures in the animals with early acquisition.

DISCUSSION

The emergence of the group B *Streptococcus* as a significant neonatal pathogen has led to intensive study of the natural history of this organism. Previous work has shown that the clinical manifestations of disease caused by this organism can be divided into early-onset disease (fulminant, septic presentation) and late-onset disease (usually meningitis) (2, 3, 17, 21).

Attempts to study the epidemiology of the clinical manifestations of this organism have been primarily aimed at early-onset disease. It has been found that the mothers of newborn infants with early-onset disease carry the same serotype of group B streptococci in their genital tracts (3, 21). These infants also have a higher incidence of obstetrical complications, such as prolonged rupture of the membranes and premature labor (2, 3, 21). The same is not true of late-onset disease (3), but the epidemiology of this manifestation of streptococcal disease is not well understood. Nosocomial spread of group B streptococcal is known to occur (1, 2, 15, 19), but the relationship of this type of transmission to the development of disease is unclear.

In the study of the pathogenesis of this disease, several animal models have been developed. These include a chicken embryo model (20), an adult mouse model (22), a newborn mouse model (10), and a newborn rat model (7, 9). The design of these models allows the study of the natural history, progression, and pathology of group B streptococcal disease but gives limited information concerning its epidemiology. The mouse model described by Furtado (10) demonstrated that group B streptococci could colonize mucosal surfaces, such as vagina and pharynx, of these rodents, but there was no investigation of neonatal acquisition (in contrast to disease) or of mother-infant transmission of group B streptococci.

Using an atraumatic technique to introduce group B streptococci into the vaginas of virgin or pregnant rats, colonization rates were achieved that are similar to those encountered in the human populations studied to date (2, 3, 8, 21). There were animals who maintained vaginal carriage of these organisms for as long as 3 to 5 weeks. None of the experimental animals developed a vaginal discharge or other clinical evidence of infection.

Several factors were evaluated which might influence vaginal carriage in adult rats. The stage of estrous cycle, repeated inoculations, and other vaginal flora did not appear to bear any relationship to achieving colonization or to prolonged carriage of group B streptococci. Larsen et al. have shown that vaginal bacterial counts are maximal at estrus, the estrogen-dominated part of the cycle (12). In other studies, they demonstrated that ovariectomized rats had a less diverse genital microbial flora (13), but none of these experiments dealt with introduction of exogenous bacteria into the genital tract. It is not certain that any phase of the estrous cycle has a preferential effect on any single species of vaginal bacteria.

The offspring of the pregnant animals with positive vaginal cultures acquired the organisms intially by contact with the contaminated birth canal. In this study, factors in the adult rat, such as duration of vaginal carriage before or after delivery and other vaginal flora, did not influence streptococcal acquisition by newborn pups,

nor, as in the human situation (2, 3), did the serotype of group B streptococci carried in the vagina. Since 20% of all pups were culture-positive within the first 24 h of life, intrapartum acquisition was extremely important.

Among the colonized animals in the litter exchange studies who were born to culture-positive mothers, over 50% developed their first positive cultures more than 24 h after birth. This finding suggests that later acquisitions were secondary either to multiplication of small numbers of bacteria acquired at birth, but initially undetectable, or to intralitter or environmental sources.

Thirteen percent of animals born to culture-negative mothers where the only source of group B streptococci was a culture-positive suckling mother eventually did develop positive cultures. Therefore, late postnatal transmission of the organisms from a culture-positive mother is possible. Whether all these pups acquired their organisms directly from the adoptive mother, through environmental contamination, or from one of the preceding two sources through a littermate cannot be determined.

Nosocomial transmission has been described in newborn nurseries (1, 15, 19), but the relative importance of this mode of transmission to the development of disease is unknown. Nosocomial transmission in humans has been traced to nonmaternal human sources (1, 8) as well as environmental contamination (6). The contribution of maternal intrapartum genital transmission versus postpartum transmission to late neonatal acquisition of group B streptococci is not clear in humans.

There has been recent interest in prevention of all forms of group B streptococcal disease by using either passive immunization approaches or antibiotic administration (2, 3). The effects these approaches may have on colonization and disease are unknown. The rate model possesses many similarities to the human modes of transmission and can be used to study factors and patterns involved in acquisition and transmission of group B streptococci.

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