INFECTION AND IMMUNITY, Aug. 1996, p. 3174–3179 Vol. 64, No. 8
0019-9567/96/$04.00+0

Target Organs of Infection in Guinea Pigs with Acquired or Congenital Syphilis

KONRAD WICHER,[1]* FRANK ABBRUSCATO,[1] VICTORIA WICHER,[1]
ROBERT BAUGHN,[2] AND GERDA T. NOORDHOEK[3]

Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201-0509[1]; Department of Microbiology and Immunology, Baylor College of Medicine & Syphilis Research Laboratory, Veterans Administration Medical Center, Houston, Texas 72211[2]; and Laboratory of Public Health in Friesland, PB 21020-8900 J. A. Leewurden, The Netherlands[3]

Received 12 February 1996/Returned for modification 25 March 1996/Accepted 29 May 1996

The target organs of infection in guinea pigs with asymptomatic acquired or congenital syphilis were identified by PCR and in some cases by rabbit infectivity test (RIT). The prevalence of *Treponema pallidum* DNA was examined in the following seven organs: the inguinal and mesenteric lymph nodes, spleen, liver, kidney, heart, and brain. Test samples consisted of 95 organs from two genetically different strains of female guinea pigs (C4-deficient and Albany) with different susceptibilities to cutaneous infection by *T. pallidum* and 195 organs from their asymptomatic offspring. Twenty organs from dams of both strains injected with heat-killed *T. pallidum* and 19 organs from their progeny served as negative controls. The infections of mothers and neonates were documented by PCR, RIT, and serology. Though any of the organs tested could be infected, there was a spirochetal predilection for some anatomical locations, such as the lymph nodes, heart, and brain, regardless of the strain, route of maternal infection, and age. None of the 49 organs collected from control animals were positive by PCR. In infected C4-deficient dams, one to four organs were positive by PCR, whereas the organs of 7 of their 27 (25%) asymptomatic offspring were treponemal DNA negative, despite evidence of immunoglobulin M treponemal antibodies. Comparative analysis done by both PCR and RIT on a limited number of samples showed 90% agreement between results. An examination of multiple samples obtained from single organs demonstrated that even within 24 h of spirochetemia, when most organs appeared to be infected, not all samples from an individual organ were positive by PCR. A specific immunological response in guinea pigs with congenital syphilis was a more consistent parameter of vertical transmission than was an analysis of *T. pallidum* DNA.

Clinical and pathological observations have indicated that in cases of both acquired syphilis (AS) and congenital syphilis (CS), any organ can be infected (14, 25, 28). In many situations, however, an association between the presence of treponemes and histopathological changes or functional abnormalities has been difficult to demonstrate unequivocally (25). The techniques available for the detection of *Treponema pallidum* in tissues are insensitive (immunofluorescence), nonspecific (silver staining), or impractical (rabbit infectivity test [RIT]). This is compounded by the fact that the etiologies of anatomic lesions and organ dysfunctions may include direct damage by treponemes and be associated with the host response (immune complexes and delayed type of hypersensitivity) or antibiotic therapy (i.e., Herxheimer reaction). Similar reasons may account for the lack of a more comprehensive study on the prevalence of *T. pallidum* in the organs of asymptomatic infected individuals. This is critical, as the presence of *T. pallidum* in certain anatomical sites may constitute potential reservoirs of infection and pathogenicity. The identification of those organs would certainly shed light on the natural history of the disease. It would help to explain the reason(s) why some organs in the natural host more so than others have the tendency to show histological or functional abnormalities after a prolonged period of latency.

In the present study, we attempted to fill this gap in information by using PCR, one of the most sensitive and specific tests available for the detection of *T. pallidum* in tissues and body fluids (6, 29, 35). We took advantage of the availability of two strains of guinea pigs which as adults show opposite susceptibilities to cutaneous infection by *T. pallidum* (38) and the lack of obvious clinical signs of infection in serologically reactive, congenitally infected offspring of both strains (34).

The recent introduction of PCR has found wide application in the diagnosis of infectious diseases. In the particular case of syphilis, the detection of *T. pallidum* DNA has been applied by several investigators for the diagnosis of neurosyphilis (6, 15, 17, 24, 29), primary syphilis (20, 35), and CS (6, 16, 29).

Using the guinea pig model of AS (mothers) and CS (offspring), we explored the applicability of PCR for the detection of *T. pallidum* DNA in various organs. On the basis of the clinical history and frequent histopathological findings reported for human syphilis (14, 25, 28), our first priority was the examination of the inguinal lymph nodes (ILN), heart (HRT), and brain (BRN), followed by the mesenteric lymph node (MLN), spleen (SP), liver (LIV), and kidney (KID). The infections of adult animals and transplacentally infected pups were further confirmed by immunological responses. RIT was used to confirm infection.

(This work was presented in part at the American Association of Immunologists Meeting, Atlanta, Ga., 9 to 13 April 1995.)

* Corresponding author. Mailing address: Wadsworth Center for Laboratories and Research, New York State Department of Health, David Axelrod Institute, P.O. Box 509, Albany, NY 12201-0509. Phone: (518) 473-8660. Fax: (518) 473-1326.

TABLE 1. Guinea pigs used in this study

Strain	Maternal infection or injection			No. of pregnant sows[a]	No. of offspring	Organ collection postinfection or after birth (mo)	
	Organism	Dose	Route			Sows	Offspring
C4D	VTP	5×10^8	i.d.	5	12	2–4	1 wk
	VTP	1×10^8	i.v.	5	15	3–5	3–4
	HKTP	1×10^8	i.v.	2	2	2–3	2–3
Alb	VTP	1×10^8	i.v.	5	6	2–4	2–4
	HKTP	1×10^8	i.v.	2	2	2–3	1–2

[a] Pregnant dams were infected at between 4 and 8 weeks gestation.

MATERIALS AND METHODS

Animals and infection. The C4-deficient (C4D) guinea pig strain, genetically related to inbred strain 13, is very susceptible (50% infective dose = 10^2 organisms), whereas the Albany (Alb) line, which is haplotype identical to inbred strain 2, is highly resistant (50% infective dose, $>10^9$ organisms) to cutaneous infection by *T. pallidum* (37, 38). The genetic backgrounds and clinical and immunological responses of these two partially inbred strains compared with those of inbred strains 2 and 13 have been previously reported (34, 35, 37). It should be stressed that C4D animals have a health status, life span, humoral and cellular immunocompetence, and susceptibilities to infectious agents similar to those of the original, complement-sufficient strain 13 (5). The female guinea pigs included in this study were 4 to 5 months old when infected.

Virulent *T. pallidum* subsp. *pallidum* Nichols (VTP), freshly prepared from orchitic rabbit testes (36, 37), was used to infect 10 C4D (five intradermally [i.d.] and five intravenously [i.v.]) and 5 Alb (i.v.) dams between 4 and 8 weeks of pregnancy (Table 1). The gestation period in the guinea pig is approximately 10 weeks. Two C4D and two Alb pregnant dams were injected i.v. with similar numbers of heat-killed *T. pallidum* (HKTP; 56°C, 2 h) organisms. The congenitally infected group consisted of 27 C4D pups from i.d.- and i.v.-infected mothers and six Alb offspring born to i.v.-infected sows. Five organs (the ILN, MLN, SP, HRT, and BRN) from four HKTP-injected dams (two C4D and two Alb) and four of their offspring were included as controls. Experimental and control animals were bled in the first week and then biweekly for 1 to 4 months. Only liveborn pups were included in this study.

The animal procedures were approved by the Institutional Animal Care and Use Committee of the Wadsworth Center for Laboratories and Research.

Organ collection and examination of *T. pallidum* DNA. A total of 115 organs, 95 from *T. pallidum*-infected and 20 from HKTP-injected sows, and a total of 214 organs from offspring, 195 born to *T. pallidum*-infected dams and 19 born to HKTP-injected dams of both strains, were collected for PCR testing. It should be stressed that only a representative number of liveborn siblings per family were tested by PCR. Thus, in Alb and C4D families with a maximum litter size of around four and seven, respectively, organs from one or two Alb and two to four C4D offspring were examined. These numbers of organs do not include those employed for multiple PCR testings or for comparative studies done by both PCR and RIT.

Most adult experimental and control animals were sacrificed, and their organs were tested by PCR after 2 to 5 months of infection, representing the latent stage of syphilis in the animal model (Table 1). To assess the presence of *T. pallidum* DNA in the organs of serologically positive (immunoglobulin M [IgM]) neonates (34, 39), pups born to i.d.-infected C4D dams were sacrificed during the first week of life. Organs from pups born to HKTP-injected Alb mothers were collected at 1 to 2 months of age, as it was very unlikely that dead organisms could cross the maternal placenta, much less persist in the progeny for a longer period.

Extreme care was taken to avoid cross-contamination during organ collection. A different set of sterile instruments was used to collect individual samples. Small organs, such as the ILN and MLN were taken as a whole, whereas approximately 1 g of tissue from various sections of large organs was taken for *T. pallidum* DNA extraction as previously reported (35). Briefly, tissues were placed into 2× TE buffer (10 mM Tris, 1 mM EDTA, pH 8), homogenized, immediately frozen on dry ice, and kept at −70°C prior to testing. For each milliliter of tissue suspension in 2× TE buffer, 20 μl of 10% sodium dodecyl sulfate and 10 μl of 10 mg/ml proteinase K were added. DNA extraction was carried out by the method of Boom et al. (4) using 50 μl of lysate adsorbed to diatoms in the presence of guanidine thiocyanate. In each experiment, *T. pallidum* at concentrations of 10^2 and 10^3 organisms per ml in TE buffer containing tRNA as a carrier was included as an extraction control. In addition, *T. pallidum* chromosomal DNA at concentrations of 2.5, 25, and 250 fg was used as a positive sensitivity control. Admixtures of PCR reagents without DNA were used as negative controls. In vitro amplification of DNA was performed by using the *bmp* (basic membrane protein) gene of *T. pallidum* (10) and primers Tp7 and Tp8 for the first PCR and primers Tp3 and Tp4 for the second PCR (nested PCR). The primers were produced at the Genetics Core of the Wadsworth Center for Laboratories and Research. The PCR products were analyzed in 2% agarose containing ethidium bromide. Though amplification with the second set of primers in the nested PCR does not occur if the product of the first amplification is not specific for *T. pallidum*, the specificities of the PCR products were further confirmed by Southern blot analysis with a biotinylated probe. The probe, 356-bp long, was prepared by PCR with internal primers Tp16 (CTG-AGT-TCG-CCA-ATT-ACG-TC) and Tp19 (TAC-TCT-TAA-TGT-CAG-CCG-TG), using biotin-16-dUTP (Boehringer Mannheim, Indianapolis, Ind.). The probe-target hybrid was detected by a BluGENE non-radioactive system of nucleic acid detection as described by the manufacturer (Bethesda Research Laboratories Life Technologies) (1a). The hybridization procedure was used occasionally, mainly to verify the specificity, not the sensitivity, of PCR.

PCR examination of multiple samples per organ. The distribution of *T. pallidum* in relatively large organs and the way in which it may be affected by the time of infection were explored in two female guinea pigs infected i.v. with freshly prepared VTP (10^8 organisms). One dam was sacrificed after 24 h, and the other was sacrificed after 5 months of infection. Multiple blood, SP, LIV, lung (LNG), HRT, and BRN samples were collected and analyzed individually by PCR.

RIT. To see whether the *T. pallidum* DNA detected by PCR was derived from live organisms, we examined by both PCR and RIT two organs (ILN and BRN) obtained from three dams infected for 6, 12, and 24 months and from two serologically positive offspring (3 to 5 months old). For RIT, tissues were teased under sterile conditions through 100-wire mesh with 2 ml of plain RPMI 1640 medium and the resulting suspension was injected intratesticularly (1 ml per testis) into Venereal Disease Research Laboratory (VDRL)-negative rabbits. The animals were regularly examined for a period of up to 3 months for evidence of infection (orchitis) and bled every 2 weeks for serological testing by the VDRL (7). Animals were considered to be positive when they developed orchitis and/or seroconverted and their testes were positive by dark-field microscopy or PCR.

Immunological examination. (i) Treponemal antibodies. Treponemal antibodies of the IgM and IgG isotypes were examined by enzyme-linked immunosorbent assay (ELISA), as previously reported (34). We used microplates coated with Percoll-purified 10% alcohol-fixed *T. pallidum* organisms as described by Zeltzer et al. (40). Briefly, 100 μl of fresh experimental and positive and negative control sera diluted 1:100 was applied to duplicate wells of *T. pallidum*-coated plates. The isotype of the bound antibodies was determined by using 100 μl of affinity-purified rabbit anti-guinea pig IgM and IgG at a 1:1,000 dilution. The reaction was developed by using 100 μl of alkaline phosphatase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa.) diluted 1:25,000. Sigma substrate 104 was used for the detection of alkaline phosphatase, and the reaction was stopped after 45 min by the addition of 3 N NaOH, with a subsequent optical density (OD) reading at 405 nm. OD values of >0.100 were considered to be positive for both antibodies. These values were >2 standard deviations, determined in sera from normal young (n = 20) and adult (n = 30) guinea pigs. Positive and negative controls consisted of individual pools of sera previously tested by ELISA and immunoblotting (34).

(ii) CIC. The levels of IgG circulating immune complexes (CIC) were determined by a modified micro-ELISA (31). Briefly, microplates coated with C1q were exposed to duplicate samples of 100 μl of individual guinea pig sera at a final dilution of 1:50 in phosphate-buffered saline–Tween. Individual pools of previously tested positive and negative sera were included in each test. Affinity-purified rabbit anti-guinea pig IgG was used as the first antibody, and alkaline phosphatase-conjugated goat anti-rabbit IgG was used as the second antibody. OD values of >0.100 (>2 standard deviations) above that of normal control sera (n = 20) were considered to be positive.

(iii) RF. The presence of 19S IgM rheumatoid factor (RF) was detected by ELISA (11) using affinity-purified guinea pig IgG-coated plates. Coated microplates were exposed to duplicate samples of 100 μl of guinea pig sera and negative and positive controls at a 1:24 dilution. Affinity-purified rabbit anti-guinea pig IgM was used as the first antibody, and alkaline phosphatase-conjugated goat anti-rabbit IgG was used as the second antibody. OD values of >0.1 (>2 standard deviations) above that of normal control sera (n = 20) were considered to be positive. The day-to-day variation in the three tests described above was from 5 to 10%. The relevance of testing for IgM antibodies, IgM RF, and CIC in congenitally infected pups has been previously discussed (34).

Statistical evaluation. The prevalence of *T. pallidum* DNA in organs and its association with the strain and route of maternal infection were determined from 2 × 2 contingency tables by using Fisher's exact test and two-tailed P values.

RESULTS

Course of infection. The numbers of experimental and control animals, sizes of inocula, routes of infection or injection, and dates of sample collection for PCR are presented in Table 1. C4D females infected i.d. developed dark-field-positive ulcerative lesions between 9 to 12 days postinfection which lasted for a period of 3 to 8 weeks. The i.v. infection of C4D or Alb dams did not produce any clinical signs of disease. None of the

TABLE 2. Target organs of infection in animals with experimental AS (mothers) and CS (offspring), as detected by PCR

Strain (*n*)	Maternal infection route[a]	No. positive/no. tested (%)[b]							
		ILN	MLN	SP	LIV	KID	HRT	BRN	Total
Mothers									
C4D (5)	i.d.	2/5	0/4	0/4	2/4	0/5	2/5	2/5	8/32 (25)
C4D (5)	i.v.	2/5	1/3	1/5	0/4	0/4	2/5	2/4	8/30 (27)
Alb (5)	i.v.	3/5	2/5	2/5	1/4	1/4	2/5	3/5	14/33 (42)
Total		7/15 (46)	3/12 (25)	3/14 (21)	3/12 (25)	1/13 (7)	6/15 (40)	7/14 (50)	30/95 (31)
Offspring									
C4D (12)	i.d.	3/12	1/10	0/8	0/9	1/8	3/12	3/12	11/71 (15)
C4D (15)	i.v.	5/15	2/12	2/11	1/10	1/8	5/15	6/15	22/86 (25)
Alb (6)	i.v.	3/6	1/6	0/5	1/5	0/4	4/6	4/6	13/38 (34)
Total		11/33 (33)	4/28 (14)	2/24 (8)	2/24 (8)	2/20 (10)	12/33 (36)	13/33 (39)	46/195 (23)

[a] A dose of 5×10^8 VTP organisms was used for i.d. infection, and a dose of 10^8 organisms was used for i.v. infection. Pregnant sows were infected at between 4 and 8 weeks gestation.

[b] Twenty organs (the ILN, MLN, SP, HRT, and BRN) from four control sows (two C4D and two Alb) injected with HKTP organisms and 19 similar organs from four of their offspring were all negative by PCR.

liveborn C4D and Alb pups developed obvious clinical signs of infection.

Detection of *T. pallidum* DNA by nested PCR. The numbers of experimental dams and offspring and the numbers of organs examined in each group are presented in Table 2. None of the 39 control organs, 20 from HKTP-injected mothers and 19 from their offspring, showed any trace of *T. pallidum* DNA (data not shown). Higher numbers of infected organs, although statistically insignificant ($P > 0.05$), were observed among i.v.-infected Alb dams (14 of 33 [42%]) and their offspring (13 of 38 [34%]) than among i.v.-infected C4D dams (8 of 30 [27%]) and their pups (22 of 86 [25%]). There were no differences in the prevalence of total infected organs between i.d.- and i.v.-infected C4D mothers (25 and 27%, respectively), though the former were infected with a fivefold-larger dose. Neither was there a significant difference ($P > 0.1$) between i.d.-infected C4D mothers' offspring (15%) sacrificed within the first week of life and i.v.-infected C4D mothers' pups (25%) sacrificed between 3 and 4 months of age.

In infected mothers and their progeny, *T. pallidum* DNA could be found in any of the seven organs examined, but the incidence tended to be higher in the BRN, (50 and 39%, respectively), ILN (46 and 33%, respectively), and HRT (40 and 36%, respectively) compared with the MLN (25 and 14%, respectively), SP (21 and 8%, respectively), LIV (25 and 8%, respectively), and KID (7 and 10%, respectively). In animals with AS, the differences between various organs were not statistically significant, with the exception of the ILN and BRN compared with the KID ($P < 0.03$). In animals with CS, however, the incidence of *T. pallidum* DNA in either the BRN, ILN, or HRT was marginally significant (BRN versus MLN [$P = 0.09$]) or statistically significant ($P < 0.05$) when compared with that in the SP, LIV, or KID.

Occasionally, the specificity of PCR was verified by hybridization; representative results for two serologically positive C4D families are shown in Fig. 1. Amplification products from the ILN, HRT, BRN, and LIV by nested PCR (Fig. 1A) and by hybridization (Fig. 1B) are shown. It should be noted that the LIV of the first mother (Fig. 1, lanes 5) was apparently negative by nested PCR and weakly positive after hybridization. Similarly, in the second family, the offspring's HRT (Fig. 1, lanes 16), negative by nested PCR, showed a very faint line after hybridization. All reagent controls were negative, whereas *T. pallidum* DNA at concentrations of 250, 25, and 2.5 fg was positive by PCR and hybridization. Lanes 24 and 25 in Fig. 1 show the results for diatom-extracted DNA from *T. pallidum* suspensions of 10^3 and 10^2, respectively.

PCR examination of multiple samples from large organs and the relationship to the time of i.v. infection showed that for the female sacrificed 24 h after infection, three of three heparinized blood, three of three SP, three of three LIV, one of three LNG, three of three HRT, two of three BRN, and zero of three KID samples and one of two eyes were positive by PCR, whereas in the second female infected for 5 months, zero of three blood, one of three SP, one of three LIV, zero of three LNG, two of three HRT, and two of three BRN samples and zero of two eyes were PCR positive (data not shown).

RIT and PCR. Examination of the ILN and BRN samples from three infected dams and two unrelated congenitally infected guinea pigs by RIT and PCR showed a 90% agreement between PCR and RIT results (Table 3). Except for one organ (the BRN of M2) which was positive by RIT but negative by PCR, seven organs were positive and two were negative by both tests. Each animal had at least one positive organ. Rabbits 1, 4, and 9 did not develop orchitis but seroconverted (VDRL 1:8 to 1:16) between 5 and 8 weeks postinjection, and their testes were positive by PCR. Rabbits 2, 3, 5, 7, and 10 developed orchitis between 3 and 5 weeks postinfection and were VDRL positive (1:8 to 1:32). Rabbits 6 and 8 neither developed orchitis nor seroconverted, and PCR examinations of their testes were negative.

Immunological response. In contrast to control animals injected with HKTP, sera from all i.d.- and i.v.-infected dams were positive for IgM (mean OD = 0.51; range, 0.37 to 0.75) and IgG (mean OD = 0.79; range, 0.58 to 0.93) treponemal antibodies. Some sows were also positive for IgG CIC (OD, >0.1), but all were negative for IgM RF (mean OD = 0.06; range, 0.03 to 0.09) at the time of delivery. In contrast, sera from all pups, regardless of the strain or route of maternal infection, showed from the first week of life high levels of IgM (mean OD = 0.44; range, 0.18 to 0.68) but no IgG (mean OD = 0.07; range, 0.02 to 0.09) treponemal antibodies. In the offspring, IgG antibodies developed after 10 to 12 weeks of life. Close to 90% of them also had a high level of IgM RF (mean OD = 0.32; range, 0.15 to 0.57). Serum samples from normal controls and from HKTP-injected mothers and their progeny were all negative by ELISA (OD, <0.1). Interestingly, while all serologically positive mothers of both strains had one to four organs positive by PCR, all the organs of 7 of 27 (25%) IgM

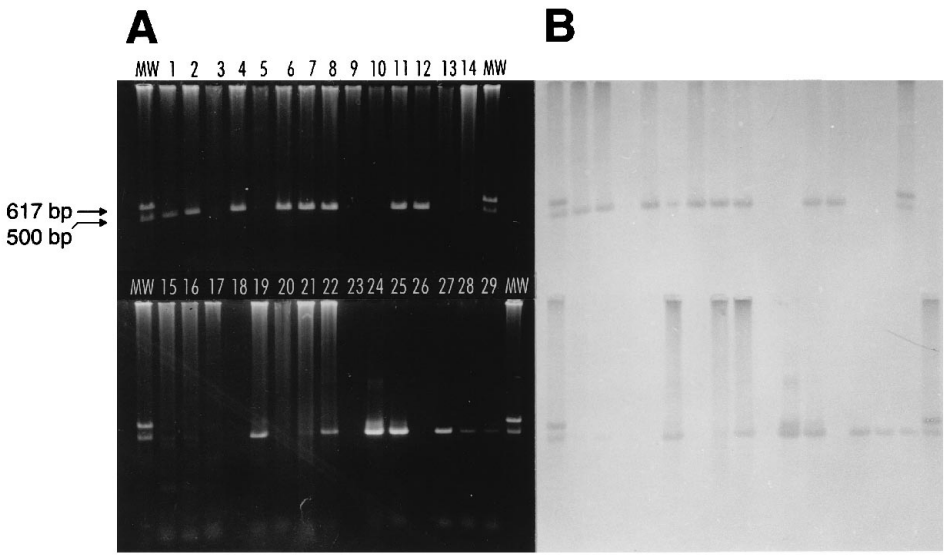

FIG. 1. Detection of *T. pallidum* DNA in the organs of two C4D infected families (families 1 [lanes 1 to 14] and 2 [lanes 15 to 22]) by nested PCR (A) and hybridization (B). Lanes MW, molecular weight controls. The amplification products from the ILN, HRT, BRN, and LIV of mother 1 and two of her pups are shown in lanes 1, 2, 4, 5, 6 through 9, and 11 through 14, respectively. Lanes 15 through 18, results from the ILN, HRT, BRN, and LIV of a serologically positive neonate from family 2; lanes 19 through 22, results from mother 2's corresponding organs; lanes 3, 10, 18, 23, and 26, reagent controls; lanes 24 and 25, diatom-extracted treponemal DNA from 10^2 and 10^3 suspensions; and lanes 27 through 29, *T. pallidum* DNA at concentrations of 250, 25, and 2.5 fg, respectively.

serologically positive C4D pups were *T. pallidum* DNA negative (data not shown).

DISCUSSION

The main objective of this study was the identification of target organs of infection in asymptomatic guinea pigs with AS and CS by the detection of *T. pallidum* DNA. Among animals with CS, only liveborns were included in this study. The variables inherent to the stillborn population, the often unknown time of in utero death or delivery and the precarious conditions of their bodies (rigid, soiled, or even cannibalized) at the time of discovery, prevented us from including it in this study.

The results showed that any of the seven organs tested per animal could be infected, but there tended to be a higher incidence of *T. pallidum* DNA in certain anatomical locations, such as the ILN, BRN, and HRT, regardless of the strain, age, or route of infection. None of the 39 organs from dams injected with HKTP or their offspring collected 1 to 3 months postinjection were positive by PCR. However, this does not exclude the possibility that treponemal DNA could be found in maternal organs within a few days of i.v. injection of HKTP. Whether HKTP is able to cross the placenta is unknown. The ability of VTP to cross the placenta was evident, however, as all progeny born to infected mothers were serologically positive (IgM) and in most of them at least one of their organs harbored treponemal DNA. These findings may have clinical and pathological relevance. Among syphilitic humans, in addition to the generalized lymphadenopathy often seen in early stages of the disease (9, 13), gummatous lesions of soft parenchyma tissues, neurosyphilis, and cardiovascular syphilis are the most common manifestations in late AS (14). In CS, with the exception of cardiovascular manifestation, which is rare (2), neurological involvement and bone and joint lesions are the most common complications of early and late manifestations of the disease (9, 13).

TABLE 3. Examination of guinea pig organs for *T. pallidum* by PCR and RIT

Guinea pig[a]	Organ	Rabbit no.[b]	RIT[c]	PCR[c]
M1	ILN	1	+	+
	BRN	2	+	+
M2	ILN	3	+	+
	BRN	4	+	−
M3	ILN	5	+	+
	BRN	6	−	−
O1	ILN	7	+	+
	BRN	8	−	−
O2	ILN	9	+	+
	BRN	10	+	+

[a] M1 through M3, mothers infected for 6, 12, and 24 months, respectively; O1 and O2, offspring infected for 3 and 5 months, respectively.

[b] Rabbits 1, 4, and 9 seroconverted (VDRL 1:8 to 1:16), and their testes were positive by PCR. Rabbits 2, 3, 5, 7, and 10 developed orchitis (dark-field microscopy positive) and seroconverted (VDRL 1:8 to 1:32). Rabbits 6 and 8 neither developed orchitis nor seroconverted.

[c] +, positive reaction; −, negative reaction.

The data also indicate that despite the relatively large litter size of the guinea pig, maternal infection by a sufficient number of VTP organisms around mid-pregnancy always resulted in congenital infection. The persistence of VTP (RIT positive) in the LN and BRN of sows infected for 6 to 24 months is also a reminder of the potential risk of fetal infection during maternal latency. The typical case in point is that reported by Fiumara (12) of a mother who was able to transmit CS to five of her eight children over a period of 10 years.

Our earlier (34, 39) and present results for cases of experimental CS suggest that a specific humoral response (IgM treponemal antibodies, followed by IgG antibodies) is a more consistent parameter of vertical transmission than is PCR analysis. In early studies (34), we presented evidence suggesting that IgM treponemal antibodies were genuinely of fetal origin and directed against exogenous treponemal antigen, not against the maternal IgG of IgG-antigen complexes.

Only one to four of the seven maternal organs tested per animal were positive by PCR, and all the organs of 25% of

serologically positive (IgM treponemal antibodies) transplacentally infected pups tested negative by PCR. Apparently, not all pups harbor the minimum number of organisms detectable by PCR. Furthermore, the distribution of the pathogen in a particular tissue may not be uniform; this may be subject to changes during the course of infection. This was inferred from an analysis of multiple samples from single large organs (the SP, LIV, LNG, HRT, BRN, and KID) and from blood from two dams infected i.v. for 24 h and 5 months. The number of PCR-positive samples per organ for the first animal was much higher than that for the second, yet only one of three LNG, two of three BRN, and zero of three KID samples and one of two eyes examined were positive within 24 h of treponemia. In fact, PCR and RIT, the two most sensitive techniques presently available for the detection of *T. pallidum*, are also restricted by the absolute minimum number of organisms present in the testing sample, more than 1 (6, 35) and more than 10 (22), respectively. In contrast, infection by *T. pallidum* generally triggers a humoral immune response, the level of which depends more on the size of the inoculum than on the particular localization of the pathogen. For humans, for which a single baby is the common rule, it has been concluded that the diagnosis of asymptomatically infected infants requires a comprehensive approach using both assays for the detection of specific IgM antibodies and PCR testing (29). Recent reports have indicated that 33 and 30% of the cerebrospinal fluid samples examined from untreated patients with acquired symptomatic neurosyphilis were positive by PCR (15) and RIT (21), respectively. Studies by Baughn (1) showed that only 15 of 54 (27%) cerebrospinal fluid samples from patients with symptomatic neurosyphilis were treponemal DNA positive. The sensitivity of our PCR was consistently at the level of 2.5 fg per test sample, and the specificity of the nested PCR was periodically confirmed by DNA-DNA hybridization. We do not know why SP and LIV samples have such a low incidence of treponemal DNA. The presence of inhibitory activity is highly unlikely. Firstly, diatomaceous earth in the presence of high concentrations of a chaotropic agent was used for the extraction and purification of DNA (35). Secondly, positive reactions were obtained after spiking the SP and LIV with various concentrations of *T. pallidum*. Thirdly, some LIV and SP samples from animals with AS and CS, as well as those obtained from dams infected i.v. for 24 h, were treponemal DNA positive.

The failure to detect *T. pallidum* DNA in the control reagents included in each test, as well as in the relatively large number of organs tested from control animals injected with or born to dams injected with HKTP, ruled out contamination and consequently false-positive reactions. Although blood is the main vehicle for early systemic dissemination of treponemes, this function seems to be curtailed after the establishment of latency. Thus, in guinea pigs sacrificed and examined after 2 months of infection, cross-contamination with blood was considered to be unlikely; in general, only a limited number of guinea pig organs tested (7 to 50%), including those well supplied with blood (the HRT, LIV, and SP), harbored treponemal DNA.

Our data indicate that the lodging of the pathogen in selective organs takes place very early in infection; it may persist for years and possibly for life without causing obvious symptomatology in the guinea pig. In humans, however, there is always the potential for reactivation. This could explain why maternal transmission of syphilis still may occur in late latency and why 15 to 30% of untreated patients develop tertiary symptoms after a variable period of latency (12). There is also evidence that VTP may persist in patients who have received what is regarded as adequate therapy for syphilis (15, 21). We have recently demonstrated by PCR and hybridization the presence of *T. pallidum* in a BRN gumma from a deceased 29-year-old human immunodeficiency virus-infected male with a history of syphilis infection and an apparent case of penicillin treatment failure (18). Asymptomatic neurosyphilis has been reported in cases of AS (21, 23) and CS (29).

We do not know the factor(s) involved in the selective targeting of organs by *T. pallidum* or its survival in those tissues. However, from clinical data and results from our own study, there is evidence that *T. pallidum* may survive at sites known to be immunologically privileged (the BRN and eyes) as well as at those without immune privilege (the LN, HRT, and SP). Several bacterial strategies have been proposed. An old theory implicates the coating of the pathogen with a protective mucoid material (32), while more recently it has been suggested that the immunologically inert treponemal outer surface is unable to trigger the host inflammatory response (26, 27, 33). Inasmuch as these strategies may help to explain persistence in asymptomatic infection, we do not have any clue as to the mechanisms determining chronicity or those involved in reactivation. In the latter, the invoked inflammatory reaction and tissue damage seem to be out of proportion to the number of treponemes found. The application of newly emerging approaches and immunological evaluation of cytokines have been critical in understanding the various manifestations of chronic infectious diseases, such as leprosy (30), leishmaniasis (3), and tuberculosis (8). They may shed light on the nature of the host-parasite relationship in cases of syphilis and its various modalities. Studies addressing this gap in information are under way in our laboratory.

The validity of this experimental model may be argued since, unlike the natural host, it is less susceptible (C4D) or even resistant (Alb) to cutaneous infection. These strains do not develop the characteristic lesions of secondary or tertiary syphilis, and their infected liveborn babies are asymptomatic. This concern, however, is not totally justified when one analyzes the clinical and laboratory findings for human syphilis, especially in the postpenicillin era. Except for the more or less consistent incidence of primary and secondary lesions, up to 70% of the untreated population with AS may remain asymptomatic for the rest of their lives (14). More than 50% of congenitally infected babies are also asymptomatic at birth, and a few of them show signs of infection after 2 years (19). Thus, the experimental host has its merits. The expression of the disease in these animals mimics that of the largest percentage of both acquired and congenitally infected populations, which by and large is subclinical.

ACKNOWLEDGMENTS

Sandy Lionarons is thanked for competent secretarial help, and Murray King is thanked for his editorial work.

This work was supported in part by grant AI21833 from the National Institute of Allergy and Infectious Diseases, by research grant 6-FY93-0080 from the March of Dimes Birth Defect Foundation (V.W.), and by funds from the U.S. Department of Veterans Affairs (R.B.).

REFERENCES

1. **Baughn, R. E.** Unpublished data.
1a. **Bethesda Research Laboratories Life Technologies.** 1993. BluGENE nonradioactive nucleic acid detection system. Bethesda Research Laboratories Life Technologies, Grand Island, N.Y.
2. **Bonugli, F. S.** 1961. Involvement of the aortic valve and ascending aorta in congenital syphilis. Br. J. Vener. Dis. **37:**257–267.
3. **Boom, H. W., L. Liebster, A. K. Abbas, and R. Titus.** 1990. Patterns of cytokine secretion in murine leishmaniasis: correlation with disease progression or resolution. Infect. Immun. **58:**3863–3870.
4. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for

purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
5. **Burger, R., and E. M. Shevach.** 1979. Evaluation of the role of C4D in the cellular immune response in vitro. J. Immunol. **122:**2388–2394.
6. **Burstain, J. M., E. Grimprel, S. A. Lukehart, M. V. Norgard, and J. D. Radolf.** 1991. Sensitive detection of *Treponema pallidum* by using the polymerase chain reaction. J. Clin. Microbiol. **29:**62–69.
7. **Centers for Disease Control.** 1969. Manual of tests for syphilis. U.S. Department of Health, Education, and Welfare publication no. 411. U.S. Department of Health, Education, and Welfare, Washington, D.C.
8. **Cooper, A. M., A. D. Roberts, E. R. Rhoades, J. E. Callahan, D. M. Getzy, and I. M. Orme.** 1995. The role of IL12 in acquired immunity to *Mycobacterium tuberculosis* infection. Immunology **84:**423–432.
9. **Crissey, J. T., and D. A. Denenholz.** 1984. Congenital syphilis, p. 143–161. *In* Clinics in dermatology, vol. 2. Lippincott, Philadelphia.
10. **Dallas, W. S., P. H. Hay, J. Leong, L. V. Stamm, and P. J. Bassford.** 1987. Identification and purification of a recombinant *Treponema pallidum* basic membrane protein antigen expressed in *Escherichia coli*. Infect. Immun. **55:** 1106–1115.
11. **Faith, A., O. Pontesilli, A. Unger, G. S. Panayi, and P. Johns.** 1982. ELISA assays for IgM and IgG rheumatoid factors. J. Immunol. Methods. **55:**169–177.
12. **Fiumara, N. J.** 1965. A legacy of syphilis. Arch. Dermatol. **92:**676–678.
13. **Fiumara, N. J.** 1973. Venereal diseases, p. 447–478. *In* D. Charles and M. Finland (ed.), Obstetrics and perinatal infections. Lea & Febiger, Philadelphia.
14. **Gjestland, T.** 1955. The Oslo study of untreated syphilis. Acta Dermato-Venereol. **35**(Suppl. 34)**:**11–368.
15. **Gordon, S. M., M. E. Eaton, R. George, S. Larsen, S. A. Lukehart, J. Kuypers, C. M. Marra, and S. Thompson.** 1994. The response of symptomatic neurosyphilis to high-dose intravenous penicillin G in patients with human immunodeficiency virus infection. N. Engl. J. Med. **331:**1469–1473.
16. **Grimprel, E., P. J. Sanchez, G. D. Wendel, J. M. Burstain, G. H. McCracken, Jr., J. D. Radolf, and M. V. Norgard.** 1991. Use of polymerase chain reaction and rabbit infectivity testing to detect *Treponema pallidum* in amniotic fluid, fetal and neonatal sera, and cerebrospinal fluid. J. Clin. Microbiol. **29:**1711–1718.
17. **Hay, P. E., J. R. Clarke, R. A. Strugnell, D. Taylor-Robinson, and D. Goldmeir.** 1990. Use of the polymerase chain reaction to detect DNA sequences specific to pathogenic treponemes in cerebrospinal fluid. FEMS Microbiol. Lett. **68:**233–238.
18. **Horowitz, H. W., M. P. Valsamis, V. Wicher, F. Abbruscato, S. Larsen, G. P. Wormser, and K. Wicher.** 1994. Cerebral syphilitic gumma confirmed by the polymerase chain reaction in a man with human immunodeficiency virus infection. N. Engl. J. Med. **331:**1488–1491.
19. **Ingall, D., S. M. Dobson, and D. Musher.** 1990. Syphilis, p. 367–394. *In* J. S. Remington, and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant, 3rd ed. W. B. Saunders, Philadelphia.
20. **Jethwa, H. S., J. L. Schmitz, G. Dallabetta, F. Behets, I. Hoffman, H. Hamilton, G. Lule, M. Cohen, and J. Fold.** 1995. Comparison of molecular and microscopic techniques for detection of *Treponema pallidum* in genital ulcers. J. Clin. Microbiol. **33:**180–183.
21. **Lukehart, S. A., E. W. Hook, S. A. Baker-Zander, A. C. Collier, C. W. Critchlow, and H. H. Handsfield.** 1988. Invasion of the central nervous system by *Treponema pallidum*: implications for diagnosis and treatment. Ann. Intern. Med. **109:**855–862.
22. **Magnuson, H. J., H. Eagle, and R. Fleischman.** 1948. The minimal infectious inoculum of Spirochaeta pallida (Nichols strain) and a consideration of its rate of multiplication in vivo. Am. J. Syph. Gonorrhea Vener. Dis. **32:**1–18.
23. **Musher, D. M.** 1991. Syphilis, neurosyphilis, penicillin and AIDS. J. Infect. Dis. **163:**1201–1206.
24. **Noordhoek, G. T., E. C. Wolters, M. E. J. de Jonge, and J. D. A. van Embden.** 1991. Detection by polymerase chain reaction of *Treponema pallidum* DNA in cerebrospinal fluid from neurosyphilis patients before and after antibiotic treatment. J. Clin. Microbiol. **29:**1976–1984.
25. **Oppenheimer, E. H., and J. B. Hardy.** 1971. Congenital syphilis in the newborn infant: clinical and pathological observations in recent cases. Johns Hopkins Med. J. **129:**63–82.
26. **Penn, C. W., and J. G. Rhodes.** 1982. Surface associated antigens of *Treponema pallidum* concealed by an inert outer layer. Immunology **46:**9–16.
27. **Radolf, J. D., M. V. Norgard, and W. W. Schultz.** 1989. Outer membrane ultrastructure explains the limited antigenicity of virulent *T. pallidum*. Proc. Natl. Acad. Sci. USA **86:**2051–2055.
28. **Rosahn, P.** 1947. Autopsy studies in syphilis, p. 1–67. *In* U.S. Public Health Service venereal disease information supplement 21. Government Printing Office, Washington, D.C.
29. **Sanchez, P. J., G. D. Wendel, E. Grimprel, M. Goldberg, M. Hall, O. Arencibia-Mireles, J. D. Radolf, and M. V. Norgard.** 1993. Evaluation of molecular methodologies and rabbit infectivity testing for the diagnosis of congenital syphilis and neonatal central nervous system invasion by *Treponema pallidum*. J. Infect. Dis. **167:**148–157.
30. **Sieling, P. A., and R. L. Modling.** 1994. Regulation of cytokine patterns in leprosy. Ann. N.Y. Acad. Sci. **730:**42–81.
31. **Singh, V. K., and A. J. Tingle.** 1982. Detection of circulating immune complexes by a C1q− microplate ELISA system. J. Immunol. Methods **50:**109–114.
32. **Turner, T. B., and D. H. Hollander.** 1957. Biology of the treponematoses. World Health Organization, Geneva.
33. **Walker, E. M., G. A. Zamphighi, D. R. Blanco, J. N. Miller, and M. A. Lovett.** 1989. Demonstration of rare protein in the outer membrane of *Treponema pallidum* subsp. *pallidum* by freeze-fracture analysis. J. Bacteriol. **171:**5005–5011.
34. **Wicher, K., R. E. Baughn, V. Wicher, and S. Nakeeb.** 1992. Experimental congenital syphilis: guinea pig model. Infect. Immun. **60:**271–277.
35. **Wicher, K., G. T. Noordhoek, F. Abbruscato, and V. Wicher.** 1992. Detection of *Treponema pallidum* in early syphilis by DNA amplification. J. Clin. Microbiol. **30:**497–500.
36. **Wicher, K., and V. Wicher.** 1989. Experimental syphilis: guinea pig model. Crit. Rev. Microbiol. **16:**181–234.
37. **Wicher, K., and V. Wicher.** 1991. Median infective dose of *Treponema pallidum* determined in a highly susceptible guinea pig strain. Infect. Immun. **59:**453–456.
38. **Wicher, K., V. Wicher, and R. F. Gruhn.** 1985. Differences in susceptibility to infection with *Treponema pallidum* Nichols between five different strains of guinea pig. Genitourin. Med. **61:**21–26.
39. **Wicher, V., R. E. Baughn, and K. Wicher.** 1994. Congenital and neonatal syphilis in guinea pigs show a different pattern of immune response. Immunology **82:**404–409.
40. **Zeltzer, P. M., J. S. Pepose, N. H. Bishop, and J. N. Miller.** 1978. Microassay for immunoglobulin G antibodies to *Treponema pallidum* with radioiodinated protein A from *Staphylococcus aureus*: immunoglobulin G response in experimental syphilis in rabbits. Infect. Immun. **21:**163–170.

Editor: J. R. McGhee