INTRODUCTION

In recent years different groups have developed ELISA-type diagnostic kits for syphilis, based on the use of recombinant proteins from Treponema pallidum (1). The sensitivity and specificity of the ELISAs for the detection of syphilis is comparable to those of the most widely used methods (2). This paper describes the cloning of a 42 kDa T. pallidum inner membrane Ag (TmpA) (3), and its expression in E. coli, under the tryptophan promoter. High expression levels were achieved using a 58 aa sequence of human interleukin-2 as stabilizer. A of study of this recombinant TmpA by ELISA against a panel of VDRL+ and VDRL- sera revealed high sensitivity and specificity, indicating that this antigen is a good option for the diagnosis of syphilis.
MATERIALS AND METHODS

The gene in question was obtained through PCR, using as substrate DNA from *T. pallidum* isolated from the lymphatic fluid of a patient. The gene was inserted in an expression vector controlled by the tryptophan promoter, using a 174 bp human IL-2 stabilizer and the T4 phage transcription terminator (4). Induction of expression was performed in M9 salts minimum medium. Protein purification was done by electroelution and purity was calculated by densitometry of SDS-PAGE. The purified recombinant TmpA was evaluated against a panel of 92 VDRL+ sera, 31 sera weakly reactive to VDRL, and 274 VDRL- sera. ELISA plates were coated with 100 mL of antigen at a concentration of 5 mg/mL. A protein A-HRPO conjugate was used. OPD was employed as substrate. Sera that did not match by ELISA and VDRL were studied using a hemagglutination confirmation kit (Fujirebio Inc.).

RESULTS AND DISCUSSION

The protein was highly expressed soluble and cytoplasmic (ca. 30% of total bacterial protein). Previously, all the proteins expressed with this vector had been produced insoluble and forming inclusion bodies (4). We hypothesized that being TmpA a bacterial protein, its structure is more prone to adopt a correct conformation in the *E. coli* cytoplasmic environment, without insolubilization. The protein obtained by electroelution was 95% pure. The ELISA developed with this electroeluted antigen showed 99.2% of specificity for the VDRL- samples and 100% sensitivity with VDRL+ samples. These results indicate that the recombinant TmpA is a good option for the diagnosis of syphilis by ELISA.

REFERENCES


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