# Experimental Congenital Syphilis: Guinea Pig Model

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Neonates born to female guinea pigs of either a highly susceptible (C4D) or a resistant (Albany) strain, infected prior to or during pregnancy with a single dose of *Treponema pallidum*, showed in their sera from the first day of life immunoglobulin M (IgM) antibodies to *T. pallidum*, circulating immune complexes consisting of IgM antibodies and treponemal antigens, and IgM rheumatoid factor. Although the animals were asymptomatic for a 6-month observation period, several lines of evidence indicated that they were infected in utero. Molecular analysis of whole sera, purified serum IgM fraction, or dissociated immune complexes demonstrated IgM reactivity against one (47 kDa) or more of several *T. pallidum* peptides (15, 17, 37, 42, 45, and 87 kDa) recognized as integral membrane components. Sequential analysis of the neonates' sera by immunoblot and enzyme-linked immunosorbent assay, using alcohol-treated *T. pallidum*, *T. phagedenis* biotype Reiter, and *T. vincentii*, demonstrated early IgM antibodies followed 3 to 4 months later by IgG2- and IgG1-specific antibodies to *T. pallidum*. Moreover, an infectivity test done in five rabbits with pooled tissue extracts prepared from liveborn or stillborn animals evoked a seroconversion in two rabbits (reactive Venereal Disease Research Laboratory and fluorescent treponemal antibody tests), suggesting the presence of *T. pallidum* in the organs. Sera from neonates born to either *T. phagedenis* biotype Reiter-injected mothers or three normal pregnant females were all serologically negative. The model offers new possibilities for exploration of factors responsible for asymptomatic infection often observed in human congenital syphilis.

Congenital syphilis is a preventable disease, and yet in the last few years its incidence has increased alarmingly in some areas of the United States (4).

Maternal transmission of *Treponema pallidum* from a mother to her fetus may result in stillbirth or fatal disease, latency, or apparent absence of infection. An early clinical diagnosis of congenital syphilis is difficult because the majority (≥50%) of infants are asymptomatic and the serodiagnosis is complicated by the transplacental passage of maternal antibodies (immunoglobulin G [IgG]). The high incidence of prenatal treatment failure and the limitations of current screening tests unable to identify an asymptomatic infected infant (17, 25, 27, 36) point to the critical lack of understanding of the fetal response to infection and therapy.

For decades it was believed that *T. pallidum* does not cross the placenta until after the fourth month of pregnancy at the time when the Langhans cells in the placenta become atrophied (6, 26). This notion, however, has been challenged. It has been indicated that treponemal invasion of the fetus may occur at any stage of pregnancy (14), but signs of fetal infection become apparent only after a functional immune system develops, which in humans is approximately at 16 weeks of gestation (34). The lack of a relevant animal model has been a major obstacle in the delineation of factors contributing to the pathogenesis and immunology of both neonatal and congenital syphilis.

Several studies using the rabbit model for these purposes showed discrepancy in the results. Some investigators reported innate fetal or neonatal resistance to syphilis infection (12, 13, 20, 21, 28), whereas others reported a runting syndrome in neonatal rabbits inoculated within 15 days of

life (10). Successful induction of fetal infection has been reported by Fitzgerald (11) by giving to pregnant female rabbits multiple intravenous (i.v.) inoculations with large numbers of treponemes. Kajdacsy-Balla et al. (18) reported induction of congenital disease by infecting female Syrian hamsters with *T. pallidum* subsp. *endemicum* (Bosnia strain). As the hamster does not develop lesions in response to *T. pallidum* subsp. *pallidum* and endemic syphilis is neither a venereal disease nor known to cause congenital syphilis in humans, the relevance of the above information to human disease remains obscure.

Using the guinea pig model, we have previously demonstrated that the ontogeny of the immune response (cutaneous reaction to intradermal [i.d.] challenge) in C4D and Albany neonates, as far as *T. pallidum* infection is concerned, has a different time course (39). Here we present evidence that pups born to syphilitic mothers of either strain mount a humoral response (IgM antitreponemal antibodies, circulating immune complexes [CIC], and rheumatoid factor [RF]) closely resembling that of human infants congenitally infected in utero.

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## MATERIALS AND METHODS

**Animals and infection.** Young adult (3 to 4 months old) females of two strains of guinea pig were used. The C4D strain (genetically related to strain 13) is very susceptible to *T. pallidum* infection (50% infective dose = $10^2$ organisms) and the Albany strain (genetically related to strain 2) is resistant to a dose of as many as $10^9$ organisms (37). *T.*

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*pallidum* subsp. *pallidum* Nichols obtained from orchitic rabbit testes (39) were used for infection of female guinea pigs either i.d. (3 × $10^7$ organisms per ml) or i.v. (5 × $10^8$ organisms per ml). Infection of C4D females by the i.d. route was done 1 to 3 months prior to pregnancy; the remaining C4D or Albany animals were infected 30 to 40 days prior to parturition. Four 30- to 40-day-pregnant females, two of each strain, were injected i.v. with 5 × $10^8$ *T. phagedenis* biotype Reiter and used as controls. The guinea pig has a gestation period of 65 to 72 days. Neonates were housed together with their mothers until they reached approximately 4 weeks of age, when they were weaned and housed in groups of two per cage and then individually. The animals were kept in air-conditioned quarters (18 to 20°C) and fed antibiotic-free food. Experimental and control animals were examined and bled at regular intervals under anesthesia (Ketaset; Bristol Laboratory, Syracuse, N.Y.). At the end of the experiments they were sacrificed by i.v. injection of euthanasia agent (Somlethol; American Hoechst, Somerville, N.J.). The animal procedures have been approved by the Institutional Animal Care and Use Committee of the Wadsworth Center for Laboratories and Research.

**FTA-ABS.** The fluorescent treponemal antibody test (FTA-ABS) was done with various dilutions of heat-inactivated sera, using commercial sorbent and slides with fixed *T. pallidum* (Zeus Scientific, Raritan, N.J.). Fluorescein isothiocyanate-conjugated rabbit anti-guinea pig IgG (heavy and light chains; Zymed Laboratories, San Francisco, Calif.) was used as second antibody (38).

**ELISA.** The specificities of the antitreponemal antibodies and the immunoglobulin isotypes were examined by enzyme-linked immunosorbent assay (ELISA), using Immulon II microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) coated with Percoll-purified *T. pallidum*, well-washed *T. phagedenis* biotype Reiter, or *T. vincentii* treated with 10% ethanol (41). The ELISA technique as described previously (2) was followed by using affinity-purified rabbit IgG anti-guinea pig IgM, IgG2, and IgG1 (diluted 1:100 in phosphate-buffered saline containing 0.1% bovine IgG and 0.5% bovine serum albumin).

Optical density (OD) values of ≥0.100 were considered positive for IgM and IgG2 antibodies and values of ≥0.025 were positive for IgG1 subclass. These values were ≥2 standard deviations (SD) determined in sera from healthy young ($n$ = 20) and adult ($n$ = 30) guinea pigs.

**IB.** Immunoblotting (IB) was done similarly as reported in reference 3, using as probes affinity-purified rabbit IgG antibodies to guinea pig IgM, IgG2 and IgG1 (ICN Biomedicals, Costa Mesa, Calif.).

**CIC.** Levels of CIC in guinea pig sera were determined by the C1q solid-phase radioimmunoassay (C1q-SPA), using iodinated affinity-purified rabbit IgG specific for guinea pig IgM or IgG (3).

**DIC.** Soluble immune complexes in sera were precipitated and then dissociated as described previously (33). Briefly, 7% polyethylene glycol in 0.1 M sodium borate buffer (pH 8.4) was mixed with an equal volume of sera containing CIC or with control sera from healthy guinea pigs and incubated overnight at 4°C. After centrifugation, the pellets were washed twice with 3.5% polyethylene glycol in sodium borate buffer and resuspended to the original volume in 0.1 M sodium borate buffer, pH 10.2. Dissociated immune complexes (DIC) were examined by IB for treponemal antibodies and antigens.

For detection of antibodies, solubilized *T. pallidum* antigens were separated by sodium dodecyl sulfate-polyacryl-

TABLE 1. Experimental and control animals included in the study[a]

| Animal strain | Organism | Route | Dose | No. of animals | | |
|---|---|---|---|---|---|---|
| | | | | Mother | Liveborn | Stillborn |
| C4D | *T. pallidum* | i.d. | 3 × $10^7$ | 6 | 19 | 6 |
| | | i.v. | 5 × $10^8$ | 2 | 5 | 2 |
| | *T. phagedenis* Reiter | i.v. | 5 × $10^8$ | 2 | 4 | 1 |
| Albany | *T. pallidum* | i.d. | 3 × $10^7$ | 2 | 4 | 0 |
| | | i.v. | 5 × $10^8$ | 5 | 10 | 2 |
| | *T. phagedenis* Reiter | i.v. | 5 × $10^8$ | 2 | 4 | 0 |

[a] Except for C4D females i.d. infected 1 to 3 months prior to parturition, the remaining animals were infected or injected 30 to 40 days prepartum.

amide gel electrophoresis (SDS-PAGE) and then transferred onto nitrocellulose strips. The strips were subsequently incubated first with DIC and then with affinity-purified rabbit IgG antibodies to guinea pig IgM, IgG2, or IgG1 and further probed with horseradish peroxidase-conjugated goat anti-rabbit IgG (3). Positive human control sera were reacted with horseradish peroxidase-conjugated goat anti-human IgM or IgG.

For detection of treponemal antigens, DIC were first separated by SDS-PAGE and then transferred onto nitrocellulose paper and probed with sera from human secondary syphilis or sera from *T. pallidum*-infected rabbits. The reaction was developed with horseradish peroxidase-conjugated goat anti-human or anti-rabbit IgG.

**RF.** The presence of 19S IgM RF was detected by a microtiter radioimmunoassay (8), using plates coated with affinity-purified guinea pig IgG (0.5 μg per well). Dilutions of the test sera were used as first antibody, and iodinated rabbit anti-guinea pig IgM (100,000 cpm per well in phosphate-buffered saline containing 0.5% bovine serum albumin) was used as second antibody. After washings, the wells of the plates were cut out and radioactivity was measured in a gamma counter. Results were expressed as cpm, and those with a value of ≥2 SD (≥100 cpm) above control sera ($n$ = 30) were considered positive.

## RESULTS

**Course of infection.** The numbers of experimental and control animals, doses of organisms, and routes of infection or injection are presented in Table 1. Between 9 and 12 days after infection, all six C4D females infected i.d. (four at 3 months and two at 1.5 months prior to fertilization) developed dark-field-positive ulcerative lesions (10 to 15 mm in diameter) which lasted for 10 to 14 weeks. In the i.d. infected females, lesions were present at the time of fertilization or during pregnancy. The i.v. infections, carried out between weeks 4 and 6 of pregnancy, did not produce any clinical signs of disease in the animals. The eight C4D-infected females gave birth to 24 liveborn and 8 stillborn infants. The Reiter-injected females delivered four healthy newborns and one stillborn animal.

Intradermally infected Albany females responded at between 9 and 11 days with dark-field-positive, nonprogressive, indurated papules (5 to 7 mm in diameter), which disappeared within 12 days. The i.v. infected females did not show any clinical signs of infection. Seven infected Albany females gave birth to 14 liveborn and 2 stillborn infants. The

TABLE 2. Treponemal antibodies (IgG FTA-ABS), CIC, and RF in families consisting of a mother and three offspring[a]

| Strain | Infection route (dose) | Bleeding postpartum (wk) | Mother | | Offspring | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | FTA-ABS[b] | IgG CIC[c] | FTA-ABS | | | IgM CIC | | | RF (cpm)[d] | | |
| | | | | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| C4D | i.d. (3 × 10⁷) | 1 | 640 | <2 | 320 | 320 | 640 | 3.3 | <2 | <2 | 335 | 258 | 573 |
| | | 4 | 160 | 3.2 | 40 | 80 | 40 | <2 | 4.4 | 3.2 | 170 | 221 | 301 |
| | | 8 | 160 | 2.7 | <10 | <10 | <10 | <2 | <2 | <2 | 186 | 172 | 173 |
| | | 12 | 160 | <2 | <10 | <10 | <10 | <2 | <2 | <2 | 49 | 92 | 75 |
| | i.v. (5 × 10⁸) | 1 | 40 | <2 | <10 | <10 | <10 | 3.3 | 3.1 | 3.3 | 1,084 | 869 | 1,086 |
| | | 4 | 160 | <2 | <10 | <10 | <10 | 3.5 | 3.3 | 2.7 | 167 | 233 | 630 |
| | | 8 | 640 | <2 | <10 | <10 | <10 | <2 | <2 | <2 | 143 | 151 | 218 |
| | | 12 | 1,280 | <2 | <10 | <10 | <10 | <2 | <2 | <2 | 85 | 65 | 138 |
| Albany | i.d. (3 × 10⁷) | 1 | 40 | <2 | 10 | 10 | 10 | 12.2 | 12.3 | 9.4 | 473 | 439 | 1,034 |
| | | 4 | 80 | 3.6 | <10 | <10 | <10 | 9.0 | 4.5 | 6.8 | 175 | 149 | 867 |
| | | 8 | 160 | 5.2 | <10 | <10 | <10 | <2 | <2 | <2 | 75 | 64 | 112 |
| | | 12 | 160 | 2.5 | <10 | <10 | <10 | <2 | <2 | <2 | 68 | 47 | 65 |
| | i.v. (5 × 10⁸) | 1 | 160 | <2 | 20 | 40 | 40 | 7.3 | 5.8 | 5.8 | 969 | 534 | 954 |
| | | 4 | 320 | 4.9 | 20 | 20 | 20 | 5.8 | <2 | <2 | 356 | 278 | 525 |
| | | 8 | 640 | 9.0 | 20 | 20 | 20 | <2 | <2 | <2 | 112 | 98 | 135 |
| | | 12 | 640 | 3.5 | 10 | 10 | 10 | <2 | <2 | <2 | 45 | 35 | 45 |

[a] Except for the C4D female i.d. infected 45 days prior to pregnancy, the remaining females were infected 30 to 40 days prior to parturition.
[b] FTA-ABS, the endpoint titer, was defined as the highest dilution giving positive fluorescence.
[c] CIC data are expressed as the number of SD above the mean of 30 normal sera.
[d] Values of ≥2 SD (≥100 cpm) above 30 control sera are considered positive.

latter two were underdeveloped as compared with live littermates (55 and 65 g versus 98 to 115 g) but their organs were negative for T. pallidum by dark-field microscopy and silver staining. The two Reiter-injected Albany females gave birth to four healthy newborn infants.

None of the 24 C4D and 14 Albany offspring developed obvious clinical signs of syphilis during 6 months of observation. Histologic examination and Warthin-Starry silver staining (9) of thymus, lung, heart, pancreas, spleen, liver, and kidney from 10 stillborn animals and from three pups sacrificed on the 2nd, 20th, or 30th day of age did not reveal the presence of treponemes or obvious abnormalities when compared with those of age-matched healthy controls or of animals born to T. phagedenis biotype Reiter-injected females.

CIC. The C1q-SPA test, using affinity-purified specific antibodies to guinea pig IgG and IgM, was used to evaluate levels of CIC in sera of neonates and their mothers. In infected sows, CIC developed after several weeks of infection and contained only IgG antibodies. In 20 of 24 (83%) C4D and in 9 of 14 (64%) Albany pups, CIC were detectable as early as 24 h of life and consisted exclusively of IgM antibodies and antigens. Moreover, 18 of 24 (75%) C4D and 9 of 14 (64%) Albany neonates produced IgM RF detectable by a radioimmunoassay. In congenital syphilis, formation of CIC and, most of all, RF has been in part associated with fetal response to maternally transmitted antibodies. However, the results presented in Table 2 show that pups born to mothers infected late during pregnancy, while containing substantial levels of CIC and RF, have very low levels or were unreactive by the (IgG) FTA-ABS test. Injection of T. phagedenis Reiter into 30- to 40-day-pregnant females induced in the mothers a mild seroconversion (IgG FTA-ABS, 1:20 to 1:40) at the time of parturition, whereas their offspring had nonreactive FTA-ABS tests. Moreover, these four mothers and their eight neonates were all negative for CIC and RF.

Molecular analysis of CIC components and whole neonatal sera. To characterize the specificity of the neonatal IgM, DIC were subjected to Western immunoblot analysis. Blots of T. pallidum were probed with serum from secondary syphilis or human congenital syphilis or with polyethylene glycol-precipitated and alkaline DIC from eight experimental pools and a normal control pool of neonatal sera. The results presented in Fig. 1 show that a pattern of IgM reactivity could be identified only in DIC from neonates born to
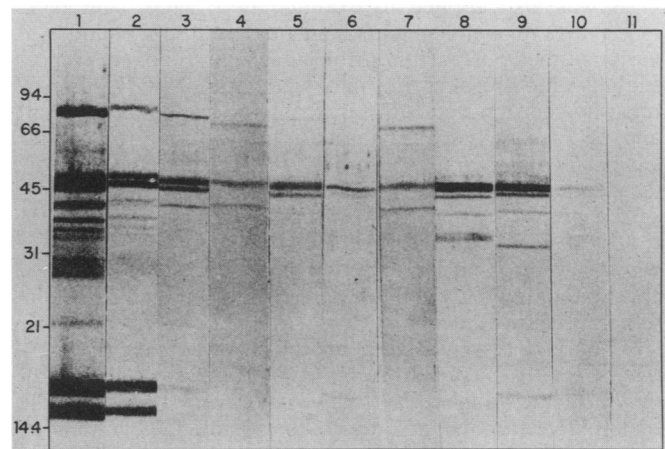


FIG. 1. Blots of T. pallidum were probed with a 1:100 dilution of serum obtained from secondary (lane 1) or congenital (lane 2) syphilis, with a 1:50 dilution of DIC prepared from sera of guinea pigs born to syphilitic mothers (lanes 3 to 10), or from control pups (lane 11). The reaction with human sera was developed with peroxidase-conjugated goat anti-human IgG (secondary syphilis) or goat anti-human IgM (congenital syphilis). The reaction with guinea pig DIC was developed with rabbit anti-guinea pig IgM and peroxidase-conjugated goat anti-rabbit IgG.

TABLE 3. Molecular analysis of IgM, IgG2, and IgG1 reactivities in sera of C4D guinea pig mother-offspring pairs taken 1 week postpartum[a]

| Route of maternal infection | Animal | Immuno-globulin isotype | T. pallidum polypeptides (kDa) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 15 | 17 | 21 | 35 | 37 | 39 | 42 | 45 | 47 | 54 | 62 | 75 | >100 |
| i.d. | Mother (no. 131) | IgM | + | + | | | | | + | + | + | + | + | | |
| | | IgG2 | + | + | + | + | + | | + | + | + | + | + | | |
| | | IgG1 | + | + | | | + | | + | + | + | | + | | |
| | Offspring (no. 131A) | IgM | | | | | | | + | | + | | | | + |
| | | IgG2 | | + | | | + | | + | + | + | | | | |
| | | IgG1 | | | | | | | | + | + | | | | |
| i.v. | Mother (no. 151) | IgM | | + | | | | | + | + | + | + | + | | |
| | | IgG2 | | + | | | | | + | + | + | + | + | + | |
| | | IgG1 | | + | | | | | + | + | | | | | |
| | Offspring (no. 151B) | IgM | | | | | | + | | | + | + | | | |
| | | IgG2 | | | | | | | + | | + | + | + | | |
| | | IgG1 | | | | | | | | | | | | | |

[a] Blots of T. pallidum were probed first with guinea pig sera and then with affinity-purified rabbit IgG antibodies to guinea pig IgM, IgG2, and IgG1.

syphilitic mothers and positive controls. The profile of the IgM response in the experimental animal was very similar to that of the human congenital syphilis, consisting of antibodies reacting with one or more T. pallidum peptides of molecular masses 15, 17, 37, 42, 45, 47, and 87 kDa. The IgM reactivity against the 47-kDa peptide was present in all DIC. On the other hand, when blots of DIC were reacted with serum obtained either from a patient with secondary syphilis or from a syphilitic rabbit, several lines of precipitation were seen, suggesting that the immune complexes contained treponemal antigens (data not shown). Precise determination of the molecular masses of antigens in the DIC, however, was not attempted since there seemed to be some changes in the peptides' electrophoretic charges possibly associated with the alkaline medium (pH 10.2) used for dissociation of CIC.

IB was also applied to examine IgM, IgG1, and IgG2 reactivities in over 20 neonatal and maternal sera obtained at various times postpartum. Molecular analysis of two representative mother-offspring pairs bled within 1 week postpartum showed that neonatal sera contained IgM (fetal origin) and IgG1 and IgG2 (maternally transmitted) reactivities against T. pallidum the patterns of which were not necessarily identical to those of their mothers (Table 3).

The possibility that RF (30) could account for the IgM reactivity was excluded by parallel examination by IB of whole serum or its IgM fraction obtained by ion-exchange chromatography (Isolab column). Both gave similar results (data not shown).

While IgM reactivity in neonatal sera was highly suggestive of fetal exposure to treponemal antigens, the physical nature of the antigen was totally unknown. It was feasible that soluble treponemal antigens or soluble CIC containing treponemal antigens could be maternally transmitted, eliciting an early fetal IgM response. Alternatively, the fetus was congenitally infected in utero.

In the first situation, we could expect that a fetal exposure to soluble treponemal antigens would trigger a limited immune response mostly of the IgM type (primary immune response) with no further implications. On the other hand, if the neonate had been congenitally infected, the continuous exposure to antigens provided by the growing virulent organism would have the potential for elicitation of a primary (IgM) and a secondary (IgG) immune response.

To assess both possibilities, pups born to syphilitic mothers were examined for a period of 5 months by IB and ELISA, the latter using 10% ethanol-treated treponemes (T. pallidum, T. phagedenis biotype Reiter, and T. vincentii). The chosen time was far beyond the point where CIC and RF could be produced by the host, but sufficient to allow full maturation of the immunocompetence in the young and further propagation of the pathogen. A 5-month period unfolding immune response to T. pallidum in neonates born to an i.v. infected C4D mother (the same as shown in Table 2) is presented in Fig. 2. These neonates had at birth an unreactive IgG FTA-ABS but were positive for CIC and RF for a period of 1 to 3 months, respectively. Blots of T. pallidum were reacted with a pool of the three neonates' sera collected at 1, 12, and 20 weeks of age. The reaction was developed with peroxidase-conjugated antibodies specific for guinea pig IgM, IgG2, and IgG1. At the first week of life, IgM reactivity (fetal origin) to three peptides (39, 47, and 54 kDa) and IgG2 reactivity (maternal origin) to four peptides
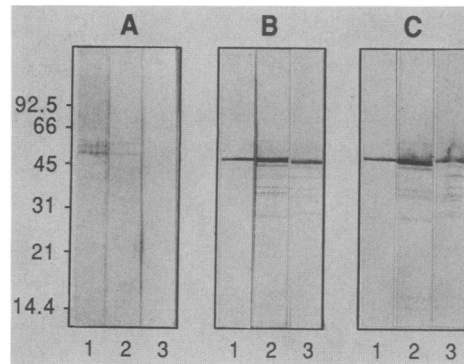


FIG. 2. Blots of T. pallidum were reacted with a pool of sera collected from three littermates, born to an i.v. infected C4D mother, at 1 (A), 12 (B), and 20 (C) weeks of age. The reaction was developed with peroxidase-conjugated antibodies specific for guinea pig IgM (lane 1), IgG2 (lane 2), and IgG1 (lane 3).
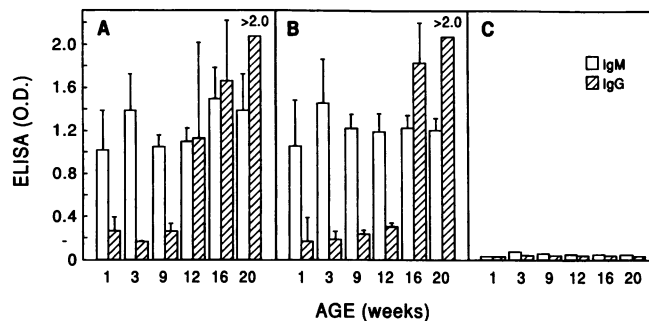
FIG. 3. ELISA-*T. pallidum*: IgM and IgG antibodies reacting with 10% alcohol-treated *T. pallidum*, produced during a period of 20 weeks in C4D offspring. The results express the mean OD ± SD of five pups born to i.v. infected sows (A), eight pups born to i.d. infected mothers (B), and four pups born to mothers i.v. injected with *T. phagedenis* biotype Reiter (C).

(42, 47, 54, and 62 kDa) were observed. No IgG1 anti-treponemal antibodies were detected. Between 12 and 20 weeks of age, the offspring sera still showed IgM antibodies to 47-kDa antigen and an increasing number of IgG2 and IgG1 antibodies (offspring origin) reacting with at least 10 *T. pallidum* antigens of molecular masses ranging from 15 to 62 kDa. Sera from neonates born to *T. phagedenis* biotype Reiter-injected mothers were all negative.

**ELISA.** To explore whether the IgM antitreponemal antibodies produced in the neonates could be detected by a serologic technique other than FTA-ABS, a solid-phase assay with alcohol-fixed *T. pallidum*, *T. phagedenis* biotype Reiter, or *T. vincentii* (2) was explored. A total of 57 serum specimens from 16 offspring born to syphilitic mothers and 24 serum specimens from offspring born to *T. phagedenis* Reiter-injected dams collected for a period of 5 months were examined for IgM and IgG reactivities against pathogenic (*T. pallidum*) and nonpathogenic (*T. phagedenis* biotype Reiter and *T. vincentii*) treponemal antigens. The reactivity against *T. pallidum* of representative pools of sera from five C4D offspring born to i.v. infected mothers, eight offspring born to i.d. infected mothers, and four offspring born to sows i.v. injected with Reiter is presented in Fig. 3. It may be seen that, while IgM antibodies were consistently detected in both experimental groups in moderate to relatively high levels (OD, 0.200 to 1.300), IgG reactivities started to be detected in levels even higher than those of IgM between 12 and 16 weeks of age, when an OD of ≥2.000 was recorded. More interestingly, the same sera examined by ELISA-*T. phagedenis* biotype Reiter or ELISA-*T. vincentii* were totally negative, with OD ranging between 0.040 and 0.067, significantly lower (Student's *t* test, $P < 0.001$) than those recorded with *T. pallidum*. Sera from control offspring were unreactive with all antigens (OD, <0.020) throughout the experimental period.

**Infectivity test.** Five Veneral Disease Research Laboratory-negative healthy rabbits were used as recipients (intratesticular injection) of organ extracts prepared from stillborn infants or neonates born to syphilitic dams. Two rabbits were injected with extract of spleen and liver obtained from two different pairs of stillborn animals (one pair of each strain of guinea pig), the third rabbit was injected with spleen and liver from an apparently healthy 1-day-old C4D neonate, and the fourth and fifth rabbits were injected with an extract of brain, lymph node, spleen, and liver from 3- or 16-day-old

apparently healthy C4D pups. Only the last two recipient rabbits seroconverted (reactive Venereal Disease Research Laboratory test and FTA-ABS) after 8 and 9 weeks, respectively, but none developed orchitis.

## DISCUSSION

In this report offspring born to *T. pallidum*-infected C4D or Albany strain mothers showed in their sera, as early as 24 h after birth, IgM antibodies specific against *T. pallidum* antigens, IgM CIC, and IgM RF. Also, the kinetics of the specific humoral response to *T. pallidum* (IgM isotype early in life followed by production of IgG antibodies after 3 months of age) and the positive infectivity test elicited by two of five organ extracts prepared from liveborn animals are all taken as evidence suggestive of infection in utero. To our knowledge this is the first report of an experimental model for congenital syphilis in which the humoral response mimics so closely that of infants congenitally infected with *T. pallidum* (1, 7, 8, 22, 31).

A bulk of evidence supports the assumption that IgM antitreponemal antibodies were genuinely of fetal origin and directed against exogenous treponemal antigens, not against maternal IgG or IgG-antigen complexes. (i) IgM antibodies are not maternally transmitted and colonization of neonates with nonpathogenic treponemes so early in life is very unlikely. (ii) As shown in Table 2, neonates born to mothers with unreactive FTA-ABS, and therefore lacking maternally transmitted immune antibodies, showed in their sera free or complexed IgM antitreponemal antibodies. (iii) Removal of IgG from whole serum by ion-exchange chromatography yielded an enriched IgM fraction that reacted as the whole sera did, with one (47 kDa) or more *T. pallidum* peptides recently identified (5, 29) as integral membrane components (15, 17, 37, 42, 45, and 87 kDa). Thus, as suggested by other investigators (7, 22, 31), interference of fetal RF in the detection of IgM reactivities by IB seems to be highly unlikely. (iv) The spectrum of IgM reactivities displayed by sera from stillborn or neonate guinea pigs did not necessarily reflect the pattern of maternal IgM reactivities. This has also been observed in clinical cases (7, 22, 31). (v) Offspring born to syphilitic mothers, but not those born to *T. phagedenis*-injected females, elicited after 3 months of age increasing IgG2 and IgG1 reactivities in IB against *T. pallidum*. This was further confirmed by ELISA with alcohol-treated *T. pallidum* but not with similarly treated *T. phagedenis* biotype Reiter or *T. vincentii*. (vi) Maternally transmitted IgG antibodies are not detectable in the offspring's circulation after 2 months of age (16), and IgG2 and IgG1 responses in guinea pigs do not appear to be induced before 1 and 2 to 3 weeks of age, respectively (35). We may assume, therefore, that both IgG reactivities detectable after 3 months of age by IB and ELISA against *T. pallidum* are genuinely produced by an infected host.

The roles of CIC and RF in the pathogenesis of experimental congenital syphilis have, so far, not been explored. In human congenital syphilis, CIC have been associated with glomerulonephritis (19, 23, 32, 40), and IgM antibodies directed against IgG (RF) have been detected in several congenital infections including congenital syphilis (7, 8, 30).

In the past, repeated failure to obtain a reactive IgM fluorescent treponemal antibody test with normal or immune guinea pig sera led us to state that, in this host, treponemal antibodies were exclusively of the IgG type (16). The present results obtained by more sensitive techniques, such as IB and ELISA with alcohol-treated treponemes, showed that

this is not the case. We do not have a readily available explanation of why IgM antitreponemal antibodies are undetectable by the fluorescent treponemal antibody test but do react with 10% alcohol-treated treponemes. It may relate to the level or to the specificity of the IgM antibodies. Alcohol treatment may induce some changes on the treponemal surface enhancing or exposing epitopes recognized by the sensitized guinea pig.

Although the number of Albany pups examined was smaller than those of the C4D strain, most of them showed either a very similar or an even higher level of CIC and RF than C4D newborns. The relatively prominent humoral immune response displayed by Albany neonates against *T. pallidum* is consistent with our previous studies on neonatal syphilis (39). Whereas C4D neonates showed a temporary resistance (2 to 4 months) to development of cutaneous lesions, 68% of Albany neonates responded to *T. pallidum* infection with large papular or typical ulcerative lesions never seen in adult animals of the same strain. Histologic examination of lymphoid tissues indicated that the maturation of B-cell areas in C4D animals lagged far behind that of the Albany strain; no differences were noticed in the T-cell areas. Phenotypic studies with monoclonal antibodies have recently confirmed that the number of B cells in neonates' spleen is significantly lower in C4D as compared with Albany strain animals (unpublished data). While the role of B cells or their products in the pathogenesis of cutaneous lesions remains speculative, all of the above clearly reflect a changing pattern in the immunological recognition of *T. pallidum*. This is not restricted to the guinea pig, however; newborn rabbits also display a temporary resistance (4 to 6 weeks) to lesion development after cutaneous challenge with *T. pallidum* (12). Obviously, the availability of two strains of guinea pigs with quite different genetic backgrounds offers the advantage of a different expression of an evolving immune response.

The overwhelming evidence of congenital infection provided by the immunological results was matched neither by the rate of organ infectivity done in rabbits nor by the clinical symptoms or histologic studies. Owing to the lack of clinical symptoms, we chose for infectivity tests organs freshly prepared from 1-, 3-, or 16-day-old pups or extracts prepared with a pool of organs from animals found dead (stillborn) after parturition. The latter were chosen in the hope that they would be the most affected by the infection. Only two of three rabbits injected with freshly prepared organ extracts, but none of those injected with extract from stillborn animals, seroconverted. It is likely that in the dead hosts the pathogen had very little chance of survival. Moreover, centrifugation of minced tissues to remove most cellular debris may have also removed tissue-attached treponemes from the supernatant.

Experiments under way in our laboratory, using the polymerase chain reaction, indicate that a large percentage of treponemes seeded in EDTA-whole blood are lost after centrifugation (300 × g) of the blood cells (unpublished data). Estimating the sensitivity of the rabbit infectivity test for examination of cerebrospinal fluid, Lukehart et al. (24) reached the same conclusion: although a rabbit infectivity test provides definite proof of the presence of viable *T. pallidum*, a negative infectivity test does not exclude the presence of treponemes in an organ.

Failure to produce symptomatic infection in neonates does not preclude congenital infection. Rather, it indicates that a low number of treponemes have reached the fetus(es), sufficient to elicit an immunologic response but insufficient to induce obvious symptomatology. In humans, over 50% of infants born to syphilitic mothers are asymptomatic and only a very low percentage of them show any sign of infection within 2 years of age or later. Moreover, in congenital syphilis the pathology of organs is ambiguous; it depends, among other factors, on the gestational age at the time of infection and the age of the child when it is examined (15). In the present study, only one dose of *T. pallidum* was used, which was 50 to 100 times lower than that needed to induce high mortality and histologic changes in rabbits born to syphilitic mothers (11). We may anticipate that a higher dosage of treponemes will have a more detrimental effect on the course of pregnancy, particularly since the guinea pig has a gestation period of 65 to 72 days, longer than that of rabbits (26 to 29 days).

In conclusion, the availability of an experimental congenital syphilis model in which asymptomatic infection occurs may serve the purpose of delineation of factors responsible for asymptomatic infection frequently observed among human infants.

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