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**USE OF *SHIGELLA* INVAPLEX TO
TRANSPORT FUNCTIONAL PROTEINS AND
TRANSCRIPTIONALLY ACTIVE NUCLEIC
ACIDS ACROSS MAMMALIAN CELL
MEMBRANES IN VITRO AND IN VIVO**

This application is based on provisional application Ser. No. 60/524,639 filed on Nov. 25, 2003. The content of the provisional application are expressly incorporated herein by reference.

FIELD OF THE INVENTION

The present invention provides for the in vivo and in vitro use of Invaplex to transport materials including functional proteins and biologically active nucleic acids across mammalian cell membranes compositions. The present invention also relates to adjuvants, immunogenic compositions and methods useful for polynucleotide-based vaccination.

BACKGROUND OF THE INVENTION

Invaplex as a Vaccine

The pathogenesis of *Shigella* spp. is attributed to this organism's ability to invade, replicate intracellularly, and spread intercellularly within the colonic epithelium. Several highly conserved, virulence plasmid-encoded proteins, called the invasion plasmid antigens (IpaA, IpaB, IpaC, and IpaD) (1), are essential participants in the invasion process. Upon contact or attachment to host cells, the *Shigella* invasins are released (22) by a type III secretion apparatus (8) and induce a phagocytic event resulting in engulfment and internalization of the bacterium by the host cell (7). The active components include an IpaB:IpaC complex that integrates into the host cell membrane, forming a channel by which other *Shigella* proteins gain entry into the host cell (16).

Recently, we have isolated an invasin protein-LPS complex from intact, virulent *Shigella* cells (20). The invasin complex or Invaplex is the subject of several issued or pending WRAIR patents (11-14). The invasin complex (Invaplex) binds to surfaces of epithelial cells and quickly becomes internalized, presumably by an endocytic process (Oaks and Kaminski, unpublished data). The ability to bind to a eukaryotic host cell surface and induce a phagocytic event indicates that the Invaplex maintains an active, native virulence structure similar to that found on the surface of invasive *Shigella*. In fact, many of the key, antigenic components found in Invaplex 24 and Invaplex 50 (see below) are located on the *Shigella* surface. These antigens include IpaB, IpaC, IpaD and LPS (all of which are present in both Invaplex 24 and Invaplex 50) and also the newly described protein antigens 72 kDa, 84 kDa, and a 60 kDa protein found exclusively in Invaplex 50 (Oaks & Turbyfill, unpub data). More recently a highly purified form of Invaplex (HP Invaplex) has been isolated by size-exclusion chromatography (SEC). The HP Invaplex 24 consists of IpaB, IpaC and LPS and has an estimated mass of about 1 MDal. Experiments in mice have determined that the *S. flexneri* HP-Invaplex 24 or HP-Invaplex 50 offer enhanced immunogenicity and efficacy over the parent Invaplex indicating that the active components of Invaplex are, at a minimum, IpaB, IpaC and LPS.

The ability to isolate a putative native surface structure such as Invaplex, which exhibits activities and immunogenicity similar to invasive shigellae, has significant implications in vaccine design and development. First, the putative native structure may enhance delivery to the appropriate portal of entry (M-cells), similar to that targeted by live-attenuated

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vaccine strains. In the case of Invaplex, this has allowed the use of relatively small doses for intranasal immunization due to its delivery efficiency. Similar to live, attenuated vaccines, immunization with the isolated subcellular, native Invaplex structure that contains all known *Shigella* antigens results in an immune response equivalent to that produced during natural infection, including recognition of epitopes found only in native structures. The immunity stimulated by Invaplex is highly protective in mice and guinea pigs (12, 20).

10 Invaplex as an Adjuvant

The delivery of antigens in a manner, which safely stimulates a protective mucosal immune response, is critical to the successful development of enteric vaccines. As an alternative to live attenuated vaccines, which are often difficult to construct, standardize and deliver without the risk of side effects, subunit vaccines offer the promise of chemically defined, well-standardized products. Unfortunately, the rapid increase in potential subunit vaccines arising from recombinant, synthetic, or subunit purifications, including both protein and DNA vaccines, has outpaced the ability to deliver these novel vaccines safely and effectively. A major obstacle has been the failure to develop an adjuvant, which effectively stimulates the mucosal immune system in a safe, non-toxic manner. One highly effective mucosal adjuvant is cholera toxin (CT), another is *E. coli* heat-labile enterotoxin (LT); unfortunately, both are extremely toxic molecules that require substantial detoxification by genetic manipulations. Before genetically modified forms of CT or LT become available as adjuvants for human use, they will require extensive safety testing.

A unique property of the Invaplex is that it enhances the immune response to substances that are not very immunogenic (11). The adjuvanticity of Invaplex has been demonstrated with several proteins including ovalbumin, the recombinant Sta56 (56K) protein of *Orientia tsustusugamushi*, the FlaA protein of *Campylobacter jejuni*, colonization factors of enterotoxigenic *E. coli* and the PA (protective antigen) of *Bacillus anthracis* (Oaks and Kaminski, unpublished data). The immunogenicity and adjuvanticity of the Invaplex is likely due to its ability to target and induce uptake by immune cells, possibly M cell equivalents in the mucosa. Stimulation of a mucosal immune response often requires uptake of the antigen or pathogen by M cells in the gut or comparable cells in other mucosal tissue. The M cells lie over an area of cells called the mucosa associated lymphoid tissue (Peyer's patches in the gut). Upon uptake of antigen, the M cell is capable of translocating the antigen to the lymphoid tissue consisting of lymphocytes, macrophages, and dendritic cells. These cells serve to present the antigen to antigen-specific lymphocytes resulting in stimulation, expansion, and expression of specific immune effectors. In the mucosa, this process leads to the development of IgA-producing B cells.

The adjuvanticity of Invaplex is likely mediated by IpaB and IpaC, which are crucial virulence proteins involved in the invasiveness of *shigellae*. Very little is known about the effect Invaplex has on host cells.

DNA Transfection and DNA Vaccines.

The process of delivering transcriptionally active DNA into eukaryotic cells is called transfection. The result of transfection is heterologous gene expression in vitro or in vivo. Transfection is often used as a means to study the function of specific proteins expressed in the transfected cell. Although physical methods such as microinjection and electroporation can be used to shuttle DNA into eukaryotic cells, methods more amenable to in vivo work have been developed. Transfection of cells in vivo has extended the in vitro functional analysis into live animals and has also allowed the immunogenicity of the expressed protein to be evaluated if the levels