THE *Neisseria meningitidis* OUTER MEMBRANE PROTEIN P1 PRODUCED IN *Bacillus Subtilis* AND REFOLDED *in vitro* AS A VACCINE CANDIDATE.

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INTRODUCTION

Single component vaccine have many advantages: low probability of adverse reactions and high immunogenicity, with the antibody response directed to the protective component. It is essential, however, that the component is chosen correctly and presented in a form preserving the native three-dimensioned structure of the epitopes that serve as targets of protective antibodies.

There is definitive clinical need for efficacious vaccines against *Neisseria meningitidis* of group B (MenB). Previous work had established that the capsular polisacharide of MenB is unlikely to serve as a basic of a vaccine because of its structural and antigenic similarity with certain glycoproteins in human tissues (Finne *et al.*, 1983). In order to identify a potential alternative vaccine component, our laboratory has developed an experimental animal model for MenB disease (Saukkonen *et al*, 1987). In this model we could show that antibodies to the class 1 protein (P1) of the meningococcal outer membrane were protective.

Because of the anticipated difficulties in purification of the P1 protein from MenB, especially in removal of the endotoxin (LPS) associated with it, we expressed P2 in a heterologous host, the Gram-positive, therefore LPS-free, *Bacillus subtilis* by methods developed in our laboratory (Palva *et al.*, 1982). The protein, called BacP1, was produced in high yield as inclusion bodies (Nurminen *et al.*, 1992).

When the protein was solubilised in urea or SDS and to immunize mice, the antibodies elicited did, however, not bind to meningococci. We belived that this was due to the denatured conformation of the protein under these conditions, and procceded to find ways of refolding it so that native-like epitopes were fermed. We initially succededin this by refolding in the presence of LPS (Nurminen *et al.*, 1992). Because of the undesirability of LPS in a vaccine we then looked for alternative procedures, and report here that BacP1 can be reconstituted into phospholipid vesicles (liposomes) which provoke antibodies that are bactericidal for MenB bacteria and protective in the infant rat model of meningitis.

EXPERIMENTAL PROCEDURE

BacP1 was expressed in *B. subtilis* from a bacillar promoter; the peptide consisted of the precise sequence of the mature P1 of MenB, precceded at the N terminus by 11 amino acids encoded by the linker and a truncated bacillar signal sequence (Nurminen *et al.*, 1992). The protein was solubilized with SDS, and then diluted into an excess of octylglucoside (OG). Micelles of phosphatidyl choline were prepared in OG and added, and the mixture subjected to dialysis or gel filtration. This procedure led, as expected (Eisele and Rosenbusch, 1990) to the formation of unilamellar liposomes upon removal liposomes (at 1-20 μg protein/dose, two subcutaneous doses). The sera were analyzed by enzyme immunoassay (EIA) with denatured BacP1 or native meningococcal cells (MenB) as antigen. They were also analyzed for bactericidal activity towards MenB bacteria and for their ability to confer passive protection in the meningitis model (Saukkonen *et al.*, 1987).

RESULTS AND DISCUSSION

The liposomes were highly immunogenic without the need of adjuvants. Analysis of the sera of the immunized mice indicated the presence of native/like epitopes in the BacP1 in the liposomes: the reacted to a high titer with native MenB bacterial both in EIA and bactericidal assays, and were protective in the experimental infection model. All these reactions were specific to the subtype of the P1 of the meningococci from which the gene was derived (P1.7,16 or P1.15), further indicating the formation of the specific surface epitopes (Van der Ley *et al.*, 1991).

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