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The Biology of *Treponema pallidum* and Syphilis

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Advances in diagnosis and treatment of syphilis have reinforced the idea that *Treponema pallidum* is an extraordinary bacterial pathogen, whose unique biologic properties permit its continued success as an infectious agent.

Organism

Treponemes are relatively long, thin, spiral-shaped, highly motile bacteria with characteristic axial fibrils that extend between the protoplasmic cylinder and the outer envelope. These filamentous organelles are flagellalike and are considered partly responsible for the organism's fascinating spirochetal movement, which is described as rotational, flexing, undulating, serpentine, and translational. For visualizing motility, darkfield or high resolution phase microscopy is required. In contrast to the "typical" flagellated bacteria, spirochetes can penetrate high viscosity environments (11). During the mid-1970s, the classification of *T. pallidum* as an anaerobe was questioned when metabolic studies (anabolic and catabolic) of organisms freshly extracted from infected rabbit tissue demonstrated O_2 consumption, a functioning electron transport system, oxidative phosphorylation, and dependence on O_2 for macromolecular synthesis. Recently, these data have been extensively reviewed and lend support to the aerobic or facultative nature of *T. pallidum* (9).

Host Response

How does the natural host, the human being, respond immunologically to *T. pallidum?* Clearly, the classical syphilitic infection is complex, characterized by intermittent periods of latency and different stages of development (primary, secondary, and tertiary illness [14]). Certain individuals become completely immune relatively early in the infection, preventing disease progression. In other patients, the disease continues, and although these patients manifest later stages of disease they are refractory to reinfection (i.e., cannot reacquire primary syphilis). This situation is analogous to the concomitant immunity observed with other well known parasitic infections (8). The pathophysiology of syphilis is undoubtedly influenced both by humoral and cell-mediated mechanisms as reflected in 1) the relatively early appearance of antitreponemal and anticardiolipin antibodies, 2) an inflammatory response consisting of lymphocytic, plasma cell, and macrophage activation and infiltration, 3) immunocomplex formation, 4) appearance and resolution of lesions, and 5) latency.

Numerous reports indicate that the temporal appearance of humoral antibody and cell-mediated immunity during *T. pallidum* infection is abnormal (6, 10). Yet other investigations suggest that the immune system operates maximally during the host–parasite interaction (7, 16). Nevertheless, syphilis remains a common infectious disease, and individuals with high titers of antitreponemal antibodies and "functioning" immune mechanisms can be totally protected, or continue to harbor *T. pallidum* in a latent state, or manifest clinical complications. How can the accumulated information and apparent inconsistencies be resolved?

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Experimental Strategies

We have undertaken a biologic–chemical–immunologic dissection of *T. pallidum* in order to define the virulence determinants of this pathogen. Our goal has been to understand the pathogenesis of syphilis and develop ways of interrupting and controlling the disease.

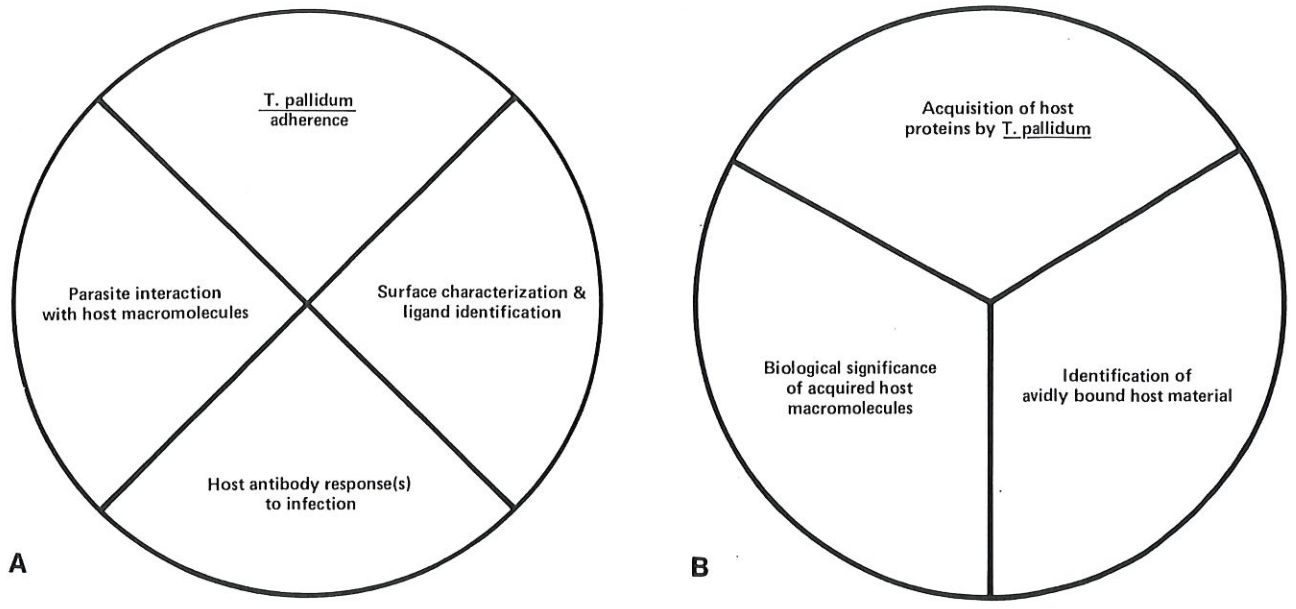
Treponema pallidum cannot be grown satisfactorily in vitro; consequently, intratesticular passage in rabbits is necessary. To overcome this technical limitation we took advantage of our earlier observation that *T. pallidum* exhibits polar attachment to eukaryotic cell monolayers during coincubation (12). The spirochetes adhere irreversibly to host cell membranes by their tapered ends and remain vigorously motile. The avirulent treponeme, *Treponema phagedènis* biotype Reiter, is unable to attach. These observations provided a focus for our investigations of treponemal pathogenicity (Figure 1A, clockwise from top).

We characterized the outer membrane of *T. pallidum* and identified three surface proteins (proteins 1, 2, and 3 with molecular weights of 89,000, 29,000, and 25,000, respectively), which function as ligands mediating surface parasitism (2, 5). The proposed biologic role of these proteins was reinforced by the observation that antibody raised against them markedly impaired treponemal attachment without decreasing motility (4, 5, 15). Treponemal immunogens that stimulate protective antibody had not been previously described; therefore, we examined sera from syphilitics and experimentally infected rabbits. A strong antibody response to the surface proteins was readily detected, and the kinetics of the response suggested a correlation with host immunity (3, 4). We detected similar antibodies in sera from patients with yaws. (The causative agent, *Treponema pertenue,* is serologically indistinguishable from *T. pallidum* (5, 17).) Using the purified protein ligands as antigens, we have devised an enzyme-linked immunosorbent assay, which shows promise as a new serologic test (13).

In other studies (1), we demonstrated a surface coat of host macromolecules on freshly extracted *T. pallidum* (Figure 1B, clockwise from top). Specific host proteins were bound avidly to *T. pallidum* and could be removed only by trypsin treatment—a procedure that also resulted in digestion of treponemal proteins. The intimacy of this binding was confirmed further by the discovery on treponemes of noncompetitive binding sites for these host macromolecules.

How do these biologic observations relate to treponemal virulence? The existence of host proteins at the outer envelope of *T. pallidum* may mask major treponemal immunogens, reducing the foreignness of the parasite during host immune surveillance (a type of biologic mimicry). This surface coat might also contribute to the pathophysiology of disease by inducing autoimmune reactions to host–treponemal complexes. To examine the possible role of host macromolecules that bind avidly to *T. pallidum,* we recently evolved a strategy using a variety of experimental approaches and techniques (Figure 1B). Iodinated Cohn fractions prepared from normal human plasma were incubated with *T. pallidum*. Fractions enriched for fibronectin selectively adsorbed to the outer envelope of *T. pallidum* and not to the avirulent Reiter treponeme. Further investigations demonstrated the tip-oriented adherence of *T. pallidum* to fibronectin-coated glass surfaces; pretreatment of fibronectin-coated coverslips with antifibronectin antibody prevented attachment (15). In parallel experiments we showed that cellular fibronectin, which is present on eukaryotic cell surfaces either as granulelike packets (on epithelial cells) or as fibrous, matrixlike patterns (on fibroblasts), is the apparent receptor for *T. pallidum*. Using fibronectin-Sepha-

Figure 1. Strategy for studying T. pallidum *virulence and major surface immunogens.*



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rose affinity chromatography and detergent-solubilized (^{35}S)methionine-labeled *T. pallidum* preparations, we detected selective binding of three prominent treponemal proteins to the columns (15). These proteins were identical to the treponemal ligands (proteins 1, 2, and 3) implicated in receptor-mediated binding to host cells (5). Essentially, we have traversed Figures 1A and B in identifying *T. pallidum* major surface protein immunogens and virulence determinants. As an added bonus, based on radioimmunoprecipitation and immunoblotting techniques, we found that *T. pertenue* not only possesses similar or identical surface macromolecules to *T. pallidum* but also shares many antigens. A discussion of syphilis should therefore mention the other treponematoses, yaws and pinta, because they are serologically identical and probably are variants of a single prototype organism. They exhibit clinically dissimilar diseases as a result of host or environmental influences.

The experimental sequence of events outlined in Figure 1 establishes specific approaches for studying microbial virulence, and for developing rapid diagnostic probes and potential immunogens for vaccines. The pathogenesis of the treponematoses will remain a fascinating mystery until the numerous technical obstacles are removed. In the meantime, specific limitations can be circumvented by logical and/or fortuitous discoveries that disclose the biologic sophistication of the virulent spirochetes.

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Summary of Microbiology Workload Recording Survey

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Response to the microbiology workload recording survey was excellent; 497 surveys were returned. The overwhelming response, not to mention malfunctioning disk drives, prevented the timely preparation of this summary, for which I apologize.

Responses were received from all regions of the United States and Canada. As anticipated, the majority of responses were from voluntary (nonprofit) hospitals; however, university affiliated, private, and city, county, or state hospitals were also represented. Among the responses 85% came from hospitals with 100 to 749 beds (questions 1–4). Because a wide variety of hospitals participated in the survey, I think the data will be very useful for setting up a new microbiology workload system.

As stated in the article that accompanied the survey (*Clin. Microbiol. Newslet.* 5(6): 37–38, 1983) the two major objectives were to determine: 1) if the majority of laboratories inocu-

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late the same number of plates and tubes for the same specimen type; 2) if there is any consistency for reading time of various cultures. Both objectives were fulfilled.

Primary Setup (Question 12)

The number of plates and tubes set up for each specimen type was consistent among most laboratories. In more than 60%, there was a difference of only one plate in the setup protocol. Recent time studies indicate that the time needed to set up one additional plate or tube is negligible. Using the survey data as our base, therefore, a unit value can be easily determined for each specimen type.

The same consistency was seen for routine subcultures or tubes. Most laboratories indicated that they subculture only tubes that have visible growth or organisms that, by Gram stain, appear different from those growing on the primary plates.

Reading Time (Question 13)

Little difference exists in reading time among the various specimen types. Of the institutions responding to the survey, 65 to 88% indicated that all specimens are read two to three times before tubes and plates are discarded. There was a difference in the reading of midstream and catheterized urine specimens; 70% of the laboratories read the plates twice and 30% read them only once.

No appreciable difference was noted in reading time of cultures with "No growth," "Normal flora," and "Insignificant growth." The same unit value, therefore, could be assigned to all cultures having such reports.

Several interesting analyses could be made using the data from questions 4, 5, and 7, i.e., a comparison between the number of beds vs. workload, number of beds vs. number of full-time equivalents (FTE), and number of FTE vs. workload (Tables 1–3). The information in Tables 1 and 2 show that hospitals with a bed size of less than 100 have a workload of 10,000 to 24,999 units/month (42%) and 1 to 3 FTE (74%). Hospitals of bed size 100 to 249 have a workload between 10,000 to 24,999 (40%) and 25,000 to 49,000 (38%) and 1 to 3 FTE (61%). Hospitals with 250 to 399 beds have a workload of 25,000 to 49,999 (45%) and 4 to 6 FTE (49%). Hospitals with 400 to 749 beds showed more workload variation ranging from 50,000 to greater than 100,000 with the number of FTE ranging from 7 to more than 10. As might be expected, hospitals with greater than 750 beds had a workload greater than 100,000 and more than 10 FTE. The correlation between workload and FTE is seen in Table 3. It is important to realize that the workload figures include both inpatient and outpatient work, whereas the bed size deals only with inpatient work.

The data gleaned from the survey will be extremely valuable for creating a new and better microbiology workload system. In addition, the data will provide the guidelines for evaluating the geographic location and type of time studies to be performed.

Table 1
Comparison of Number of Beds Vs. Workload

	Workload (units/month)					
Number of Beds	*<10,000 (%)*	*10,000–24,999 (%)*	*25,000–49,999 (%)*	*50,000–74,999 (%)*	*75,000–99,999 (%)*	*≥100,000 (%)*
<100	25	42	13	4	8	8
100–249	5	40	38	11	3	3
250–399	4	13	45	21	10	7
400–749	1	5	11	25	19	39
>750	0	13	6	13	0	68

Table 2
Comparison of Number of Beds Vs. Number of FTEs

	Number of FTEs				
Number of Beds	*1–3 (%)*	*4–6 (%)*	*7–10 (%)*	*>10 (%)*	*Other (%)*
<100	74	9	9	0	6
100–249	61	28	12	2	1
250–399	15	49	21	15	0
400–749	0.5	16	37	46	0.5
>750	7	7	7	72	7

Table 3
Comparison of Number of FTEs Vs. Workload

	Workload (units/month)					
Number of FTEs	*<10,000 (%)*	*10,000–24,999 (%)*	*25,000–49,999 (%)*	*50,000–74,999 (%)*	*75,000–99,999 (%)*	*≥100,000 (%)*
1–3	17	52	30	1	0	0
4–6	1	12	56	27	2	2
7–10	3	8	12	35	28	14
>10	1	5	5	9	18	62
Other	33	0	0	0	0	67

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Total Number of Responses = 497

1. Geographic location of facility.

12% Region I: ME, VT, NH, MA, CT, RI, NY, NJ
25 Region II: DE, DC, MD, WV, VA, KY, OH, IN, MI, PA
17 Region III: NC, SC, GA, FL, AL, MS, LA, AR, OK, TX, TN
19 Region IV: IL, WI, MN, IA, MO, KS, NE, SD, ND
15 Region V: AZ, CA, NV, HI, CO, NM, UT
8 Region VI: WY, MT, ID, OR, WA, AK
4 Region VII: Canada

2. Type of Laboratory.

92% Hospital
2 Commercial laboratory (Skip to question 5)
2 Group practice (Skip to question 5)
1 Private (Skip to question 5)
3 Other (Skip to question 5)

3. If hospital laboratory, specify type.

41% Voluntary
12 University affiliated
19 City, county, state
19 Proprietary
2 Military
4 V.A. or other government hospital
3 Other

4. If hospital laboratory, how many beds?

8% <100
31 100–249
28 250–399
27 400–749
6 >750

5. How many full-time equivalents (FTEs) are employed in microbiology, include all three shifts?

30% 1–3 FTEs
27 4–6 FTEs
20 7–10 FTEs
20 >10 FTEs
3 Other

6. Do you use the CAP Workload Recording Method in microbiology?

75% Yes **25%** No (Skip to Question 8)

7. What is your total CAP *monthly* workload in microbiology?

6% <10,000 units/month
21 10,000–24,999 units/month
29 25,000–49,999 units/month
17 50,000–74,999 units/month
10 75,000–99,999 units/month
17 ≥100,000 units/month

8. How long are *Mycobacterium* cultures routinely held before releasing a final report?

0.8% 4 Weeks
0.2 5 Weeks
28 6 Weeks
1 7 Weeks
51 8 Weeks
19 Other, specify sent to reference lab

9. How long are mycology cultures routinely held before releasing a final report?

58% 4 Weeks
2 5 Weeks
24 6 Weeks
0 7 Weeks
2 8 Weeks
14 Other, specify sent to reference lab

10. Are some mycology cultures held for different lengths of time (either longer or shorter) as compared to routine mycology cultures?

26% Yes
74% No (Skip to question 12)

11. Indicate below the types of mycology cultures that would be held for different lengths of time and indicate the lengths of time held before releasing a final report.

Mycology Culture

Type of Culture	*Length of Time Held*
Candida	1 week = 43%
Dermatophytes	3 weeks = 39%, 2 weeks = 23%
Throat	1 week = 40%, 2 weeks = 30%
Systemic	6 weeks = 38%, 8 weeks = 31%
Urine	1 week = 29%, 2 weeks = 29%
Stool	1 week = 43%, 2 weeks = 29%
Tissue	8 weeks = 47%, 7 weeks = 41%
Vaginal	1 week = 62%
CSF	1–2 weeks = 50%

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12. Indicate on the table below the number of primary plates and tubes set up for each specimen type for a bacteriologic culture. Then indicate if the tubes are routinely subcultured and if so, to how many different plates.

Primary Set-Up

Specimen	Number of Plates		Number of Tubes	Are Tubes Routinely Subcultured		If Tubes Are Routinely Subcultured	
	CO_2/O_2	ANA[a]		No	Yes	Number of Tubes Subcultured	Number of Plates Used for Subcultures[b]
Urine—midstream	2 = 65% 3 = 25	0 = 100	0 = 97			N/A	N/A
Urine—catheterized	2 = 61% 3 = 28	0 = 100	0 = 91			N/A	N/A
Throat	2 = 38% 1 = 24 3 = 22	0 = 80 1 = 20	0 = 88 1 = 12			N/A	N/A
Strep only	1 = 64% 0 = 21	0 = 79 1 = 20	0 = 93			N/A	N/A
Sputum	3 = 62% 4 = 21	0 = 89 1 = 10	0 = 84 1 = 16			N/A	N/A
CSF	2 = 47% 3 = 34	0 = 73 1 = 21	1 = 79	67%	33%	1 = 89	2 = 50 1 = 25
Pleural fluid	3 = 45% 4 = 24 2 = 20	0 = 37 1 = 32 2 = 16	1 = 77 2 = 18	64%	34%	1 = 85	2 = 48 3 = 21
Peritoneal fluid	3 = 48% 4 = 27	0 = 32 1 = 31	1 = 76 2 = 18	66%	34%	1 = 82	2 = 45 3 = 23
Eye	3 = 47% 2 = 27	0 = 87 1 = 10	1 = 74 0 = 21	75%	25%	1 = 97	2 = 45 1 = 26
Ear	3 = 54% 4 = 27	0 = 86 1 = 11	1 = 69 0 = 28	80%	20%	1 = 98	2 = 45 3 = 24
Genital	4 = 40% 3 = 23 5 = 22	0 = 90 1 = 7	0 = 57 1 = 41	89%	11%	1 = 98	2 = 43 3 = 33
Gonococcus only	1 = 51% 2 = 38	0 = 99	0 = 93	98%	2%	1 = 100	1 = 40 2 = 40
Stool	4 = 33% 5 = 28	0 = 96	1 = 66 2 = 28	7%	93%	1 = 69 2 = 28	2 = 45 1 = 25 3 = 21
Tissue	3 = 45% 4 = 26	0 = 33 1 = 27 2 = 19	1 = 75 2 = 18	65%	35%	1 = 87	2 = 58 3 = 15
Aspirated abscess	3 = 45% 4 = 27 2 = 16	2 = 25 1 = 24 0.3 = 23	1 = 75 2 = 21	61%	39%	1 = 81 2 = 19	2 = 45 3 = 22
Superficial wound	3 = 49% 4 = 20	0 = 74 1 = 16	1 = 83	74%	26%	1 = 97	2 = 50 3 = 23

[a] ANA = Anaerobic. [b] If more than one tube is subcultured, give the total number of plates used in subculturing all tubes.

13. Indicate on the table below the number of times the plates and tubes are read before discarding.

Reading Time

	Number of Times Plates and Tubes Are Read before Discarding		
Specimen	*No Growth*	*Normal Flora, No Additional Work-Up*	*Insignificant Growth No Additional Work-Up*
Urine—midstream	once = 34 twice = 64	N/A	once = 35 twice = 47
Urine—catheterized	once = 25 twice = 71	N/A	once = 21 twice = 42 N/A = 36
Throat	twice = 57 N/A = 30	once = 23 twice = 68	N/A
Strep only	twice = 50 N/A = 32	once = 29 twice = 59	N/A
Sputum	twice = 61 N/A = 28	twice = 75	N/A
CSF	2 = 25 3 = 41	N/A	N/A
Pleural fluid	twice = 34 three = 33	N/A	N/A
Peritoneal fluid	twice = 35 three = 33	N/A	N/A
Eye	twice = 56 three = 29	twice = 35 N/A = 46	twice = 36 N/A = 43
Ear	twice = 58 three = 24	twice = 39 N/A = 41	twice = 40 N/A = 40
Genital	twice = 43 three = 38	twice = 41 three = 32 N/A = 22	twice = 32 three = 26 N/A = 39
Gonococcus only	twice = 41 three = 43	twice = 29 three = 30 N/A = 35	twice = 27 three = 25 N/A = 43
Stool	twice = 44 three = 25 N/A = 22	twice = 48 three = 27	twice = 31 N/A = 43
Tissue	twice = 35 three = 31	N/A	N/A
Aspirated abscess	twice = 37 three = 32	N/A	N/A
Superficial wound	twice = 52 three = 27	twice = 31 N/A = 47	twice = 33 N/A = 42

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Workshops and Meetings

Keystone Summit on Allergy, Immunology, Pulmonology, and ENT.
Keystone, Colorado.
January 29–February 2.
Contact: Helga W. Cole, Department of Pediatrics, National Jewish Hospital and Research Center, 3800 E. Colfax Ave., Denver, CO 80206.

Advanced Microbiology: Recent Developments and New Frontiers.
Dallas, Texas.
February 6–10.
Contact: Regional Educational Activities, 2100 W. Harrison St., Chicago, IL 60612.

South Central Association for Clinical Microbiology.
Cincinnati, Ohio.
April 5–7.
Contact: SCACM, 3901 N. Meridian St., Suite 235, Indianapolis, IN 46208.

Update in Clinical Microbiology and Immunology, 7th Annual.
Park City, Utah.
January 30–February 3.
Contact: Constance W. Ward, Course Coordinator, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT, 84132.

ASM Annual Meeting, 84th. St. Louis, Missouri,
March 4–9.
Contact: Meetings Department, American Society for Microbiology, 1913 I St., N.W., Washington, DC 20006.

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