CLONING, EXPRESSION AND CHARACTERIZATION OF THE P64k OUTER MEMBRANE PROTEIN FROM *N. meningitidis.*

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INTRODUCTION

Meningococcal meningitis as an endemic disease occurs with a frequency of approximately 0.5-5 per 100 000 per year in a great part of the world. Epidemic disease has been reported from all continents in the last few years.

The serogroups A, B and C are the main cause of the disease. The group B *N. meningitidis* polysaccharide (PS) in contrast to group A, C, and others is a poor immunogen in humans due to immunological tolerance and structural similarities that have been found in developing fetal brain tissue (1-2). Therefore, the strategy of vaccine against serogroup B should be based in strains of *N. meningitidis*, could be an effective approach to confer protection against meningococcal disease in humans.

EXPERIMENTAL PROCEDURES

Restriction enzymes, T4 DNA ligase, Klenow fragment of DNA polymerase I and Exonuclease III were obtained from Heber Biotec (Cuba). The oligonucleotides and the adapter were synthesized at Heber Biotec. The [α^{32}-P]dATP, the Multiwell microtitre plate DNA sequencing system-T7 DNA plolymerase, peroxidase-conjugated streptavidin and anti-human, rabbit and mouse biotinilated antibodies were purchased from Amersham (UK). The chemicals were of the highest grade commercially available. All the procedures were performed following manufacturing instructions or as in (3). The complete amino acid sequence of P64k was obtained using automatic sequencing and mass-spectrometry.

RESULTS AND DISCUSSION

The gene M-6, encoding the high molecular weight protein P64k, was isolated from a genomic library of the *N. meningitidis* strain B385, constructed in the EMBL-3 vector. This library was screened using a rabbit hyperimmune serum raised against a purified fraction of high molecular weight proteins. The clone 31 was selected for further study on western blotting, with a pool of sera from convalescent people to meningococcemia.

The DNA insert containing the entire M-6 gene was subcloned and sequenced. Then the M-6 gene was expressed in *E. coli* as a fusion protein with the N-terminal of interleukine-2, under the control of tryptophan promoter. P64k was also produced as a non-fusion protein.

The expression of P64k was shown on PAGE and Western-blotting using rabbit polyclonal antibodies against the whole outer membrane mixture from *N. meningitidis*. MAbs obtained against the recombinant protein recognized the natural protein on Western-blotting. The recombinant protein is also recognized by serum from convalescent patients to meningococcal disease.

The MAb 114 was assayed in ELISA with a panel of 85 *N. meningitidis* strains. The protein was recognised in 81 strains (95.3%). The strains that were not recognised, were neither epidemic nor isolates from systemic disease. The predicted amino acid sequence of the M-6 gene showed complete homology when compared with the amino acid sequence from the recombinant protein.

The P64k protein has been expressed in *E. coli*. None of other antigens: Tbp1, class 1, class 3 and class 5C has been possible to express as a non-fusion protein at high levels, under the control of tryptophan promoter in our laboratory.

REFERENCES

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