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

Pirjo H. Makela, Sarah Butcher, Ilona Idanpan-Heikkila, Marjatta Nurminen, Susanna Mutilaine, Kate Runeberg-Nyman, Matti Srvas and Eva Wahistr.

National Public Health Institute, Mannerheimintie 166, FIN/00300 Helsinki, Finland.

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 polysaccharide 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 believed that this was due to the denatured conformation of the protein under these conditions, and proceeded to find ways of refolding it so that native-like epitopes were formed. We initially succeeded in 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, preceded 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 phosphatidylcholine 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).

REFERENCES