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Review

Mucosal adjuvants

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Abstract

Induction of immune responses following oral immunization is frequently dependent upon the co-administration of appropriate adjuvants that can initiate and support the transition from innate to adaptive immunity. The three bacterial products with the greatest potential to function as mucosal adjuvants are the ADP-ribosylating enterotoxins (cholera toxin and the heat-labile enterotoxin of *Escherichia coli*), synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG ODN), and monophosphoryl lipid A (MPL). The mechanism of adjuvanticity of the ADP-ribosylating enterotoxins is the subject of considerable debate. Our own view is that adjuvanticity is an outcome and not an event. It is likely that these molecules exert their adjuvant function by interacting with a variety of cell types, including epithelial cells, dendritic cells, macrophages, and possibly B- and T-lymphocytes. The adjuvant activities of CpG and MPL are due to several different effects they have on innate and adaptive immune responses and both MPL and CpG act through MyD88-dependent and -independent pathways. This presentation will summarize the probable mechanisms of action of these diverse mucosal adjuvants and discuss potential synergy between these molecules for use in conjunction with plant-derived vaccines.

Keywords: Adjuvant; Oral immunization; Enterotoxin; CpG; MPL

Contents

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1. Introduction

Recently, a great deal of effort has been directed towards replacement of existing whole cell or formalin-inactivated vaccines with subunit vaccines that may be safer and more effective than existing vaccines. Still other efforts are directed at developing alternatives to traditional vaccine delivery, including mucosal (oral) delivery of plant-derived vaccines. Mucosally delivered vaccines offer a number of potential advantages over traditional vaccines including (1) the potential to confer mucosal as well as systemic immunity, (2) increased stability, (3) increased shelf-life, and (4) elimination of needles and the need for specially trained healthcare specialists to administer vaccines. A major limiting factor for the development of plant-derived oral vaccines is the availability of safe, effective adjuvants that function mucosally.

Induction of immune responses following mucosal immunization is usually dependent upon the co-administration of appropriate adjuvants that can initiate and support the transition from innate to adaptive immunity. While a number of substances of bacterial origin have been tested as mucosal adjuvants, the three bacterial products with the greatest potential to function as mucosal adjuvants are the ADP-ribosylating enterotoxins (cholera toxin (CT), produced by various strains of *Vibrio cholerae*, and the heat-labile enterotoxin (LT) produced by some enterotoxigenic strains of *Escherichia coli* [1–5]), synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG ODN) [6], and monophosphoryl lipid A (MPL) [7–9]. There are a limited number of reports of these or related adjuvants having been examined in conjunction with plant-derived vaccines.

The mechanism of adjuvanticity of the ADP-ribosylating enterotoxins is the subject of considerable debate. Our own view is that adjuvanticity is an outcome and not an event. It is likely that these molecules exert their adjuvant function by interacting with a variety of cell types, including epithelial cells, dendritic cells, macrophages, and possibly B- and T-lymphocytes. This complex and dynamic interaction changes the context in which antigen is processed and presented during the initiation phase of the immune response. LT and CT elevate intracellular cAMP in a variety of cell types and their adjuvanticity is at least, in part, related to that function. The adjuvant activities of CpG and MPL are due to several different effects they have on innate and adaptive immune responses and both MPL and CpG act through MyD88-dependent and -independent pathways. Below we summarize the probable mechanisms of action of these diverse mucosal adjuvants and discuss potential synergy between these molecules for use in conjunction with mucosal vaccines.

2. Mucosal immunization

The first productive interaction between most infectious agents and the human host is with mucosal surfaces, specifically, the nasal, oropharyngeal, respiratory, genitourinary, and gastrointestinal mucosa. Traditional vaccine strategies that involve parenteral immunization with inactivated viruses or bacteria or subunits of relevant virulence determinants of those pathogens do not prevent those initial interactions. In fact, traditional vaccine strategies do not prevent infection but instead resolve infection before disease ensues. In some cases, once the pathogen crosses the mucosal surface and enters the host cell, be that a macrophage, a dendritic cell, an epithelial cell, or a T-cell, the host–parasite relationship is moved decidedly in favor of the pathogen. Moreover, many bacterial toxins bind to and interact with mucosal epithelial cells, in which case significant damage to the host may ensue before serum antibodies can play a role in protection.

A great deal of attention has focused on mucosal immunization as a means of inducing secretory IgA (S-IgA) antibodies directed against specific pathogens of mucosal surfaces. These antibodies may block attachment of bacteria and viruses, neutralize bacterial toxins, and even inactivate invading viruses inside of epithelial cells. In addition, the existence of a *Common Mucosal Immune System* permits immunization on one mucosal surface to induce secretion of antigen-specific S-IgA at distant mucosal sites. Mucosal immunization can be an effective means of inducing not only S-IgA, but also systemic antibody and cell-mediated immunity, and frequently requires less antigen and fewer doses than does parenteral immunization.

3. Mucosal adjuvants

Different strategies have been developed to facilitate and enhance the immune response obtained after mucosal immunization, including the use of attenuated mutants of bacteria (i.e., *Salmonella* spp., *Shigella* spp.) as carriers of heterologous antigens, encapsulation of antigens into microspheres, gelatin capsules, different formulations of liposomes, adsorption onto nanoparticles, use of lipophilic immune stimulating complexes, and addition of bacterial products with known adjuvant properties. As mentioned above, the three bacterial products with the greatest potential to function as mucosal adjuvants are the ADP-ribosylating enterotoxins, LT and CT [1–5]), synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG ODN) [6], and monophosphoryl lipid A [7–9]. Our laboratory has extensively studied the use of ADP-ribosylating enterotoxins as adjuvants and this review will principally focus on the mechanisms of toxicity and adjuvanticity of those molecules.

3.1. ADP-ribosylating enterotoxins as adjuvants

In order to understand the adjuvant properties of the ADP-ribosylating enterotoxins, it is important to first understand the mechanisms through which these molecules function as enterotoxins. Both LT and CT are synthesized as multisubunit toxins with A and B components. The A-subunit is the