INFECTION AND IMMUNITY, Jan. 1980, p. 260-263
0019-9567/80/01-0260/04$02.00/0

Vol. 27, No. 1

Concanavalin A-Mediated Affinity Film for *Treponema pallidum*

J. B. BASEMAN,* Z. ZACHAR, AND N. S. HAYES

Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Freshly extracted *Treponema pallidum* bound to glass cover slips preexposed to specific lectins, permitting biological testing in the absence of complex tissue fluids.

Although considerable data exist concerning the biology of virulent *Treponema pallidum*, numerous experimental problems remain. These spirochetes still cannot be readily grown in vitro, and when multiplication is observed treponemal cell densities that are achieved do not even closely approximate values obtained during the direct isolation of *T. pallidum* from infected rabbit tissue (10, 14).

A major technical limitation is the continual exposure of *T. pallidum* during in vitro incubation to tissue extract contaminants. In almost all cases, *T. pallidum* is harvested at peak orchitis, usually 8 to 12 days after intratesticular inoculation of rabbits with 10^7 to 10^8 treponemes per testis. A tissue extract containing treponemes and soluble testicular components is prepared relatively free of host cells and cellular debris. It has been suggested that this tissue milieu resulting from the mincing of infected and inflamed testes improves in vitro survival of treponemes (7, 12, 15). We have considered the possibility that along with beneficial factors the testicular extract may also contain biologically active substances which are detrimental to *T. pallidum*, thus complicating the interpretation and duplication of experimental data.

Our previous attempts to purify *T. pallidum* from tissue extract by velocity sedimentation in Hypaque resulted in loss of viability (6). Also, concentration of *T. pallidum* by centrifugation procedures that pellet treponemes decreases in vitro survival when compared with uncentrifuged controls (data not shown). Because of these difficulties, we have sought procedures that provide purified and metabolically active virulent *T. pallidum* and avoid potentially stressful manipulations such as high-speed centrifugation or extended exposure of treponemes to testicular fluids.

Several years ago, we described the specific orientation of virulent *T. pallidum* by its terminal structures to tissue cells in monolayer culture (2, 11). Because our data implicated the existence of receptor-like molecules on both pathogen and host cell (3, 11), we decided to explore these observations further. Lectins will bind noncovalently to glass and plastic surfaces (13), and we exploited this property by establishing lectin films on cover slips (9 by 35 mm) in Leighton tubes. Commercially available lectins were dissolved at 500 μg/ml in phosphate-buffered saline (pH 7.0) and filter sterilized. One-milliliter volumes were added per tube, and incubation was continued for 10 to 30 min at room temperature. The length of incubation did not alter the results. Virulent *T. pallidum* was extracted in treponemal medium (4) with or without glucose, and host cells and cellular debris were removed by Methocel-Hypaque gradient centrifugation (5). The lectin solution was aspirated, and 5×10^7 treponemes in 0.5 ml of testicular extract were added for 2 h at 33°C unless otherwise noted. Then, the treponemal suspension was removed, and the cover slip was washed three times in phosphate-buffered saline before counting by dark-field microscopy.

T. pallidum interacted best with concanavalin A (ConA) of the lectins tested (Table 1). It is not known whether ConA bound more efficiently to the glass surface to permit the formation of a more extensive or accessible film in contrast to other lectins. The observation that wheat germ agglutinin promotes treponemal attachment is consistent with the reported agglutination of *T. pallidum* by this lectin (8). The temperature of incubation of the treponemal suspension and the concentration of ConA used to form the lectin film were important (Table 2). Also, the number of treponemes attaching per cover slip was directly proportional to the initial cell density of the treponemal inoculum (Table 2). Thus, the ultimate efficiency of treponemal binding to lectin-treated glass surfaces is dependent upon several experimental parameters. It was interesting that *T. pallidum* attached to the lectin film by its terminal structures (11). It is unclear, however, whether this interaction was

TABLE 1. *Affinity of T. pallidum for various lectin films*

Lectin[a]	No. of treponemes[b] per microscopic field[c]	
	Expt 1	Expt 2
PBS	2	3
PWM	3	4
PHA	17	38
WGA	21	23
ConA	61	65

[a] Lectins were dissolved at a concentration of 500 μg/ml in phosphate-buffered saline (PBS, pH 7.0). PWM, pokeweed mitogen (Sigma Chemical Co., St. Louis, Mo.); PHA, phytohemagglutin (Sigma); WGA, wheat germ agglutinin (Sigma); ConA, was from Miles Laboratories, Elkhard, Ind.

[b] Each number is the average of triplicate samples from separate experiments. Within each experiment, individual samples varied less than 15% from the mean value.

[c] Ten random fields per cover slip were counted by dark-field microscopy at a magnification of 500×.

TABLE 2. *Parameters influencing T. pallidum attachment to ConA affinity film*

Experimental condition	No. of treponemes[a] per microscopic field	
	Expt 1	Expt 2
Temperature (°C)		
4	4	8
26	34	36
33	56	63
Concn of ConA (μg/ml)		
0	3	1
100	14	16
200	37	26
300	36	43
400	60	66
500	69	60
T. pallidum inoculum (treponemes/ml)		
3.5×10^7	32	34
7×10^7	59	54
1.4×10^8	87	83
2.8×10^8	120	134

[a] Values were determined as described in the footnotes of Table 1.

mediated by a treponemal or host component (1) associated with the surface of *T. pallidum*. Alternatively, soluble tissue components in the testicular extract may combine with ConA to generate a functional affinity film. The extent to which soluble tissue components adsorb to the lectin-coated glass has not been determined, although carbohydrate-containing host molecules with appropriate lectin affinity may bind along with molecules that display less-specific interactions with the glass surface.

To clarify the specificity of this interaction, a range of sugars considered to be inhibitors or noninhibitors of ConA binding was monitored for ability to alter *T. pallidum* attachment. As seen in Table 3, sugars known to interact directly with ConA prevented treponemal binding to the lectin film. Since α-methyl mannoside does not decrease ConA binding to glass or plastic surfaces (13), our data suggest that specific sugars (α-methyl mannoside, mannose, glucose) at relatively high concentrations (9) blocked ConA-reactive sites necessary for the interaction of lectin with the carbohydrate moieties of the implicated treponemal or tissue component(s). Once *T. pallidum* bound to the affinity film, attachment could be reversed, although not totally, by the addition of 0.1 M α-methyl mannoside but not by 0.1 M galactose, further supporting the selectivity of the association. It is important to note that attachment of *T. pallidum* to rabbit testicular cells in monolayer culture (11) is not prevented by coincubation of animal cells and treponemes with ConA (500 μg/ml) or mannose (0.1 M) or glucose (0.1 M). These data suggest the possibility that treponemal binding to the lectin affinity film may be an in vitro phenomenon.

After attachment of *T. pallidum* to the affinity film, the cover slip can be removed or the fluid can be decanted and the bound treponemes can be rinsed free of residual tissue fluid. Under these conditions metabolic and growth potential of *T. pallidum* can be examined free from the complexities of undefined medium constituents such as inflamed tissue extract (Fig. 1). Treponemes remain actively motile for days either attached to the film or having detached, moving

TABLE 3. *Specificity of ConA affinity film as determined by competition with various sugars*

Sugar[a]	No. of treponemes[b] per microscopic field	
	Expt 1	Expt 2
None	64	68
Galactose	53	54
Lactose	51	61
Ribose	43	50
Mannose	5	2
α-Methyl mannoside	6	1
α-Methyl glucoside	3	2

[a] Sugars at a final concentration of 0.1 M were dissolved directly in ConA solution (500 μg/ml) before addition to Leighton tube.

[b] Values are the average of triplicate samples (see footnotes of Table 1).

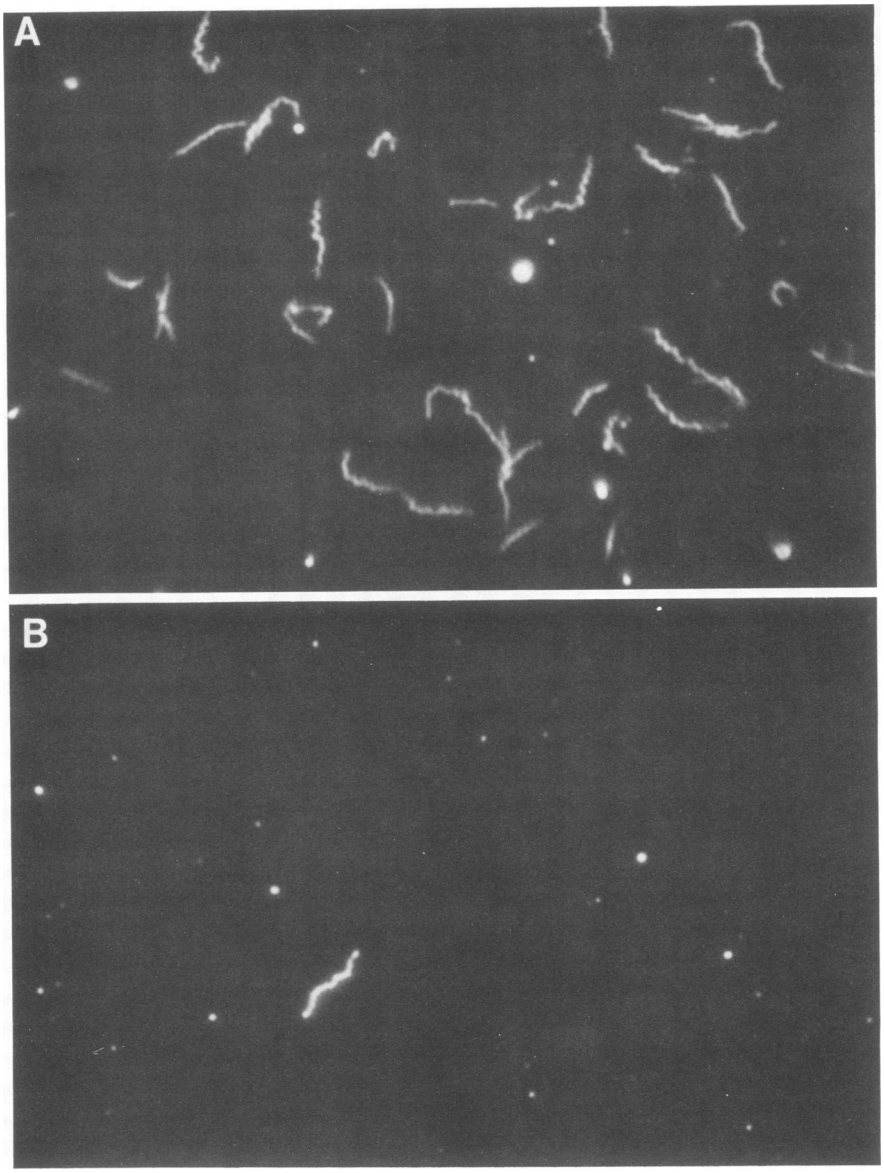

FIG. 1. *Attachment of T. pallidum to lectin affinity film as viewed by dark-field microscopy. Cover slips were removed from Leighton tubes and rinsed in phosphate-buffered saline before microscopic analysis. (A) ConA-mediated film; (B) pokeweed mitogen-mediated film. Magnification, 900×.*

freely in the medium, and the influence of a spectrum of nutrients, cofactors, enzymes, and other variables can be monitored as the varibles affect the metabolic state of *T. pallidum*.

This work was supported by Public Health Service grant AI-15036 and research career development award 1-K04-AI-00178 from the National Institute of Allergy and Infectious Diseases to J.B.B.

LITERATURE CITED

1. **Alderete, J. F., and J. B. Baseman.** Surface-associated host proteins on virulent *Treponema pallidum*. Infect. Immun. **26**:1048–1056.
2. **Baseman, J. B.** 1977. Report of a workshop: the biology of *Treponema pallidum*. J. Infect. Dis. **136**:308–311.
3. **Baseman, J. B., N. S. Hayes, J. K. Spitznagel, and E. C. Hayes.** 1979. Virulence determinants among the spirochetes, p. 203–208. *In* D. Schlessinger (ed.), Micro-

biology—1979. American Society for Microbiology, Washington, D.C.

4. **Baseman, J. B., J. C. Nichols, and N. S. Hayes.** 1976. Virulent *Treponema pallidum*: aerobe or anaerobe. Infect. Immun. **13**:704–711.
5. **Baseman, J. B., J. C. Nichols, and S. Mogerley.** 1979. Capacity of virulent *Treponema pallidum* (Nichols) for DNA synthesis. Infect. Immun. **23**:392–397.
6. **Baseman, J. B., J. C. Nichols, J. W. Rumpp, and N. S. Hayes.** 1974. Purification of *Treponema pallidum* from infected rabbit tissue: resolution into two treponemal populations. Infect. Immun. **10**:1062–1067.
7. **Fieldsteel, A. H., F. A. Becker, and J. G. Stout.** 1977. Prolonged survival of virulent *Treponema pallidum* (Nichols strain) in cell-free and tissue culture systems. Infect. Immun. **18**:173–182.
8. **Fitzgerald, T. J., and R. C. Johnson.** 1979. Surface mucopolysaccharides of *Treponema pallidum*. Infect. Immun. **24**:244–251.
9. **Goldstein, I. J.** 1976. Carbohydrate binding specificity of concanavalin A, p. 55–65. *In* H. Bittiger and H. P. Schnebli (ed.), Concanavalin A as a tool. John Wiley and Sons, New York.
10. **Hardy, P. H., Jr., and E. E. Nell.** 1975. Isolation and purification of *Treponema pallidum* from syphilitic lesions in rabbits. Infect. Immun. **11**:1296–1299.
11. **Hayes, N. S., K. E. Muse, A. M. Collier, and J. B. Baseman.** 1977. Parasitism by virulent *Treponema pallidum* of host cell surfaces. Infect. Immun. **17**:174–186.
12. **Nelson, R. A., Jr.** 1948. Factors affecting the survival of *Treponema pallidum* in vitro. Am. J. Hyg. **48**:120–132.
13. **Phillips, P. G., and M. Lubin.** 1977. Influence of serum factors on the interaction of concanavalin A with inert and biological surfaces. Exp. Cell Res. **106**:31–38.
14. **Turner, T. B., and D. H. Hollander.** 1954. Studies on the mechanism of action of cortisone in experimental syphilis. Am. J. Syph. **38**:371–387.
15. **Weber, M. M.** 1960. Factors influencing the in vitro survival of *Treponema pallidum*. Am. J. Hyg. **71**:401–417.