Human bactericidal antibody response to meningococcal outer membrane protein vaccines

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Abstract. Several different meningococcal outer membrane protein vaccines have been prepared and used in human safety and immunogenicity studies. The results of these studies have led to some general conclusions regarding the human antibody response to these vaccines. A review of these conclusions, however, indicates that a number of important questions and problems still need to be addressed. Two of these are the determination of the protective level of bactericidal antibody in human serum and the impact of phase variation of surface antigens on vaccine strategy. Bactericidal assays using intrinsic complement and high concentrations of serum suggest that the level of natural immunity to group B meningococci is quite high, but is increased by vaccination with outer membrane protein vaccine. Phase variation in meningococcal surface antigens including capsule, class 1 protein, class 5 protein, and lipopolysaccharide was demonstrated using colony blotting with monoclonal antibodies. Phase variation resulted in differences in susceptibility to the bactericidal activity of human sera.

Introduction

Most of the meningococcal OM protein vaccines that have been tested for safety and immunogenicity in human volunteers have consisted of noncovalent complexes of lipopolysaccharide(LPS)-depleted OM proteins and capsular polysaccharide(s) (Frasch et al. 1985, 1986; Zollinger et al. 1979, 1980, 1985, 1986; Froholm et al. 1986). The binding of the polysaccharide to the proteins is hydrophobic in nature and is thought to be mediated by a lipid moiety attached to the end of the polysaccharide (Gotschlich et al. 1981). The binding of capsular polysaccharide to the OM proteins results in their solubilization and in a significant enhancement of their immunogenicity (Zollinger et al. 1980). Several different methods have been used to prepare group B OM protein vaccine and have resulted in products with variable amounts of residual LPS (2–15% relative to protein) and different relative amounts of the class 1, class 2 or 3, and class 5 major outer membrane proteins. In spite of such variations, a number of general characteristics of the human antibody response to meningococcal outer membrane protein vaccines have emerged. Although a considerable amount of data that has accumulated, many important questions regarding the production,
standardization, and effectiveness of meningococcal OM protein vaccines persist. The studies we report here relate to several of these questions.

Methods

Bactericidal assays were performed as described (Zollinger et al. 1986a) except that when exogenous complement was not used the reaction mixture contained 10–80 μl serum (handled to preserve complement), 20 μl organisms (about 1000 cells), and buffer with 1% gelatin to a total volume of 100 μl.

Colony blots were done using monoclonal antibodies specific for the antigen of interest (Zollinger et al. 1984). After one to two days of growth, isolated colonies of the strain to be tested were blotted onto nitrocellulose filter disks. The disks were then reacted with monoclonal antibody (Zollinger et al. 1984) and developed using alkaline phosphatase or horseradish peroxidase-conjugated anti-mouse IgG, IgA, and IgM (Kirkgaard & Perry Laboratories, Inc., Gaithersburg, Maryland, USA) and an appropriate substrate system e.g. Naphthol mx and Fast Red for alkaline phosphatase or 4-chloro, 1-napthol and hydrogen peroxide for the peroxidase.

Results and discussion

One of our goals has been to attempt to prepare immunogenic OM protein vaccines that are substantially free of LPS (less than 1%). These studies have led to the development of new methods for the isolation of OM proteins which result in much reduced LPS levels (0.4% to 2%) and equivalent immunogenicity. We have previously reported on human studies with OM proteins prepared in this way and complexed to a tetravalent ACYW mixture of capsular polysaccharides (Zollinger et al. 1984, 1986; Frøholm et al. 1986). Additional studies with sera from one of these studies were done to determine the levels of bactericidal activity in normal adult sera and the bactericidal antibody response to vaccination using only the intrinsic complement in the individual's serum.

The bactericidal activity of a series of 54 normal human sera was determined for two different group B strains as a function of serum concentration over the range from 10% to 80% serum. The sera had been quick frozen and stored at −70°C to preserve complement activity. No exogenous complement was added. The percentage of organisms killed after 1 h at 37°C is plotted in Fig. 1 against the percentage of serum in the assay. The values for the individual sera as well as the mean are given. It is apparent that as the serum concentration approached 100% a high percentage of the sera were able to kill all the organisms. The percentage of sera that killed at least 95% of the organisms was significantly greater