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Review

Non-toxic derivatives of LT as potent adjuvants

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ABSTRACT

The heat-labile enterotoxin of *Escherichia coli* (LT) consists of an enzymatically active A subunit (LTA) and a pentameric B subunit (LTB). LT has been extensively studied as a potent modulator of immune responses but wild-type LT is toxic and therefore unsuitable for clinical use. Approaches pursued to avoid the toxicity associated with the use of the native toxin while retaining its adjuvant properties have included isolation of subunit B (LTB) and construction of non-toxic LT AB complex mutants, such as LTK63 mutant. Here we review the immunomodulatory characteristics of LTB and LTK63 and their potential as mucosal and parenteral vaccine adjuvants.

Contents

1. Introduction

Vaccination is undoubtedly the intervention with the greatest impact on global health [1]. New generation vaccines, particularly subunit vaccines, based on recombinant or purified proteins and synthetic peptides, are less reactogenic but poorly immunogenic in comparison with whole-organism vaccines which contain many immunostimulatory components. Thus, subunit vaccines are typically administered with adjuvants to amplify and direct vaccine-specific immunity [2,3].

Adjuvants are defined as pharmacological or immunological agents that can stimulate the immune system and increase, modulate and/or prolong the intrinsic immunogenicity of co-administered antigens, thereby enhancing vaccine efficacy. The word "adjuvant" comes from the Latin word *adjuvare*, meaning to help or aid [4]. Adjuvants are a heterogeneous group of compounds and can be divided in two groups according to their dominant mechanisms of action. They can operate via activation and potentiation of innate immunity either directly or via pattern-recognition receptors (PRRs) to generate robust and long-lasting adaptive immune response. Alternatively, delivery systems may concentrate and display antigens in repetitive patterns, target vaccine antigens to antigen-presenting cells (APCs)

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and help localize antigens and immune potentiators to ensure that the vaccine is delivered to the right place at the right time [5,6]. These activities are not mutually exclusive and some adjuvants exhibit both properties [7]. In addition, immune polarization of the vaccine-induced response is an important consideration [8].

A range of compounds including emulsions, saponins, bacterial products, Toll-like receptor (TLR) agonists, nucleic acids, virosomes, liposomes and a combination of some of them have been shown to display potent adjuvant activity in animal models [2,3,5,7]. However, only a few vaccine adjuvants are licensed for use in humans. For more than 70 years, aluminum mineral salts was the only vaccine adjuvant approved worldwide for clinical use and remained the only one licensed for human use by the U.S. Food and Drug Administration (FDA) until 2009. In the past decade, two oil-in water emulsions (MF59 and AS03), one recombinant toxin (cholera toxin B subunit, CTB), a virosome (immunopotentiating reconstituted influenza virosomes, IRIV) and one TLR-4 agonist (monophosphoryl lipid A formulated in aluminum hydroxide, AS04) were licensed by the European Medicines Agency (EMA) as vaccine adjuvants for human use. Recently, the USA FDA approved the adjuvant AS04 for clinical use [2,3,9]. The need for new adjuvants has been led mainly by the shortcoming of the currently approved ones in eliciting the desired immune response against different target pathogens.

A number of novel adjuvants are in development and evaluation and some have demonstrated profound effects on vaccine potency in preclinical models. These include synthetic TLR agonists, such as unmethylated viral or bacterial CpG DNA and oligonucleotides [10], lipopeptides such as tripalmitoyl-S-glyceryl cysteine (Pam3Cys) [11], water-in-oil emulsions such as Montanide ISA 720 [12], saponins such as QS21 [7], and bacterial enterotoxins such as the heat-labile enterotoxin from *Vibrio cholerae* (cholera toxin, CT) and *Escherichia coli* (LT) [13–15].

Of particular interest are products of the heat-labile enterotoxin of *E. coli* (LT) including subunit B (LTB) and non-toxic LT AB complex mutants such as LTK63. These enterotoxins have demonstrated potent immunomodulatory activity with a range of antigens in different animal models, with enhanced immunogenicity as well as protective efficacy, and are considered potent mucosal and parenteral adjuvants [13]. Herein, we review the immunomodulatory characteristics of LTB and LTK63 and provide examples of how these properties have been exploited for vaccine development.

2. Heat-labile enterotoxin of *E. coli* (LT): structure and activity

Certain enterotoxigenic strains of *E. coli* bacteria produce two types of toxins: heat-stable (ST) and heat-labile (LT). The heat-labile enterotoxin of *E. coli* (LT) is a bacterial adenosine phosphate (ADP)-ribosylating exotoxin. Two major LT families are known, LT-I and LT-II, however most available information about LT relates to the LT-I family. The LT protein is composed of two subunits coded by an operon; subunit A (LTA) is a 28-kDa enzyme and subunit B (LTB) is a 60-kDa protein, composed of five identical polypeptides (11.6 kDa) [16]. Each polypeptide is produced separately, with a leader sequence which allows it to be transported into the cell periplasm where it is cleaved and a toxin unit is assembled [17]. The non-toxic B subunit is assembled as a highly stable pentameric structure arranged in a cylinder-like structure with a central cavity [18]. The A subunit is composed of two domains: (1) A_1, a globular structure with ADP-ribosylating activity; and (2) A_2, a long α-helix. The two domains are linked by a trypsin-sensitive loop and by a disulphide bridge between A_1 and A_2. The enzymatic activity is dependent on the proteolytic cleavage of the loop and reduction of the disulphide bridge [19,20]. The A subunit is linked to the B subunit by the trypsin-sensitive loop and the long α-helix, the C terminus of which enters into the central cavity of the B subunit, thus anchoring the A subunit to the B pentamer structure (AB_5 complex or holotoxin) [19] (Fig. 1). The B pentamer structure contains the cellular receptor binding function. The principal receptor for LTB is the ganglioside GM1, a glycosphingolipid found ubiquitously on the cell surface of mammalian cells. Hydrogen bond interactions within each of the five pockets formed by the B subunit pentamer allow LTB to crosslink GM1 with extremely high affinity. The receptor binding activity of LTB is required for the uptake and internalization of the AB_5 complex by host cells. The A subunit is the toxic portion of LT and is internalized and subsequently stimulates the cellular adenylate cyclase-cyclic AMP system, leading to dramatic and unregulated elevation of intracellular cAMP. In intoxicated gut epithelial cells, cAMP elevation results in massive secretion of electrolytes and water into the gut lumen, clinically manifested as diarrhea. Therefore, the A subunit is responsible for the toxicity of LT [16,19,21–24].

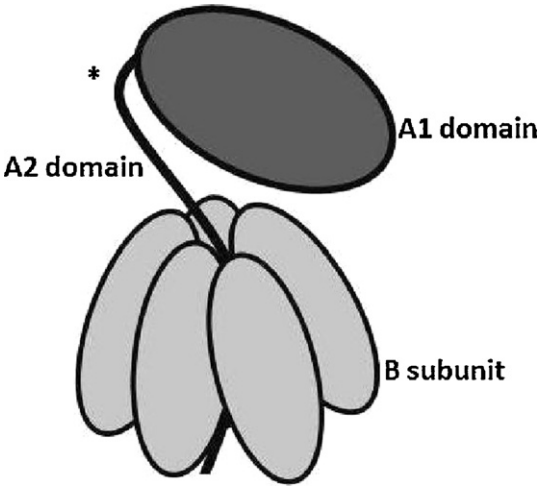

Fig. 1. Schematic structure of heat-labile enterotoxin produced by *E. coli*. The globular structure of A subunit (A_1 domain) is linked to the B subunit by a trypsin sensitive loop (*) and a long α-helix (A_2 domain), the C-terminal of which enters the central cavity of the B pentamer.

3. Immunomodulating properties of LT

LT has been extensively studied for immunomodulatory properties which enhance immunogenicity and protective efficacy. Although the mechanisms by which the enterotoxin-based adjuvant exerts immunomodulating effects are not well characterized, enhancement of inflammatory cytokine and chemokine production and transient recruitment of immune effectors cells to the site of immunization have been implicated [25]. LT is also known to influence dendritic cell maturation [26], antigen presentation and T-cell activation and promote the induction of antigen-specific cytotoxic T lymphocyte (CTL) responses in mouse models [27,28]. The use of LT as an adjuvant has resulted in a balanced cytokine response, involving the production of both Th1 and Th2 cytokines [29] and several antibody classes in mice [30] and in humans [31]. Both the ADP-ribosylation activity of LTA and GM1 binding of LTB have been proposed to be involved in immune stimulation. Results by Domingos et al. [32] suggest that both A and B subunits are required to induce TNF-α release by macrophages. Other studies have proposed that there are multiple immune-modulating pathways triggered by LT, including mechanisms independent of both ADP-ribosyl-transferase activity [22,33] and GM1-binding affinity [22].