SHORT COMMUNICATION

Phenotypic Expression of the Major 47 kDa Surface Immunogen of *Treponema pallidum* in Virulent, Tissue-cultured Treponemes

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Specific monoclonal antibody and Western blot analysis were used to examine the phenotypic expression of the major 47 kDa surface immunogen of *Treponema pallidum* among organisms cultivated *in vitro*. Tissue-cultured treponemes synthesized the 47 kDa immunogen as well as, or better than, organisms cultivated *in vivo* (rabbit testicles).

INTRODUCTION

Considerable progress has been made recently in identifying antigens or immunogens of *Treponema pallidum* that may significantly influence the developing immune response to treponemal infection (for review, see Jones et al., 1984; Marchitto et al., 1986). Of particular interest to us has been the major, immunodominant, 47 kDa surface immunogen that appears to be specific to virulent subspecies of *T. pallidum* (Jones et al., 1984; Marchitto et al., 1986). Monoclonal antibodies directed against this antigen are reactive in the microhaemagglutination assay for *T. pallidum* antibodies (MHA-TP) and in the *T. pallidum* immobilization (TPI) assay (Jones et al., 1984). They also partially block the attachment of *T. pallidum* to rabbit host cells *in vitro*, and apparently neutralize virulent *T. pallidum* (Jones et al., 1984), as assayed by the *in vitro–in vivo* neutralization test of Bishop & Miller (1976). These observations allowed potential biological importance to be assigned to the 47 kDa surface immunogen of *T. pallidum*; it may be the major surface immunogen of the organism (Jones et al., 1984; Penn et al., 1985). The biological significance of this antigen, however, has been inferred primarily on the basis of experiments using indirect strategies; purified 47 kDa immunogen has not yet been tested directly to confirm the hypothesis.

One of our laboratories (M. V. N.) has been interested in exploiting monoclonal antibodies directed against the 47 kDa immunogen in immunoaffinity chromatography as a strategy for affinity-purifying the 47 kDa immunogen of *T. pallidum*. Results of initial antigen purification experiments revealed certain potential limitations in utilizing *in vivo*-cultivated *T. pallidum* as a starting source of treponemal antigens. In this regard, results of earlier studies (Alderete & Baseman, 1979; Christiansen, 1963), as well as recent work performed in this laboratory, suggest that various host-derived molecules associate with the surface of *T. pallidum* during infection of the host and growth of the organism *in vivo*. These host-derived molecules include specific anti-*T. pallidum* polyclonal antibody (Logan, 1974; K. S. Marchitto & M. V. Norgard, unpublished).
In *vivo* cultivation of *T. pallidum* offers a strategy for cultivating *T. pallidum* in the absence of a specific humoral immune response by the host against the infecting organism, thereby circumventing the binding of at least one major class of host-derived molecules (specific immunoglobulins) to the surface of the organism. A limited *in vitro* cultivation system for *T. pallidum* has been described using tissue culture (Fieldsteel *et al.*, 1981); the efficacy of this *in vitro* system for cultivating virulent *T. pallidum* has been independently confirmed (Norris, 1982). *T. pallidum* cultivated *in vitro* retains its infection and virulence capabilities (Fieldsteel *et al.*, 1981). This system has now been developed to the stage where large-scale cultivation of the organism is potentially feasible (Fieldsteel *et al.*, 1982). However, before *in vitro* cultivation is effectively utilized as a source of starting material for treponemal antigen purification, it must be established that an alteration in the phenotypic expression of individual antigens of *T. pallidum* does not occur in this artificial cultivation system. We have examined the phenotypic expression of the major 47 kDa surface immunogen of *T. pallidum* in tissue-cultured treponemes using specific anti-47 kDa monoclonal antibodies in *Western blot* analysis.

**METHODS**

The Nichols strain of *T. pallidum* was cultivated in the laboratory of D.L.C. using the *in vitro* tissue-culture system (Fieldsteel *et al.*, 1981, 1982). Rabbit tissue culture cells (SF1Ep) were removed from the disrupted cell suspension by differential centrifugation (500 g for 10 min), followed by collection of the treponemes by centrifugation at 13000 g for 15 min. Treponemes were washed twice by centrifugation in sterile phosphate-buffered saline (0.85% NaCl in 0.01 M-sodium phosphate, pH 7.2; PBS), concentrated in PBS, and enumerated by darkfield microscopy. The *T. pallidum* suspension was then transferred on ice within 1 h to the laboratory of M.V.N.; treponemes were counted again by darkfield microscopy. Additional *T. pallidum* cells were cultivated *in vivo* (rabbit testicles) and were isolated by routine techniques described previously (Robertson *et al.*, 1982); they also were purified by differential centrifugation similar to the method used for *in vitro*-cultivated treponemes.

Both preparations of bacteria were used in Western blot analysis as described previously (Marchitto *et al.*, 1984). Monoclonal antibody 11E3 (IgG2a), directed specifically against the major 47 kDa surface immunogen of virulent subspecies of *T. pallidum*, has been well characterized (Jones *et al.*, 1984; Marchitto *et al.*, 1984, 1986) and was used to detect the presence of the 47 kDa immunogen in Western blots of treponeme preparations. The binding of monoclonal antibody 11E3 to 47 kDa immunogen in the Western blot assay was determined by using a probe of horseradish peroxidase-conjugated goat anti-mouse IgG (heavy and light chain specific) (Cappel-Cooper Biomedical) as previously described (Marchitto *et al.*, 1984).

**RESULTS AND DISCUSSION**

Fig. 1 shows results of a Western blot using both *in vivo*- and *in vitro*-cultivated *T. pallidum* and monoclonal antibody 11E3 to detect the presence of the 47 kDa immunogen in the two treponeme preparations. In this particular experiment, there had been a 28.3-fold increase in the number of motile treponemes at the time that the *in vitro* *T. pallidum* culture was terminated and used as a source of *T. pallidum* (lanes 3 and 4) in the Western blot. It was necessary to address the possibility that the 47 kDa antigen among *in vitro*-cultivated *T. pallidum* might represent the proportion of 47 kDa antigen of *T. pallidum* harvested from rabbit tissue that was used as the starting inoculum for the *in vitro* culture. Inasmuch as 2 x 10^6 *T. pallidum* were used in *Western blotting* to assay for the presence of the 47 kDa immunogen, 1/28 of this number of cells (7.1 x 10^4) potentially represented a source of *in vivo*-synthesized 47 kDa immunogen in the *in vitro*-cultivated *T. pallidum* preparation. To exclude this possibility, 1 x 10^5 rabbit-cultivated *T. pallidum* were used in the Western blot to compare the level of 47 kDa immunogen in this amount of material with that found in 2 x 10^6 *in vitro*-cultivated organisms. The result shown in lane 1 of Fig. 1 represented the critical control for the experiment and demonstrated that anti-47 kDa monoclonal antibody 11E3 was barely capable of detecting the presence of the 47 kDa antigen in only 1 x 10^5 rabbit-cultivated *T. pallidum* cells. However, the monoclonal antibody distinctly revealed the presence of the 47 kDa antigen in 2 x 10^6 rabbit-cultivated *T. pallidum* cells (lane 2). As predicted by the result shown in lane 1, 1 x 10^5 *in vitro*-cultivated *T. pallidum* also barely revealed the 47 kDa antigen. In contrast, lane 4 of Fig. 1 shows strong reactivity of...
the monoclonal antibody with the 47 kDa antigen of $2 \times 10^6$ *T. pallidum* cultivated *in vitro*.

As indicated, lane 1 of Fig. 1 provided the key control to conclude that *in vitro*-cultivated *T. pallidum* does, indeed, phenotypically express the major 47 kDa immunogen of *T. pallidum* (lane 4). Inasmuch as this control contained a greater number ($1 \times 10^5$) of rabbit-cultivated *T. pallidum* potentially contaminating the *in vitro*-cultivated *T. pallidum* preparation ($7.1 \times 10^4$), a firm conclusion could be drawn. Furthermore, the findings were consistent with other data obtained regarding the *in vitro* synthesis of 47 kDa surface immunogen of *T. pallidum*; we recently established that the 47 kDa immunogen can be radiolabelled *in vitro* with $[^{35}S]$methionine in the presence of 100 μg cycloheximide ml$^{-1}$ (Stamm & Bassford, 1985) using a recently described *T. pallidum* labelling medium (Moskophidis & Müller, 1984) (K. S. Marchitto, M. A. Swancutt & M. V. Norgard, unpublished data). The radiolabelled 47 kDa product can be immunoprecipitated with monoclonal antibody 11E3 (unpublished data).

In comparing the results of lanes 2 and 4 in Fig. 1, it may be significant that *in vitro*-cultivated *T. pallidum* seemed to express the 47 kDa antigen as well as, or better than, *in vivo*-cultivated *T. pallidum*. 

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**Fig. 1.** Western blot of *in vitro*- and *in vivo*-cultivated *T. pallidum* probed with anti-47 kDa immunogen monoclonal antibody 11E3. Abbreviations: rab, rabbit-cultivated; T. pal., *T. pallidum*. 

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organisms. This result was reproducible. The 47 kDa immunogen band shown in lane 4 appeared to contain about twice as much antigen in comparison with that visualized in lane 2. However, precise quantification of the differences in the amount of antigen expressed among the two populations of *T. pallidum* is difficult, due to potential errors in counting treponemes by darkfield microscopy, and in using the Western blot assay, which is not strictly quantitative.

In any event, it is clear that the 47 kDa immunogen of *T. pallidum* is phenotypically expressed among *in vitro*-cultivated organisms at a level equal to or greater than that of *T. pallidum* propagated *in vivo*. This finding has provided impetus for exploiting the use of *in vitro*-cultivated *T. pallidum* as a source of constituent antigen for immunoaffinity purification of the major 47 kDa surface immunogen.

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REFERENCES


