Journal of
Clinical & Cellular Immunology

Nashar, J Clin Cell Immunol 2014, 5:3
DOI: 10.4172/2155-9899.1000225

Commentary Open Access

The Quest for an HIV-1 Vaccine Adjuvant: Bacterial Toxins as New Potential Platforms

Toufic O Nashar*

College of Veterinary Medicine, Nursing & Allied Health, Department of Pathobiology, Tuskegee University, Tuskegee, AL 36088, USA

***Corresponding author:** Toufic O Nashar, Assistant Professor, College of Veterinary Medicine, Nursing & Allied Health, Department of Pathobiology, Tuskegee University, Tuskegee, AL 36088, USA, Tel: 334-727-8366; Fax: 334-724-4110; E-mail: tnashar@mytu.tuskegee.edu

Received date: February 25, 2014, **Accepted date:** June 10, 2014, **Published date:** June 17, 2014

Abstract

While tremendous efforts are undergoing towards finding an effective HIV-1 vaccine, the search for an HIV-1 vaccine adjuvant lags behind and is understudied. More recently, however, efforts have focused on testing adjuvant formulations that can boost the immune response and generate broadly neutralizing antibodies to HIV-1 ENV (gp160). Despite this, there remain a number of challenges towards achieving this goal. These include safety of adjuvant formulations; stability of the incorporated antigens; maintenance of ENV immunogenicity; optimal inoculation sites; the effective combination of adjuvants; stability of ENV neutralizing epitopes in some adjuvant formulations; mucosal immunity; and long-term maintenance of the immune response. A new class of adjuvants for HIV-1 proteins is suggested to overcome many of the limitations of some other adjuvants. Type 1 (LT-I) and type 2 (LT-II) human *E. coli* enterotoxins (HLTs) and their non-toxic B-subunits derivatives are strong systemic and mucosal adjuvants and effective carriers for other proteins and epitopes. Their stable molecular structure in the presence of fused proteins and epitopes, and their ability to target surface receptors on antigen presenting cells make them ideal for the delivery of HIV-1 ENV or HIV other proteins. Importantly, unlike some other adjuvants, HLTs and derivatives have well-defined modes of immune system activation. The challenges in finding optimal HIV-1 vaccine adjuvant formulation and the important properties of HLTs are discussed.

Keywords: Adjuvants; Enterotoxins; LT; LTB; EtxB; CT; HIV-1; ENV; Vaccines

Challenges in the selection of an HIV-1 vaccine adjuvant

Diligent efforts are currently being made in the search of an HIV-1 vaccine adjuvant although this has not been at pace with efforts in finding a vaccine against HIV-1. There were probably good reasons for the slow progress. Besides the disappointing earlier clinical trial with recombinant ENV protein (VAX 003, VAX 004) and lack of numerous accessible neutralizing epitopes on the protein, which in the majority of cases are shielded by glycans, much needed to be understood about the intrinsic properties of the immunogen [1-3] before finding a suitable adjuvant. These included finding suitable conformation of the trimeric ENV protein that could stimulate broadly neutralizing antibodies, and the nature of epitopes that form the basis for the strong and broad neutralizing ability of some anti-HIV-1 polyclonal and monoclonal antibodies. The current interest in finding suitable adjuvants may have been accelerated by the findings that certain antibody responses correlated with protection from HIV-1 acquisition in the recent RV144 phase III trial. Adding to this is the discovery of a series of potent and broad spectrum neutralizing antibodies that have been previously isolated from infected individuals [4]. In the RV144 trial, antibodies correlated with reduced risk from HIV-1 infection. Thus, currently the challenge is to mimic those antibodies in a vaccine against HIV-1. Generating broadly neutralizing antibodies either synthetically or following immunization would have tremendous impact on HIV-1 infection either therapeutically to lower viral load in infected individuals, or in the prevention of HIV-1 infection, respectively. However, to achieve these goals a greater understanding of suitable adjuvant platforms for the vaccine is required. A number of challenges exist in this regard. These include: 1- The issue of toxicity of some adjuvants such as some cytokines or adjuvants not suitable for use in human. 2- Stability of the desired conformation of ENV in some adjuvant formulations [5]. 3- The site of inoculation where ENV and adjuvant work best [6]; these include subcutaneous, intramuscular, intranasal or oral. 4- A large dose of ENV is required for immunization [7]; hence, an adjuvant that results in a strong humoral immune response is required. 5- Appropriate combination of adjuvants might be required to enhance immunogenicity of ENV [8]. 6- Selection of adjuvant formulations that do not result in destruction of the broadly neutralizing epitopes before binding to the B cell receptor [9]. 7- Finding adjuvants that do not alter antigen processing of the neutralizing ENV epitopes in a way that alters re-elicitation of the same type of antibodies to the initial epitope. 8- Selection of the right combination of adjuvant formulations and inoculation sites in prime/boost regimens. 9- Finding suitable adjuvants that incorporate ENV fragments and maximize responses to neutralizing antigenic determinants in the absence of effects from non-essential dominant epitopes. 10- Finding adjuvant formulations able to induce high level of protective mucosal IgA antibody [10]. 11- Finding appropriate adjuvants able to stimulate T cell-mediated immunity to HIV-1 proteins or epitopes other than ENV namely, HIV-1 Gag, Pol and Nef [8,11,12].

E. coli Heat-Labile Enterotoxins as Potential Adjuvants

To be highly effective, adjuvants should trigger a multitude of biological processes in antigen presenting cells (APCs) and be able to direct the immune response to relevant epitopes. A new class of bacterial toxins adjuvants may prove to be highly effective in priming

the immune response to HIV-1 ENV, Gag, Pol and Nef proteins or derived epitopes. This refers to the family of type 1 (LT-I) and type 2 (LT-II) human *E. coli* enterotoxins (HLTs). HLTs contain an enzymatically active A1 domain responsible for toxicity, and the A2 domain that allows for non-covalent interaction of the A subunit and the non-toxic B-subunit pentamer to give holotoxin (Figure 1). LT-I, LT-II and their non-toxic B subunits derivatives modulate immune responses to other antigens by a number of mechanisms. These include effective targeting of fused proteins and epitopes to surface of APCs, alteration of cytokine production towards either T helper I (Th1), T helper II (Th2) or both, increased expression of co-stimulatory molecules on APCs, and expansion of T cells [13-19]. Recently, we demonstrated the potential role of LT-I nontoxic B-subunits in APC targeting and induction of T cell responses to HIV-1 gag p24 [20]. Many of the stimulatory effects of HLTs and their derivatives to other proteins are attributed to binding to surface receptors, such as gangliosides and Toll-like receptor 2 (TLR-2). Thus binding of LT-I to ganglioside G_{M1} receptor [13,16,17,21], a component of lipid rafts, directly activates B cells [14] by increased levels of PI3K and MAP/ERK kinases [22]. The outcome of these signals is an upregulation of co-stimulatory molecules including MHC class II, B7-2, CD25, CD40 and ICAM-1 [14]. Non-toxic derivatives of LT-I also act on dendritic cells for stimulation of $CD4^+$ T cells and secretion of cytokines [25,26], and potentiate antigen- or virus-specific CTLs (23-25) independent of IL-12 and IFN-γ(24). Unlike CpG1826, non-toxic mutants of LT-I enhance germinal center reaction and prolong persistence of antibody-secreting cells in the bone marrow [26], properties that may be essential in broadening antibody specificities and memory to HIV-1 ENV. Further, LT-I or LT-IB subunits can be used to adjuvant a variety of soluble antigens [25,27,28], and plasmids encoding these molecules are strong adjuvants for the weakly immunogenic DNA vaccines [19]. Targeting of LT-IB fusion proteins to G_{M1} on APCs significantly enhances their presentation to T cells and immunogenicity [16,21]. These findings are explained by the ability of LT-IB to deliver antigen cargo to MHC-I and MHC-II compartments [16,29], and to a depot effect [21] in the APCs. Non-toxic mutants of LT-I conjugates also boost immune responses to a variety of polysaccharides [30,31] while DNA vaccines are unable to express these molecules. Further, HLTs and recombinant fusions can be expressed in a variety of hosts including bacteria, yeast and plants [32-34]. In comparison to LT-I, research in Terry Connell laboratory (University of Buffalo, NY) demonstrated unique properties of LT-II and their derivatives. There are three types of LT-II namely, LT-IIa, LT-IIb and LT-IIc [18,35] wherein LT-IIxB designates their B subunits pentamers. LT-IIaB binds to Toll-Like Receptor 2 (TLR-2) on mouse and human monocytes and induces secretion of TNF-α, IL-1, IL-6 and IL-8 by activation of NF-κB [36]. In contrast to LT-1, LT-IIaB upregulates expression of CD80 but not CD86 on mouse B cells [36]. LTIIaB also acts on dendritic cells by increasing their migration in nasal mucosa by upregulation of CCR7, enhancing uptake and presentation of co-administered antigen, and inducing maturation of dendritic cells by increased expression of CD80, CD86, and CD40 [36]. LT-IIaB effects on dendritic cells and other APCs occur following binding to TLR-2 [36]. LT-IIaB also augments antigen-specific $CD4^+$ T cells proliferation, IgA and IgG antibodies [36].

Despite that HLTs can exert their adjuvant function both in a mixture or when conjugated to other proteins, there are more advantages in using chemical or genetic fusions [20]. These include stability and more efficient targeting of epitopes into pathways of antigen processing and presentation. The ease of HLTs genetic manipulation can be exploited to engineer chimeras with HIV epitopes either by using their non-toxic B subunits or modified holotoxins that have reduced or no toxicity. Both strategies have been proven successful with a number of proteins [37-40]. For LT-I, these include fusing an epitope of the *Bordetella pertussis* p69 antigen to LT-IB [39]. Fusions to LT-IIa and cholera toxin of a large protein, the saliva-binding region from the streptococcal adhesin AgI/II (SBR, ~ 42KD) were also made [40,41]. Other chimeras include MBP of *E. coli*, HBsAg of hepatitis virus, and a surface antigen of *Haemophilus influenzae* (Connell, personal communication). Chemical conjugations of antigens to LT-IB and to the structurally and functionally related cholera toxin (CT) or cholera toxin B subunits (CTB) were also made and shown to boost immune responses to the coupled bacterial proteins and provide protection against the pathogens [42-47]. These include conjugation to CTB of a streptococcal surface protein (*S. mutans* antigen), ovalbumin, and keyhole limpet hemocyanin (KLH), and conjugation of CT to Sendai virus.

In other laboratories, bacterial toxins were used to boost immune responses to HIV-1 antigens. In some cases, the A1 subunit was used as an adjuvant combined with HIV-1 gag or HIV-1 gp120 [48]. CTB was also used as a scaffold for the V3 loop of HIV-1 gp120 [49]. In addition, two copies of the D-fragment of *Staphylococcus aureus* protein A fused to CTA1 (CTA1-DD) was also used to adjuvant monomeric and trimeric HIV-1 gp120 [50]. Moreover, CTB was fused to drug escape variant of HIV reverse transcriptase epitope [51]. In all these strategies, components of cholera toxin were shown to adjuvant immune responses to HIV-1 proteins.

The structural and functional properties of LT-I and LT-II and their derivatives will provide several advantages to ENV epitopes. The pentameric structure of the B subunits (Figure 1) allows fusions of 5 moieties of the desired epitopes thus maximizing the dose of antigen delivered. The toxic A1 subunit can be removed and replaced by ENV epitopes fused to the A2 subunit thus pointing away from the receptor binding site of the B subunits (Figure 1). This strategy has been successfully used with other proteins [40,41]. Alternatively, fusions of the ENV epitopes can be engineered in the absence of the A1 and A2 subunits either at the C- or N-termini of the B subunits. The advantage of the latter approach is that it generates recombinants that allow fusion of 5 moieties of the desired epitopes. An additional approach would be to engineer ENV epitopes to mutants of the holotoxins (A1+A2+B) that have reduced or no toxic activities [52-54]. However, due to the large size of the hexameric ENV protein, a reductionist approach should be made by for example engineering monomeric fragments or fragments containing several B cell and Th epitopes so that the structural integrity of the toxins and their receptor binding site are preserved. Alternatively, chemical fusions could be generated with trimeric gp120 or gp41 or hexameric ENV to test their immunogenicity. Finally, different HIV epitopes including ENV antigens each loaded to either HLT-I or HLT-II can be used in a mixture to boost immunity against HIV.

In conclusion, HLTs may overcome several issues associated with the use of ENV and other HIV proteins in some adjuvant formulations. These include lack of stability of the antigens, reduced immunogenicity, limited use of inoculation sites (HLTs are effective at mucosal and in systemic compartments), destruction of neutralizing epitopes by adjuvants, inability to target epitopes to surface receptors on APCs, inability to induce long-term memory, and failure to

generate quantitative and qualitative immune responses that can be maintained (Th1, Th2 or both). They may hold a great promise as adjuvants and carriers for HIV-1 proteins and epitopes particularly when used in combination with recombinant viral vaccines expressing HIV-1 proteins in prime/boost regimens.

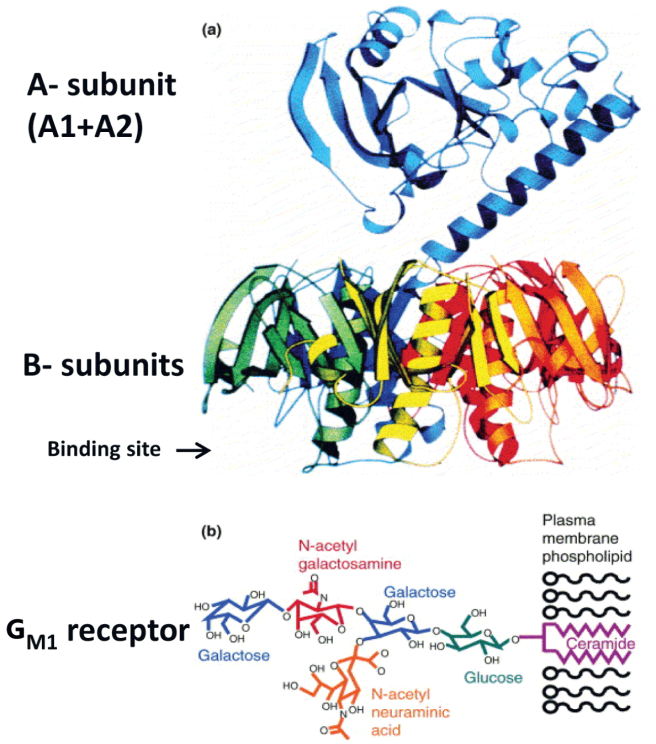

Figure 1: The molecular structure of HLT-I [similar to HLT-II except for the type of receptor (55)]. The family of LT and LT-II toxins contains an enzymatically active A1 domain and the A2 domain that allows for non-covalent interaction of the A subunit and the B-subunit pentamer to give holotoxin. Importantly, this molecular structure can be exploited to engineer chimeras with other proteins or epitopes. In the native structure of the holotoxin, A1 points away from the A2-B pentamer complex. Inserts can be engineered at the N terminus of A2 subunit to replace A1 (responsible for toxic activity) with the target of interest and thus still allow appropriate assembly. Such chimeric proteins assemble, do not interfere with the immunological function of the binding subunits of the toxins, and are non-toxic. Alternatively, fusions can be made directly to the C-terminus of the B subunits in the absence of the A subunits (A1 and A2). The binding site of the B subunits cross-links five moieties of G_{M1} via interaction with their pentasaccharide moieties exposed upon the lymphocyte cell surface. G_{M1} is integrated into the plasma membrane through its hydrophobic ceramide moiety. Diagram: a) A1/A2-B5 holotoxin; b) G_{M1} receptor.

Funding

This work was supported by a grant from US DOEd # P031B120901 and RCMI # G12MD00758-23.

References

1. Schiffner T, Sattentau QJ, Dorrell L (2013) Development of prophylactic vaccines against HIV-1. Retrovirology 10: 72.
2. Mascola JR, Montefiori DC (2010) The role of antibodies in HIV vaccines. Annu Rev Immunol 28: 413-444.
3. Kwong PD, Mascola JR (2012) Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. Immunity 37: 412-425.
4. Binley JM, Lybarger EA, Crooks ET, Seaman MS, Gray E, et al. (2008) Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C. J Virol 82:11651-11668.
5. Nkolola JP, Cheung A1, Perry JR1, Carter D2, Reed S2, et al. (2014) Comparison of multiple adjuvants on the stability and immunogenicity of a clade C HIV-1 gp140 trimer. Vaccine 32: 2109-2116.
6. Buffa V, Klein K, Fischetti L, Shattock RJ (2012) Evaluation of TLR agonists as potential mucosal adjuvants for HIV gp140 and tetanus toxoid in mice. PLoS One 7: e50529.
7. van Gils MJ, Sanders RW (2013) Broadly neutralizing antibodies against HIV-1: templates for a vaccine. Virology 435: 46-56.
8. Moore AC, Kong WP, Chakrabarti BK, Nabel GJ (2002) Effects of antigen and genetic adjuvants on immune responses to human immunodeficiency virus DNA vaccines in mice. J Virol 76: 243-250.
9. Yu B, Fonseca DP, O'Rourke SM, Berman PW (2010) Protease cleavage sites in HIV-1 gp120 recognized by antigen processing enzymes are conserved and located at receptor binding sites. J Virol 84: 1513-1526.
10. Hur EM, Patel SN, Shimizu S, Rao DS, Gnanapragasam PN, et al. (2012) Inhibitory effect of HIV-specific neutralizing IgA on mucosal transmission of HIV in humanized mice. Blood 120: 4571-4582.
11. Cox KS, Clair JH, Prokop MT, Sykes KJ, Dubey SA, et al. (2008) DNA gag/adenovirus type 5 (Ad5) gag and Ad5 gag/Ad5 gag vaccines induce distinct T-cell response profiles. J Virol 82: 8161-8171.
12. Kalams SA, Parker S, Jin X, Elizaga M, Metch B, et al. (2012) Safety and immunogenicity of an HIV-1 gag DNA vaccine with or without IL-12 and/or IL-15 plasmid cytokine adjuvant in healthy, HIV-1 uninfected adults. PLoS One 7: e29231.
13. Nashar TO, Williams NA, Hirst TR (1998) Importance of receptor binding in the immunogenicity, adjuvanticity and therapeutic properties of cholera toxin and Escherichia coli heat-labile enterotoxin. Med Microbiol Immunol 187: 3-10.
14. Nashar TO, Hirst TR, Williams NA (1997) Modulation of B-cell activation by the B subunit of Escherichia coli enterotoxin: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1. Immunology 91: 572-578.
15. Williams NA, Hirst TR, Nashar TO (1999) Immune modulation by the cholera-like enterotoxins: from adjuvant to therapeutic. Immunol Today 20: 95-101.
16. Nashar TO, Betteridge ZE, Mitchell RN (2001) Evidence for a role of ganglioside GM1 in antigen presentation: binding enhances presentation of Escherichia coli enterotoxin B subunit (EtxB) to CD4(+) T cells. Int Immunol 13: 541-551.
17. Nashar TO, Webb HM, Eaglestone S, Williams NA, Hirst TR (1996) Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: receptor binding is essential and induces differential modulation of lymphocyte subsets. Proc Natl Acad Sci U S A 93:226-230.
18. Connell TD (2007) Cholera toxin, LT-I, LT-IIa and LT-IIb: the critical role of ganglioside binding in immunomodulation by type I and type II heat-labile enterotoxins. Expert Rev Vaccines 6: 821-834.
19. Arrington J, Braun RP, Dong L, Fuller DH, Macklin MD, et al. (2002) Plasmid vectors encoding cholera toxin or the heat-labile enterotoxin from Escherichia coli are strong adjuvants for DNA vaccines. J Virol 76: 4536-4546.
20. Karmarcha M, Nashar TO (2013) E. coli Heat-labile Enterotoxin B Subunit as a Platform for the Delivery of HIV Gag p24 Antigen. J Clin Cell Immunol 4:1-7.

21. Nashar TO, Betteridge ZE, Mitchell RN (2002) Antigen binding to GM1 ganglioside results in delayed presentation: minimal effects of GM1 on presentation of antigens internalized via other pathways. Immunology 106: 60-70.
22. Bone H, Eckholdt S, Williams NA (2002) Modulation of B lymphocyte signalling by the B subunit of Escherichia coli heat-labile enterotoxin. Int Immunol 14: 647-658.
23. Fu N, Khan S, Quinten E, de Graaf N, Pemberton AJ, et al. (2009) Effective CD8(+) T cell priming and tumor protection by enterotoxin B subunit-conjugated peptides targeted to dendritic cells. Vaccine 27: 5252-5258.
24. Simmons CP, Mastroeni P, Fowler R, Ghaem-maghami M, Lycke N, et al. (1999) MHC class I-restricted cytotoxic lymphocyte responses induced by enterotoxin-based mucosal adjuvants. J Immunol 163: 6502-6510.
25. Partidos CD, Pizza M, Rappuoli R, Steward MW (1996) The adjuvant effect of a non-toxic mutant of heat-labile enterotoxin of Escherichia coli for the induction of measles virus-specific CTL responses after intranasal coimmunization with a synthetic peptide. Immunology 89: 483-487.
26. Bjarnarson SP, Adarna BC, Benonisson H, Del Giudice G, Jonsdottir I (2012) The adjuvant LT-K63 can restore delayed maturation of follicular dendritic cells and poor persistence of both protein- and polysaccharide-specific antibody-secreting cells in neonatal mice. J Immunol 189:1265-1273.
27. Apostolaki M, Williams NA (2004) Nasal delivery of antigen with the B subunit of Escherichia coli heat-labile enterotoxin augments antigen-specific T-cell clonal expansion and differentiation. Infect Immun 72: 4072-4080.
28. Millar DG, Hirst TR, Snider DP (2001) Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of cholera toxin. Infect Immun 69: 3476-3482.
29. Hearn AR, de Haan L, Pemberton AJ, Hirst TR, Rivett AJ (2004) Trafficking of exogenous peptides into proteasome-dependent major histocompatibility complex class I pathway following enterotoxin B subunitmediated delivery. J Biol Chem 279: 51315-51322.
30. Jakobsen H, Schulz D, Pizza M, Rappuoli R, Jónsdóttir I (1999) Intranasal immunization with pneumococcal polysaccharide conjugate vaccines with nontoxic mutants of Escherichia coli heat-labile enterotoxins as adjuvants protects mice against invasive pneumococcal infections. Infect Immun 67: 5892-5897.
31. Bergquist C, Lagergård T, Lindblad M, Holmgren J (1995) Local and systemic antibody responses to dextran-cholera toxin B subunit conjugates. Infect Immun 63: 2021-2025.
32. Fingerut E, Gutter B, Meir R, Eliahoo D, Pitcovski J (2005) Vaccine and adjuvant activity of recombinant subunit B of E. coli enterotoxin produced in yeast. Vaccine 23: 4685-4696.
33. Amin T, Hirst TR (1994) Purification of the B-subunit oligomer of Escherichia coli heat-labile enterotoxin by heterologous expression and secretion in a marine vibrio. Protein Expr Purif 5: 198-204.
34. Tokuhara D, Yuki Y, Nochi T, Kodama T, Mejima M, et al. (2010) Secretory IgA-mediated protection against V. cholerae and heat-labile enterotoxinproducing enterotoxigenic Escherichia coli by rice-based vaccine. Proc Natl Acad Sci U S A 107:8794-8799.
35. Nawar HF, Greene CJ, Lee CH, Mandell LM, Hajishengallis G, et al. (2011) LT-IIc, a new member of the type II heat-labile enterotoxin family, exhibits potent immunomodulatory properties that are different from those induced by LT-IIa or LT-IIb. Vaccine 29: 721-727.
36. Lee CH, Masso-Welch P, Hajishengallis G, Connell TD (2011) TLR2-dependent modulation of dendritic cells by LT-IIa-B5, a novel mucosal adjuvant derived from a type II heat-labile enterotoxin. J Leukoc Biol 90: 911-921.
37. Rock EP, Reich KA, Lyu DM, Hovi M, Hardy J, et al. (1996) Immunogenicity of a fusion protein linking the beta subunit carboxyl terminal peptide (CTP) of human chorionic gonadotropin to the B subunit of Escherichia coli heat-labile enterotoxin (LTB). Vaccine 14: 1560-1568.
38. Bibolini MJ, Julia Scerbo M, Peinetti N, Roth GA, Monferran CG (2012) The hybrid between the ABC domains of synapsin and the B subunit of Escherichia coli heat-labile toxin ameliorates experimental autoimmune encephalomyelitis. Cell Immunol 280: 50-60.
39. Lipscombe M1, Charles IG, Roberts M, Dougan G, Tite J, et al. (1991) Intranasal immunization using the B subunit of the Escherichia coli heat-labile toxin fused to an epitope of the Bordetella pertussis P.69 antigen. Mol Microbiol 5: 1385-1392.
40. Martin M, Hajishengallis G, Metzger DJ, Michalek SM, Connell TD, et al. (2001) Recombinant antigen-enterotoxin A2/B chimeric mucosal immunogens differentially enhance antibody responses and B7-dependent costimulation of CD4(+) T cells. Infect Immun 69: 252-261.
41. Hajishengallis G, Hollingshead SK, Koga T, Russell MW (1995) Mucosal immunization with a bacterial protein antigen genetically coupled to cholera toxin A2/B subunits. J Immunol 154: 4322-4332.
42. Katz J, Harmon CC, Buckner GP, Richardson GJ, Russell MW, et al. (1993) Protective salivary immunoglobulin A responses against Streptococcus mutans infection after intranasal immunization with S. mutans antigen I/II coupled to the B subunit of cholera toxin. Infect Immun 61:1964-1971.
43. Wu HY, Russell MW (1994) Comparison of systemic and mucosal priming for mucosal immune responses to a bacterial protein antigen given with or coupled to cholera toxin (CT) B subunit, and effects of pre-existing anti-CT immunity. Vaccine 12: 215-222.
44. Menge AC, Michalek SM, Russell MW, Mestecky J (1993) Immune response of the female rat genital tract after oral and local immunization with keyhole limpet hemocyanin conjugated to the cholera toxin B subunit. Infect Immun 61: 2162-2171.
45. Liang XP1, Lamm ME, Nedrud JG (1989) Cholera toxin as a mucosal adjuvant. Glutaraldehyde treatment dissociates adjuvanticity from toxicity. J Immunol 143: 484-490.
46. Van der Heijden PJ, Bianchi AT, Dol M, Pals JW, Stok W, et al. (1991) Manipulation of intestinal immune responses against ovalbumin by cholera toxin and its B subunit in mice. Immunology 72: 89-93.
47. Wu HY, Russell MW (1993) Induction of mucosal immunity by intranasal application of a streptococcal surface protein antigen with the cholera toxin B subunit. Infect Immun 61:314-322.
48. Bagley KC, Lewis GK, Fouts TR (2011) Adjuvant activity of the catalytic A1 domain of cholera toxin for retroviral antigens delivered by GeneGun. Clin Vaccine Immunol 18: 922-930.
49. Totrov M, Jiang X, Kong XP, Cohen S, Krachmarov C, et al. (2010) Structure-guided design and immunological characterization of immunogens presenting the HIV-1 gp120 V3 loop on a CTB scaffold. Virology 405: 513-523.
50. Sundling C, Schön K, Mörner A, Forsell MN, Wyatt RT, et al. (2008) CTA1-DD adjuvant promotes strong immunity against human immunodeficiency virus type 1 envelope glycoproteins following mucosal immunization. J Gen Virol 89: 2954-2964.
51. Boberg A, Gaunitz S, Bråve A, Wahren B, Carlin N (2008) Enhancement of epitope-specific cellular immune responses by immunization with HIV-1 peptides genetically conjugated to the B-subunit of recombinant cholera toxin. Vaccine 26: 5079-5082.
52. Douce G, Turcotte C, Cropley I, Roberts M, Pizza M, et al. (1995) Mutants of Escherichia coli heat-labile toxin lacking ADP-ribosyltransferase activity act as nontoxic, mucosal adjuvants. Proc Natl Acad Sci U S A 92: 1644-1648.
53. Pizza M, Fontana MR, Giuliani MM, Domenighini M, Magagnoli C, et al. (1994) A genetically detoxified derivative of heat-labile Escherichia coli enterotoxin induces neutralizing antibodies against the A subunit. J Exp Med 180: 2147-2153.
54. Norton EB, Lawson LB, Freytag LC, Clements JD (2011) Characterization of a mutant Escherichia coli heat-labile toxin, LT(R192G/L211A), as a safe and effective oral adjuvant. Clin Vaccine Immunol 18: 546-551.
55. van den Akker F, Sarfaty S, Twiddy EM, Connell TD, Holmes RK, et al. (1996) Crystal structure of a new heat-labile enterotoxin, LT-IIb. Structure 4: 665-678.