CULTIVATION OF PATHOGENIC TREPONEMA IN TISSUE CULTURES OF SHEP CELLS

DAVID L. COX, RANDOLPH A. MOECKLI, AND A. HOWARD FIELDSTEEL

Life Sciences Division, SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025

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SUMMARY

Recently, the successful in vitro cultivation of the Nichols strain of Treponema pallidum was achieved. Afterward, attempts were made to cultivate three other strains of T. pallidum and two strains of T. pertenue. The cultivation of the KKJ, Mexico A, and Bosnia A strains of T. pallidum was somewhat successful; the average increases were 10.8, 9.1, and 7.5-fold, respectively. The range of growth for each of these strains varied dramatically from experiment to experiment. The KKJ strain varied from 14.4 to 8.0-fold; the Mexico A strain from 12.8 to 5.4-fold; and the Bosnia A strain from 11.3 to 3.6-fold. However, the attempts to cultivate the Gauthier and the FB strains of T. pertenue were unsuccessful. The average increases were 1.7 and 1.9-fold, respectively. Although the maximum growth observed was about threefold with either of these strains of T. pertenue, over 50% of the treponemes remained motile for 10 d. These results suggest that although each of these strains of T. pallidum and T. pertenue has been shown to be genetically identical, they are very diverse biologically even among strains of the same species.

INTRODUCTION

It is generally accepted that there are three species of Treponema that cause four clinically distinct diseases in humans (1,2). Treponema pallidum is the etiological agent of venereal syphilis and endemic nonvenereal syphilis (bejel). T. pertenue is the agent of yaws and T. carateum of pinta. Although these diseases are clinically distinguishable, the causative agents are morphologically indistinguishable and immunologically related, but not identical (2). There are two theories regarding this relationship. The first is that one organism causes all four diseases and the clinical distinctions are caused by environmental conditions. The second is that the human pathogens arose from a bacterium from a nonhuman animal and that mutants were selected by environmental changes. It was proposed that T. carateum was the earliest pathogen that mutated to produce T. pertenue, which in turn mutated to produce T. pallidum.

Miao and Fieldsteel (3) have reported that the Nichols and KKJ strains of T. pallidum and the Gauthier strain of T. pertenue were genetically identical. They noted that although there was no detectable difference in the genomes of these two organisms, the limit of resolution from the saturation reassociation assays was 2% of the genome (1.8 X 10^6 daltons). This is large enough to possibly code for 180 proteins. Therefore, they warned that even if identical genomes existed, the same biochemical functions might not be expressed in the three pathogens.

Schell et al. (4) have reported that hamsters inoculated with T. pallidum Bosnia A were substantially resistant to infection with T. pallidum, Nichols and Bosnia A, and T. pertenue. Hamsters inoculated with T. pertenue displayed little resistance to infection with T. pallidum Bosnia A and those inoculated with T. pallidum Nichols displayed little resistance to infection with T. pertenue. This suggests that T. pallidum Bosnia A expresses antigens in common with or similar to those of both T. pallidum Nichols and T. pertenue that the latter two do not share with Bosnia A. Thus the in vitro cultivation of T. pallidum Bosnia A could be very important in...
the development of a vaccine that could provide protection against many strains of *T. pallidum* and *T. pertenue*.

Previously we reported the cultivation of the virulent *T. pallidum* (Nichols) in tissue cultures of SfIEp cells (5) and, in a supplementary article, some improvements in cultivation techniques (6). Next we attempted to cultivate other virulent strains of *T. pallidum* and also virulent strains of *T. pertenue* using the same cultivation techniques as used for *T. pallidum* (Nichols). This report describes those attempts.

**MATERIALS AND METHODS**

The rabbits used for passage of strains of *T. pallidum* and *T. pertenue* were 6 to 8-mo-old New Zealand white males housed at 16 to 18°C. They were free of treponemal infection as determined by Venereal Disease Research Lab (VDRL) tests. The virulent *T. pallidum* used were the Nichols, KKJ, Mexico A, and Bosnia A strains. The virulent *T. pertenue* used were the Gauthier and FB strains. The Nichols strain of *T. pallidum* was harvested after 12 d of infection. However, the other strains of *T. pallidum* and *T. pertenue* were harvested after 28 d of infection. For tissue culture studies, freshly harvested treponemal suspensions were prepared as previously described (5).

Treponemal infections of rabbit testis with strains other than Nichols are dramatically different from those with the Nichols strain. The orchitis produced by these infections is delayed and not as pronounced as those caused by the Nichols strain. Routinely, two rabbits were infected with the Nichols strain of *T. pallidum* for each experiment run. However, because there is a greater variability in the infections caused by the other strains of *Treponema*, three rabbits were routinely used for each strain to ensure that two develop adequate infections. We did not attempt to cultivate more than three strains of *T. pallidum* in one experiment because of the difficulties of harvesting and processing several inocula from so many rabbits.

BRMM (5) was prepared as previously described (6). Briefly, BRMM consisted of a modified Eagle’s MEM containing 10 mg/100 ml each of pyruvic acid and dithiothreitol (DTT), 20% fetal bovine serum (FBS), and a 1:30 dilution of fresh rabbit testis extract.

An established cell line used in these experiments was the SfIEp (epidermis, cottontail rabbit) line (American Tissue Culture Collection, Rockville, MD). In one experiment, a primary cell culture of nude mouse ear fibroblast (NME, initiated in our laboratory) was also used. The passage levels utilized were: SfIEp, 74 to 95; NME, 9 to 11. Tissue culture monolayers were established, infected, and harvested as previously described (5). The enumeration of the treponemes in the harvested suspensions was performed using dark-field microscopy.

**RESULTS AND DISCUSSION**

Several experiments were conducted to cultivate the four strains of *T. pallidum* (Table 1). In each experiment, the Nichols strain of *T. pallidum* grew significantly better than any other strain. The average growth of the Nichols strain in the five experiments was 29.4-fold. The next best growth was produced by the KKJ strain, followed by the Mexico A and Bosnia A strains, respectively. The maximum obtained with the KKJ strain was 14.4-fold, which was about half that observed with the Nichols strain. The average growth of the KKJ strain from the three experiments conducted was 10.8-fold and over 50% of the treponemes remained motile after 12 d of incubation. The Mexico A and the Bosnia A strains of *T. pallidum* averaged 9.1 and 7.5-fold increases, respectively. However, the range of growth seemed to be more dramatic; from 12.8 to 5.6-fold for the Mexico A strain and from 11.3 to 3.6-fold for the Bosnia A strain.

The growth of each of these strains was less than that of Nichols, probably because each strain has its own set of optimal conditions. Therefore, logically, the Nichols strain grew better because the medium and experimental conditions had been designed specifically for this strain. Although the other strains did not grow as well, they seemed to possess growth requirements close to those of the Nichols strain. In at least one experiment, each strain grew about 12-fold or more. It is also interesting to note that the number of motile treponemes in the Nichols cultures remained around 90% for 10 d. However, in none of the other cultures was the motility maintained at 90% even for 6 or 7 d.

Fieldsteel et al. (7) reported that *T. pallidum* (KKJ) and (Nichols) attached to SfIEp cells in a vertical oxygen gradient culture. There were concurrent increases of attached treponemes; however, the increases observed in cultures inoculated with *T. pallidum* (KKJ) were greatest at depths of 11 and 17 mm, compared with 17...