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Experimental Model of Congenital Syphilis

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Female LSH hamsters infected with *Treponema pallidum* subsp. *endemicum* before pregnancy or during early pregnancy transmit a form of syphilis to the fetus that is similar to human congenital syphilis. The offspring develops rhinitis, skin rash, failure to thrive, and hepatosplenomegaly. *T. pallidum* is detectable in their livers, spleens, and nasal secretions. Immunoglobulin M antibodies are detected in the serum.

Congenital syphilis occurs when a pregnant woman with primary, secondary, or latent syphilis transmits the infection across the placenta to the fetus during gestation. About 25% of prenatal infections result in stillbirths, while 25 to 30% of newborn infants die shortly after birth and 40% develop late syphilis (5). The risk of in utero infection with *Treponema pallidum* in untreated early maternal syphilis is estimated to be about 80 to 90% (10). Progress in the understanding of mechanisms of transplacental transmission of syphilis has been hampered by the absence of an adequate experimental model. Fitzgerald has shown the congenital transmission of syphilis in rabbits (6). In his study, pregnant rabbits given multiple intravenous injections of very high concentrations of *T. pallidum* (10^8 microorganisms) gave birth to babies with treponemas in fetal tissue. The sick newborns died within 1 or 2 days after birth. It is not clear in that model whether the mother ever acquired the disease before transmitting it to the offspring; the fetus could have received the treponemas directly through the placenta at the time of inoculation.

LSH Syrian hamsters infected with *T. pallidum* subsp. *endemicum* (Bosnia A strain), the etiologic agent of endemic syphilis (bejel), develop an infection that resembles human venereal syphilis more closely than any other animal model does (1–3, 7–9). We now describe in utero transmission of syphilis in the hamster, a model that has many similarities to human congenital syphilis.

Female and male 10- to 12-week-old LSH/Ss Lak inbred strains of Syrian hamsters (Charles River Breeding Laboratories, Wilmington, Mass.) were kept in group housing at an ambient temperature of 18°C. *T. pallidum* subsp. *endemicum* (Bosnia A strain) organisms were a generous gift from Paul Hardy, Jr., Johns Hopkins University. Hamsters were infected as described previously (7), and infections were maintained by passage in vivo. Treponemas (10^5 microorganisms in 0.1 ml) were injected intradermally at the preshaved inguinal regions of nulliparous female hamsters. Alternatively, 10^5 or 10^6 microorganisms were inoculated intraperitoneally. In preliminary studies in adult males these were the doses that induced the highest bacterial counts in lymph nodes. Control groups included animals that were not exposed to *T. pallidum* and animals injected with heat-killed (56°C for 1 h) microorganisms intraperitoneally. After a predetermined time following infection, two to three females were caged with an uninfected male for 1 to 2 days. The male was then removed, and each female was housed separately.

Tables 1 and 2 show that mothers infected before conception or during the early stage of gestation (2 days after detection of vaginal plugs) gave birth to a high proportion of babies with clinical and laboratorial abnormalities. The most significant abnormalities were found in babies born to mothers infected 2 months before conception (Table 1). Twenty-three percent (8 of 35) of the offspring born to infected mothers were stillborn or died shortly after birth. A large number exhibited skin rashes, ano-genital lesions, rhinitis, hepatomegaly, and splenomegaly (Table 1). Both the ano-genital lesions and the rhinitis were characterized by erythema and a mucous scanty exudate. The other types of skin rashes were dry, mildly scaly, erythematous, nonraised, and poorly delineated from surrounding areas. More than 50% of the babies exhibited rhinitis, and approximately a third had perianal and perigenital lesions (Fig. 1). Unlike in the infected adult hamster, perioral ulcers were absent. Most of the affected animals failed to thrive. Most animals of each group were sacrificed and underwent necropsy at 6 days after birth. Tissue samples from bone marrow, spleen, and liver were fixed in formalin and embedded in paraffin. Hematoxylin-eosin- and Warthin-Starry-stained histological sections were examined by light microscopy (Fig. 2). Treponeme counts were performed by dark-field microscopy in fresh nasal and anal secretions and in liver single-cell suspensions in chilled medium.

The average number of treponemas found in 10 high-power microscopic fields (100× oil immersion objective lens fields) was determined after 50 high-power fields were examined for each sample. For this experiment, mothers were infected intraperitoneally with *T. pallidum* (10^5 microorganisms) 3 weeks before conception. With this route of admin-

TABLE 1. Presence of various signs of syphilis in offspring born to syphilitic mothers

Sign of syphilis[a]	No. of positive animals/no. of animals examined	
	4 wk[b]	8 wk[b]
Stillborn	5/24	3/11
Rhinitis	11/19	8/8
Skin rash	11/19	5/8
Hepatosplenomegaly	9/19	8/8
Ano-genital lesions	6/19	6/8

[a] Signs noticed at birth or within 3 weeks of birth.
[b] Mothers were intradermally inoculated either 4 or 8 weeks before conception.

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TABLE 2. Presence of serum treponema-specific IgM antibodies in newborn (1-week-old) hamsters

Group	Type of inoculation	Time from conception	No. positive/ total no. tested
A	10^5 live treponemas, inguinal	8 weeks before	26/28[a]
B	10^5 live treponemas, inguinal	3–5 days before	9/12[a]
C	Heat-killed treponemas, inguinal	3–5 days before	0/6
D	10^6 live treponemas, intraperitoneal	0–1 day before	6/6
E	10^6 live treponemas, intraperitoneal	5–7 days after	0/22
F	None (normal control)		0/11

[a] The remaining samples were weakly positive.

istration, mothers had no apparent signs of syphilis but had positive serology for syphilis at about 6 weeks after inoculation. Motile treponemas were detected by dark-field microscopy in liver cell suspensions of 85% of the newborn babies from these mothers (Table 3). Fewer organisms were seen in the spleen and in the mucus of nasal or ano-genital secretions. Care was taken to avoid cross-contamination among different organs. A moderate increase in extramedullary hematopoiesis was seen in the liver and spleen.

Serum of newborn animals was assessed for the presence of *T. pallidum*-specific immunoglobulin M (IgM) antibodies. Treponemas isolated at peak infection (4 weeks postinfection) from inguinal lymph nodes of adult animals were smeared onto fibronectin-coated glass slides (1 μg/ml; Sigma Chemical Co., St. Louis, Mo.), air dried, fixed with absolute methanol for 10 min, and stored at −20°C until use. Fibronectin increases the adhesion of organisms to glass slides. Ten minutes before the assay, slides were dipped into phosphate-buffered saline (PBS) for 5 min. *T. pallidum* slides were exposed to 1:5-diluted test sera for 30 min, washed three times with PBS, and then incubated with rabbit anti-hamster IgM (μ-chain-specific) antibodies (1:20 dilution; Accurate Chemical, Westbury, N.Y.) for an additional 30 min. After incubation, slides were washed three times in PBS and then incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin (Accurate Chemical). Only 3+ to 4+ staining was considered positive. All incubations were done at room temperature. Serum of 89% of live babies had treponema-specific IgM antibodies (Table 2). As in humans, IgM does not cross the placenta in hamsters (4).

FIG. 1. Newborn hamster with congenital syphilis (4 days old) showing rhinitis (snuffles).

FIG. 2. Photomicrograph of *T. pallidum* subsp. *endemicum* spirochetes (arrows) in the neonatal spleen (Warthin-Starry stain; original magnification, ×630).

The animal model of congenital syphilis presented here has close similarities to the human disease, even though *T. pallidum* subsp. *endemicum* does not cause congenital syphilis in humans. This is the only animal model of congenital syphilis that has skin rashes, rhinitis (snuffles), and hepatosplenomegaly. The incidence of stillbirths in this model (23%) is similar to the incidence of stillbirths in untreated pregnancies of women infected with *T. pallidum* (25%) (5). The majority of the offspring show signs of infection both in humans and in this animal model. Many of these manifestations were present at birth, ruling out the hypothesis of intrapartum or postnatal infection.

The timing of maternal inoculation is critical in this model: if the inoculation of treponemas occurred after 5 days of gestation, the offspring did not show any clinical or serolog-

TABLE 3. Presence of treponemas in liver cell suspensions of newborn hamsters from syphilitic mothers

Newborn no.	Sign of syphilis at birth	No. of treponemas/ 10 high-power fields
1	Rhinitis (severe)	60
2	Rhinitis (moderate)	30
3	Rhinitis (moderate)	10
4	Rhinitis (moderate)	3
5	Rhinitis (moderate)	1
6	Rhinitis (moderate)	20
7	Rhinitis (mild)	2
8	Rhinitis (mild)	5
9	None	1
10	None	1
11	None	0
12	None	0

ical evidence of disease. This is probably related to the short duration of pregnancy in hamsters (18 to 22 days) and the long incubation time for the maternal infection to get established, even if the process is abbreviated by injecting treponemas intraperitoneally. The peak incidence of congenital defects occurs when inoculation is performed about 8 weeks prior to conception. This is probably due to peak treponemia in adult hamsters at 6 to 8 weeks of infection (7).

This animal model may be useful in studying mechanisms of congenital transmission, as well as the role of immune responses and vaccination in modifying the incidence and morbidity of neonatal disease.

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