CLINICAL TRIALS OF SHIGELLA VACCINES IN ISRAEL

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1. INTRODUCTION

Shigellosis or bacillary dysentery is caused by organisms belonging to genus *Shigella*, divided into four species (*S. dysenteriae*, *S. boydii*, *S. flexneri* and *S. sonnei*). With the exception of *S. sonnei* which has a single serotype, each species is divided into several serotypes according to the O-polysaccharide antigen of the cell wall (*S. dysenteriae* has 12 serotypes, *S. flexneri* has 6 serotypes, and *S. boydii* has 18 serotypes). *Shigella* spp. are invasive organisms that penetrate into the enterocytes of the colon epithelium, escape very quickly from the phagocytic vacuole and multiplicate intracellularly. Although non-motile, shigellae can move on an actin skeleton and spread to adjacent cells. The inflammatory process is usually limited to the lamina propria and does not involve the spread of *Shigella* deeper, into the submucosa. Pathogenesis in *Shigella* spp. is associated with a constellation of genes encoded on both the chromosome and a large 140 MDa virulence plasmid. These genes can be divided into two groups: regulatory genes and structural genes. The 140 MDa plasmid encodes for all the genes essential for invasion of *Shigella* into the epithelium of the colon. Regulatory genes are located on the virulence plasmid or on the chromosome.

The initial manifestations of acute shigellosis are usually fever, malaise, abdominal pain and watery diarrhea. The disease may progress, with the appearance of tenesmus, and of blood and mucus in the stool. Infection caused by *S. dysenteriae* type 1 and sometimes by *S. flexneri* may cause severe protein-loss. Extra-intestinal complications may include reactive arthritis, toxic megacolon, bacteremia and hemolytic-uremic syndrome. Shigellosis is endemic throughout the world. The disease is hyperendemic in developing countries. The annual global incidence of shigellosis is around 200 million cases, with more than 650,000 fatal cases of the disease (Institute, 1986).
Israel is highly endemic for shigellosis, having a reported incidence of disease about 20-30 times higher than that of the United States (Green, 1991). Young children, mainly of nursery school age, appear to be particularly vulnerable to the disease, which accounts for approximately 600 pediatric admissions per year. The use of antibiotic treatment in shigellosis is becoming more and more problematic in Israel, since clinical isolates are becoming increasingly resistant to antimicrobial agents (Smollan, 1992; Ashkenazi, 1993). Shigellosis causes about five deaths in Israel each year, typically in the pediatric and geriatric age groups (Green, 1991). In addition to children, soldiers serving under field conditions constitute another risk group for shigellosis (Green, 1987; Cohen, 1991). Of 420 laboratory-investigated outbreaks of diarrhea occurring in the Israel Defence Force between 1985 and 1993, 181 (43%) were caused by Shigella organisms. The relative importance of S. sonnei and S. flexneri as etiologic agents of epidemic shigellosis in the IDF is similar, each of these species being responsible for about 45% of these outbreaks. S. boydii and S. dysenteriae are involved in the rest of the outbreaks. The very low infectious dose of Shigella, of about 10^2-10^3 organisms (DuPont, 1989), and difficult field conditions facilitate the transmission of the pathogen, explaining the partial failure of routine sanitary and hygienic measures to prevent the sporadic and epidemic occurrence of shigellosis among Israeli military populations (Green, 1987; Cohen, 1991). In such circumstances, effective vaccination appears to be the only reliable means of preventing outbreaks.

2. CURRENT STRATEGIES IN THE DEVELOPMENT OF SHIGELLA VACCINES

Candidate Shigella vaccines developed in the last five decades have not reached an acceptable level of safety and efficacy. There are neither licensed vaccines for it, nor consensus on the relative importance of the host components responsible for protective immunity to shigellosis. Since there is evidence that shigellosis confers protection against recurrent disease due to the homologous Shigella group (Cohen, 1988; Herrington, 1990), it has been assumed that an efficient Shigella vaccine should induce, to a comparable extent, the same immune mechanisms as natural infection does, without causing the symptoms of diarrhea or bacillary dysentery. New generations of live-attenuated or subunit vaccines are currently employed to deliver the O-polysaccharide, the protective antigen of Shigella.

Formal and co-workers constructed an E. coli K12-S. flexneri 2a hybrid vaccine by conjugal transfer of the 140 MDa S. flexneri invasion plasmid and genes encoding for the S. flexneri 2a LPS into an E. coli K12 recipient (Formal, 1984; Newland, 1992). Lindberg and his group in Sweden induced aro mutations in Shigella spp. wild strains and obtained auxotrophic live-attenuated Shigella vaccines which showed a good level of safety and immunogenicity in humans (Lindberg, 1990; Li, 1993). Sansonetti and coworkers reached attenuation of S. flexneri 5 strain by a deletion in the plasmid ics (encoding for cell-to-cell spread) followed by an insertional mutation in the chromosomal iuc (encoding for aerobactin) (Sansonetti, 1989). Recently, deletion mutations in the chromosomal gene aroA and plasmid gene virG were induced in a wild-type S. flexneri 2a by Noriega and coworkers to engineer an oral vaccine prototype capable of penetrating the intestinal epithelial cells but incapable of extensive intracellular replication (Noriega, 1994). In view of previous studies among Israeli recruits, showing a strong association between serum anti-LPS IgG antibodies and protection against shigellosis, Robbins, Schneerson and co-workers developed at NIH Shigella conjugate vaccines capable of inducing high levels of such antibodies when administered parenterally (Robbins, 1992; Chu, 1992; Taylor, 1993). A subunit Shigella vaccine composed of S. sonnei or S. flexneri 2a hydrophobically-complexed with pro-