

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,400

Open access books available

117,000

International authors and editors

130M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# The Cholera Toxin as a Biotechnological Tool

Noelia Olivera, Maia Cédola and Ricardo M Gómez  
*Instituto de Biotecnología y Biología Molecular, CONICET La Plata  
Argentina*

## 1. Introduction

It was as early as 1886 when Robert Koch proposed that the symptoms caused by *Vibrio cholerae* were initiated by a "poison" produced by the pathogen. However, it was not until 1959 that this postulate could be demonstrated by reproducing the disease in an animal model [De, 1959]. Today, cholera toxin (CT) is known to exhibit toxic effects in human cells and produces dehydrating diarrhea in humans. It is produced almost exclusively by few serogroups of *V. cholera*, however, sometimes may be naturally produced by other organisms, as the opportunistic pathogen *V. mimicus* [Nishibuchi and Seidler, 1983; Spira and Fedorka-Cray, 1984].

CT has important immunological properties and for that reason it has been extensively used as a systemic and mucosal adjuvant because it enhances the immunogenicity of most antigens fused or co-administered with the toxin [Sanchez and Holmgren, 2008].

The aim of this chapter will be to describe the biotechnological utilities of CT, with special attention to its adjuvant effect as well as its application in the treatment of autoimmune diseases through its ability to generate oral tolerance.

## 2. Structure

CT belongs to the family of AB<sub>5</sub>-type toxins, since it is composed of two subunits in a 1:5 ratio. The A subunit (CTA), of 28 kDa, is a heterodimer associated non-covalently to a homopentamer formed by the subunits B (CTB) of 56 kDa [Merritt et al., 1994; Vanden Broeck et al., 2007]. CTA is responsible for the biological activity and CTB binds to the cell membrane receptor [Holmgren et al., 1973; Lonroth and Holmgren, 1973] (Fig. 1.).

CTA comprises 240 amino acids, and the 11.6 kDa B subunit monomers each have 103 amino acids. CTA is synthesized as a single polypeptide chain and is post-translationally modified through the action of a *V. cholerae* protease at position R192 [Mekalanos et al., 1979]. The cleavage of this amino acid, found in an exposed loop that extends from C187 to C199 residues, generates two fragments named CTA1 and CTA2, which remain linked by a disulfide bridge [Lencer and Tsai, 2003; Tsai et al., 2001]. The toxic activity (enzymatic ADP-ribosylating) activity of CTA resides in CTA1, whereas CTA2 serves to insert CTA into the CTB pentamer [Sanchez and Holmgren, 2011]. The C-terminal hydrophobic region including residues 162-192 of CTA1, plays a key role in toxicity. It triggers the ER-associated degradation (ERAD) mechanism (see section 3) and facilitates interaction with

the cytosolic ADP-ribosylation factors (ARFs) that serve as allosteric activators of CTA1 [Teter et al., 2006].

The remarkable stability of pentameric CTB is attributed to non-covalent interactions including 130 hydrogen bonds, 20 salt bridges, as well as tight packing of subunits via hydrophobic and pentamer-pentamer interactions. Consequently, the CTB pentamer is held together and remains as a complex unless boiled or monomerized by acidification at pH below 3 [Sanchez and Holmgren, 2008].

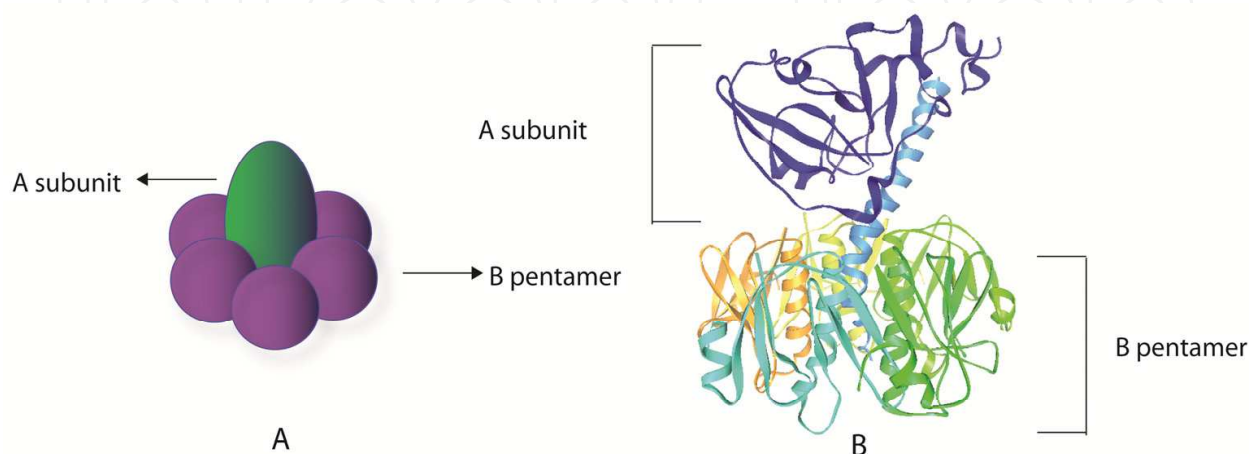


Fig. 1. Cholera toxin structure. A) Schematic model of cholera toxin. A subunit contains the toxic activity while B subunits bind to cells. B) Model based on X-ray crystallography analysis. Each subunit is represented by a different color. Adapted from Zhang et al 2005.

### 3. Binding and mechanism of action

CT is secreted through the outer membrane of *V. cholerae* and its toxic action begins when its B subunit binds to the high-affinity monoganglioside GM1 receptor. GM1 is a glycolipid commonly found in caveolae, organized membrane structures enriched in glycolipids, cholesterol and caveolin, involved in endocytosis and transcytosis, cellular transport and signal transduction [Shin and Abraham, 2001]. These membrane structures are present in various cell types, including immune cells [Thomas et al., 2004]. Each B subunit monomer has a binding site for GM1, however, the CTB pentamer has a much higher binding affinity for the receptor due to the important role played by a single amino acid from an adjacent B subunit that enhances this action [Merritt et al., 1994]. After binding to the receptor, CT enters human intestinal cells through endocytosis and is transported from early endosomes to the Golgi. Endocytosis of CT may follow one of three pathways: (i) lipid raft/caveolae mediated endocytic pathway, (ii) clathrin mediated endocytic pathway, or (iii) noncaveolar clathrin-independent pathway [Chinnapen et al., 2007]. GM1 is the vehicle for retrograde transport of the CT holotoxin from the plasma membrane to the ER [Fujinaga et al., 2003]. In the ER, the disulfide bond that links CTA1 and CTA2 to CTB is reduced and a protein disulfide isomerase mediates the dissociation of CTA1 from CTA2/CTB. CTA1 moves from the ER to the cytosol by the ERAD dislocation mechanism, which recognizes misfolded proteins in the ER and exports them to the cytosol for degradation by the 26S proteasome [Massey et al., 2009]. Once inside the host cells, CTA1 catalyzes the transfer of an ADP-

ribose unit from NAD<sup>+</sup> oxidizing agent to an arginine residue of Gs protein. This covalent modification leads to the loss of GTPase activity of the Gs protein, which remains attached to GTP, keeping the adenylate cyclase (AC) enzyme active that will produce increasing amounts of cAMP. Over 100 times the normal concentration of cAMP, the intestinal mucosa cells open a Cl<sup>-</sup> channels in the cytoplasmic membrane, resulting in an influx of ions and water to the gut lumen that causes the characteristic acute diarrhea of cholera [Spangler, 1992]. As little as 5 µg of purified CT administered orally is sufficient to induce significant diarrhea in human volunteers while ingestion of 25 µg of CT elicits a full 20 litres cholera purge [Levine et al., 1983].

#### 4. Immune properties

Adjuvants are substances that have the ability to enhance the immune response when co-administered with poor immunogenic molecules. CT is a bacterial immunogen with a great function as an adjuvant to a variety of antigens when given by systemic and mucosal route whether these are linked to or simply mixed with the toxin, generating a long-term immune response (Elson 1989; Vajdy and Lycke 1992).

These properties may be explained by three main characteristics of the molecule. First, CT is remarkably stable to proteases, bile salts and other compounds in the intestine. Secondly, its high affinity to GM1 ganglioside receptor, which is present on most mammalian cells including the M cells covering the Peyer's patches, as well as all antigen-presenting cells (APC), facilitates the uptake and presentation of the toxin to the gut mucosal immune system. Finally, CT has strong inherent adjuvant and immunomodulating activities that depend both on its cell binding capability and its enzymatic ADP-ribosylating function (Sanchez and Holmgren 2008).

Pioneer studies carried out in 1972 showed that CT delivered by the intravenous route with a foreign antigen behaved as an adjuvant [Northrup and Fauci, 1972], a fact confirmed later by several groups using a number of unrelated antigens of little immunogenicity [Bianchi et al., 1990; Elson and Ealding, 1984]. Additional studies revealed that upon co-administration of CT and antigen through parenteral, mucosal, and transcutaneous routes resulted in substantial enhancement of mucosal immunoglobulin A (IgA) and serum IgG responses to the co-administered antigen [Chen and Strober, 1990; Drew et al., 1992; Reuman et al., 1991]. In addition to enhancing humoral immune responses, CT also augmented cellular immune responses to co-administered antigens enhancing induction of CD4<sup>+</sup> T helper (Th) and class I-restricted cytotoxic T lymphocyte responses [Nurkkala et al.; Simmons et al., 1999]. In most cases, CT induced a Th2 bias response [Lavelle et al., 2004; Okahashi et al., 1996]. However, other studies have reported Th1 [Sasaki et al., 2003; Taniguchi et al., 2008] or mixed Th1/Th2 responses following oral, sublingual and intranasal immunization with antigens in the presence of CT [Cuburu et al., 2007; Fecek et al., 2010]. More importantly, subsequent studies showed that CT elicited a long-term memory response and thus was detectable long after the initial immune response [Soenawan et al., 2004; Vajdy and Lycke, 1992].

CT also acts as mucosal adjuvant against a variety of pathogens. Examples include, tetanus toxoid [Jackson et al., 1993], *Helicobacter felis* [Jiang et al., 2003], *Schistosoma japonicum* [Kohama et al., 2010], *Helicobacter pylori* [Raghavan et al., 2002], and *Sendai virus* [Liang et al., 1988]. There are many other examples where it was shown that CT has significant potential

for use as adjuvant for mucosally administered antigens [Clapp et al., 2010; Jhon Carlos Castaño Osorio, 2002].

## 5. Mechanism of adjuvant activity

The mechanism of adjuvanticity of CT is still unclear but it has been related to: (i) the induction of increased permeability of the intestinal epithelium leading to enhanced uptake of co-administered antigens; (ii) the induction of enhanced antigen presentation by various APC; (iii) the promotion of isotype differentiation in B cells leading to increased IgA formation; and (iv) exhibition of complex stimulatory as well as inhibitory effects on T cell proliferation and cytokine production. Among these many effects, those leading to enhanced antigen presentation by various APC are probably of the greatest importance [Sanchez and Holmgren, 2011].

As mentioned before, the polarity of the immune response generated by CT is a matter of debate. Some studies indicate that CT primes naïve T cells *in vitro* and drives them towards a Th2 phenotype, with production of interleukins IL-4 (a cytokine needed for B cell differentiation), IL-5, IL-6 and IL-10, but little IFN- $\gamma$  (a cytokine needed to evoke Th1 responses) and suppression of IL-12 production by dendritic cells (DC) [Braun et al., 1999; Klimpel et al., 1995; Wilson et al., 1991]. Moreover, after immunization of animals with CT co-administered antigens, IL-4 levels were significantly elevated in gut-associated tissues and in spleen, while the levels of IFN- $\gamma$  either decreased or remained static [Akhiani et al., 1997; Marinaro et al., 1995]. These results are supported by evidence of increased secretory IgA, serum IgA and IgE levels [Adel-Patient et al., 2005; Bourguin et al., 1991], and higher titers of IgG1 than IgG2a [Glenn et al., 1998; Lycke et al., 1990].

In contrast, others have reported that CT induces a mixed Th1/Th2 type of immune response with the production of IFN- $\gamma$  and IL-4 [Fromantin et al., 2001; Imaoka et al., 1998]. In addition, it has been shown that CT induces strong Th17-type responses after intranasal delivery [Datta et al.; Lee et al., 2009].

Furthermore, CT markedly increased antigen-presentation by DC, macrophages, and B cells [Bromander et al., 1991; George-Chandy et al., 2001]. Also, CT upregulates the expression of MHC/HLA-DR molecules, CD80/B7.1 and CD86/B7.2 co-stimulatory molecules, as well as chemokine receptors CCR7 and CXCR4, on both murine and human DC, among other APC [Cong et al., 1997; Gagliardi et al., 2000]. Importantly, CT also induced the secretion of IL-1 $\beta$  from both DC and macrophages. IL-1 $\beta$  not only induces the maturation of DC, but also acts as an efficient mucosal adjuvant when co-administered with protein antigens and might mediate a significant part of the adjuvant activity of CT [Staats and Ennis, 1999]. Treatment with CT has been demonstrated to induce maturation and mobilization of DC [Lavelle et al., 2003]. Also, CT interferes with the differentiation of monocytes into DC, giving rise to a distinct population (Ma-DC), which displays an activated macrophage-like phenotype, induces a strong allogeneic and antigen specific response, and promotes the polarization of naïve CD4<sup>+</sup> T lymphocytes toward a Th2 profile [Raghavan et al., 2010]. In addition, CT enhanced IL-6 secretion by peritoneal mast cell [Leal-Berumen et al., 1996] and production of IL-1 $\beta$ , IL-6, and IL-10 together with inhibition of IL-12, TNF- $\alpha$ , and nitric oxide in macrophages [Cong et al., 2001], depleted the CD8<sup>+</sup> intraepithelial lymphocyte population [Flach et al., 2005], and induced isotype differentiation of B cells acting synergistically with IL-4 [Salmond et al., 2002]. Recent studies show that CT enhances STAT3 gene expression

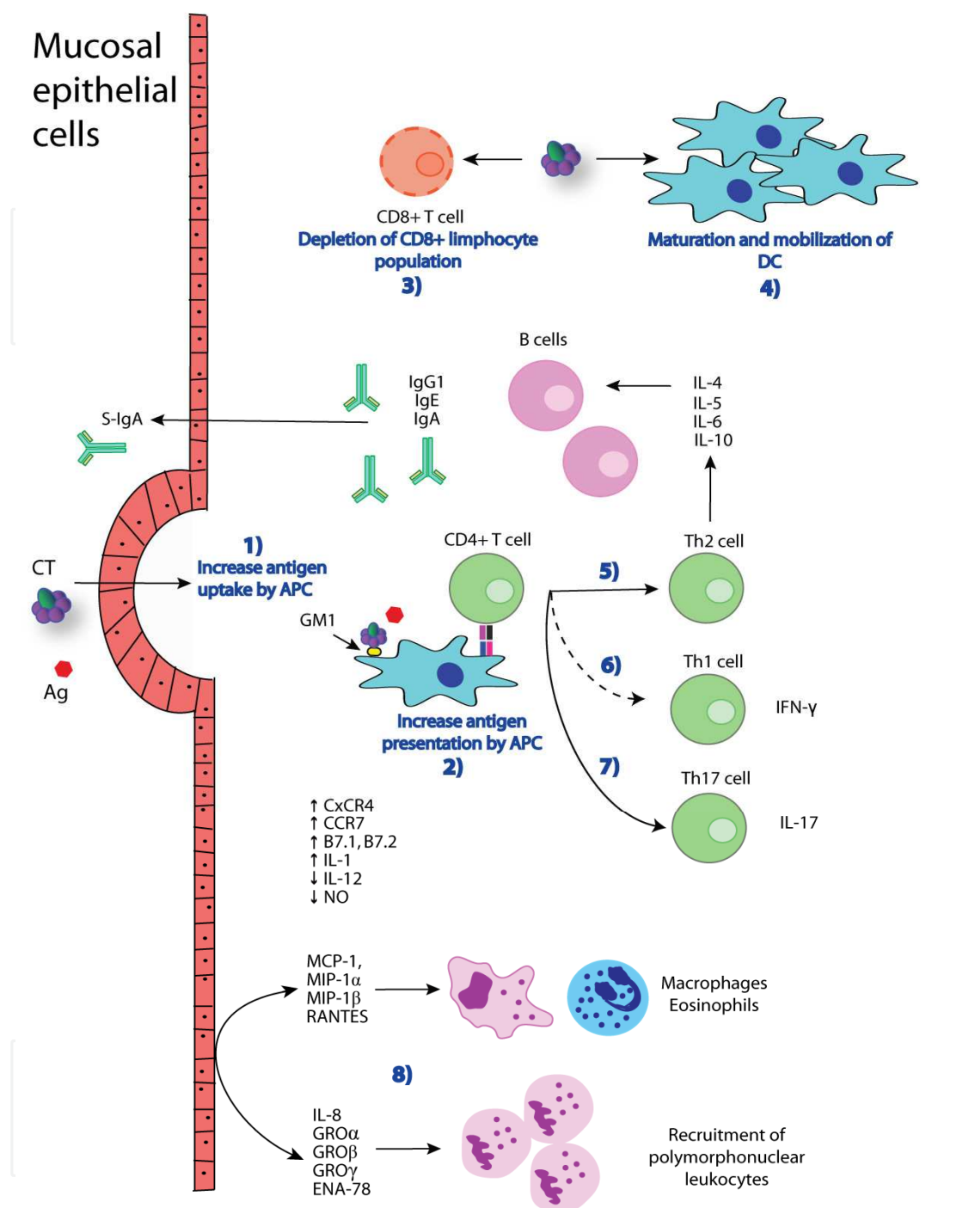


Fig. 2. Proposed mechanism of action by CT as a mucosal adjuvant. CT induces increased permeability of the intestinal epithelium leading to 1) enhanced uptake of co-administered antigens and 2) enhanced antigen-presentation by various APC. 3) It causes the depletion of CD8<sup>+</sup> lymphocyte population that may produce inhibitory cytokines, and 4) induces maturation and mobilization of DC. In addition, 5) CT promotes a strong Th2 dominant response to bystander antigens, and can either 6) induce or inhibit a Th1 response. Moreover, 7) CT induces strong Th17-type responses. Furthermore, 8) mucosal epithelial cells contribute to the adjuvant activity of CT by secreting a number of chemokines and acting on polymorphonuclear leukocytes, macrophages, eosinophils and T cells.

in murine B cells, and may critically modulate immune responses in both a pro-inflammatory and anti-inflammatory direction, depending on the circumstances and the types of cells involved Sjoblom-Hallen et al., (2010).

It has been suggested that mucosal epithelial cells may also play a role in adjuvanticity. Human epithelial cells express and secrete high levels of the chemoattractant cytokines IL-8, GRO $\alpha$ , GRO $\beta$ , GRO $\gamma$ , and ENA-78 in response to stimulation with TNF- $\alpha$ , IL-1 $\beta$ , or infection with enteroinvasive microorganisms. These chemokines attract and activate polymorphonuclear leukocytes. Activated epithelial cells also secrete MCP-1, MIP-1 $\beta$ , MIP-1 $\alpha$ , and RANTES, which variably act on monocytes/macrophages, eosinophils, and subpopulations of T-cells [Freytag and Clements, 2005]. One possibility is that CT interacts with epithelial cells triggering expression of one or more immunomodulatory factors that recruit APC and immune effector cells or activate those cells, or both [Lopes et al., 2000; Soriani et al., 2002].

A proposed mechanism of action of CT as adjuvant is shown in Fig. 2.

## 6. Genetic modifications of CT

The inherent enterotoxicity of CT has limited its widespread use as a vaccine component and adjuvant. In dogs, protection due to CT occurred only with doses that caused transient, sometimes severe, diarrhea [Pierce et al., 1982]. Moreover, murine models demonstrated that intranasal sensitization with CT as adjuvant led to increased lung inflammation with a massive recruitment of macrophages as well as accumulation in the olfactory nerves, epithelium and the olfactory bulbs of mice after binding to GM1 gangliosides [Fischer et al., 2005]. These limitations have led to mucosal strategies involving nontoxic mutants and purified B subunits.

Although early reports showed that mutants without the ADP-ribosyltransferase activity lack their adjuvant properties [Lycke et al., 1992], later studies showed that non-toxic mutants retained their adjuvant and immunogenic properties [Douce et al., 1997; Yamamoto et al., 1997] without central nervous system (CNS) toxicity [Hagiwara et al., 2006]. This suggests that the ADP-ribosyltransferase activity is not essential for its immunogenic properties, though it contributes to the adjuvant effect.

In a different approach, the CTA1 fragment linked to a synthetic analogue of *Staphylococcus aureus* protein A, the D fragment with affinity for APC, [Agren et al., 1997], proved to be non-toxic [Eriksson et al., 2004]. The fusion protein CTA1-DD binds specifically to immunoglobulins on the surface of antigen-presenting B cells through the DD polypeptide, and induces the ADP ribosylation by CTA1. Although this produces a good immune response when administered intranasally, it has been shown not to work as well after oral administration. This limitation was overcome by fusing CTA1-DD with immunostimulating complexes, such as ISCOMs (lipophilic immune stimulating complexes), producing both Th1/Th2 responses at systemic and mucosal levels [Andersen et al., 2007]. A recent report showed that CTA1 potently enhances a GeneGun-delivered DNA prime for human and simian immunodeficiency viruses antigens boost in macaques and mice [Bagley et al., 2011].

## 7. Immunological and adjuvant properties of CTB

Several studies using different conditions and routes of administration have described that CTB has several immunomodulatory properties opening many perspectives for future therapeutic and biotechnological applications. In this regard, intranasal immunization of women with CTB resulted in the production of long-lasting IgG and IgA anti-CTB in serum, nasal and vaginal secretions in a dose-dependent manner [Bergquist et al., 1997].

However, its capacity as mucosal adjuvant has proven to be much less than that of the toxin when given together with non-coupled antigens by the oral route [Sanchez and Holmgren, 2008]. Recombinant CTB has been successfully used as a mucosal adjuvant in vaccines for human use such as the cholera vaccine itself [Quiding et al., 1991], and the vaccine against enterotoxigenic *E. coli* that causes diarrhea [Peltola et al., 1991; Qadri et al., 2000]. Analogously, CTB proved to be good adjuvant for a *Streptococcus pneumoniae* cellular vaccine [Malley et al., 2004] and a severe acute respiratory syndrome-associated coronavirus vaccine [Qu et al., 2005] when administered intranasally in mice.

Given the potential of CTB as a regulator of the immune response, this subunit has been produced in various biological systems such as *Vibrio cholerae* [Sanchez and Holmgren, 1989], *Escherichia coli* [Arimitsu et al., 2009], *Bacillus brevis* [Goto et al., 2000], *Lactobacillus paracasei* and *plantarum* [Slos et al., 1998], in the yeasts *Hansenula polymorpha* [Song et al., 2004] and *Saccharomyces cerevisiae* [Mohsen and Rezae, 2005], and in silkworm [Gong et al., 2005]. In addition, CTB has been expressed successfully in tomato [Jani et al., 2002], lettuce [Young-Sook Kim, 2006], rice [Oszvald et al., 2008], tobacco [Hein et al., 1996], carrots [Kim et al., 2009], banana [Renuga et al., 2010] and potato transgenic plants, [Arakawa et al., 1997] where ubiquitin fusion enhances CTB expression [Mishra et al., 2006]. CTB may induce systemic immune responses in mice after gavage of the animals with the transgenic vegetal [Jiang et al., 2007]. The advantage of this approach is that plants present a low-cost agricultural-based effective production system. Different formulations, such as encapsulation in liposomes or microspheres with antigens [Seo et al., 2002] or combined with vesicles or liposomes containing antigens [Harokopakis et al., 1998; Lian et al., 1999] were also successfully tested.

CTB is a useful carrier protein for induction of mucosal IgA antibodies against chemically coupled antigens. In this regard, mice immunized intraduodenally with the horseradish peroxidase (HRP) covalently coupled to CTB showed a 33–120 fold higher level of IgA anti-HRP in intestinal washes as well as increased levels of serum IgG anti-HRP [McKenzie and Halsey, 1984]. In addition, CTB chemically conjugated to the protein I/II of *Streptococcus mutans* when administered in mice by oral [Russell and Wu, 1991], intranasal [Wu and Russell, 1998], and intragastric routes [Wu and Russell, 1993] results in the production of antistreptococcal IgG and IgA in serum and mucosa, as well as the presence of large numbers of antibody-secreting cells in salivary glands, mesenteric lymph nodes, and spleens. Similar results were found with CTB conjugated to human gamma globulin (HGG) and the recombinant *Neisseria gonorrhoeae* transferrin binding proteins, TbpA and TbpB. Vaginal and intranasal immunizations with CTB-HGG resulted in high levels of anti-HGG antibodies [Johansson et al., 1998], while rCTB-TbpA and rCTB-TbpB administered intranasally induced antibody responses in the serum and genital tract [Price et al., 2005]. Moreover, CTB was chemically conjugated to type III capsular polysaccharide from



*Streptococcus* group B [Shen et al., 2000] or to protein-polysaccharide conjugates [Bergquist et al., 1995] and in both cases, after subcutaneous administration, high levels of specific antibodies were detected. In addition to generating humoral response, simian immunodeficiency virus (SIV) virus-like particles (VLP) chemically conjugated to CTB showed higher levels of cytokine IFN- $\gamma$ -producing splenocytes and cytotoxic-T-lymphocyte activities of immune cells than VLPs plus CTB, indicating a generation of a Th1 response in mice by CTB-VLP [Kang et al., 2003]. Finally, CTB chemically conjugated to the *Plasmodium vivax* ookinete surface protein, Pvs25, proved to be a potent transmission-blocking antigen in both intranasal and subcutaneous routes in mice [Miyata et al., 2010], and to protect against pharyngeal colonization by group A *streptococcus* when conjugated to the widely shared C repeat region of M6 protein [Bessen and Fischetti, 1990].

	Antigen	Route	CTB administration	Reference
Proteins	Nucleoprotein of Influenza A virus	in	co-administered	[Guo et al., 2010]
	Hepatitis B virus surface antigen	in	co-administered	[Isaka et al., 2001]
	MSP4 5 malaria protein	Oral	co-administered	[Wang et al., 2003]
	OVA	im	co-administered	[Rolland-Turner et al., 2004]
	HIV-1 gp41	sl	chemically coupled	[Hervouet et al., 2010]
	Epitopes from <i>Schistosoma mansoni</i> glutathione-S-transferase	in	genetically fused	[Lebens et al., 2003]
Polysaccharide	Group B Streptococcus Type III Capsular Polysaccharide	in, oral, rectal, and vaginal	chemically coupled/co-administered	[Shen et al., 2000]
	Lipopolysaccharide from <i>V. cholerae</i> O1, serotype Inaba	sc	chemically coupled	[Gupta et al., 1998]
	<i>Pseudomonas aeruginosa</i> polysaccharide	Oral	co-administered	[Abraham and Robinson, 1991]
Micro-organisms	Measles virus	in, ig	co-administered	[Muller et al., 1995]
	Influenza virus	in	co-administered	[Yang et al.]
	Pneumocystis carinii	in	co-administered	[Pascale et al., 1999]

Table 1. Antigens towards which CT has adjuvant activity. in: intranasal, im: intramuscular, sl: sublingual, sc: subcutaneous, ig: intragastric.

Another way of using CTB as an adjuvant is in genetic constructions based on the toxin and heterologous antigens. In general, these hybrid molecules are composed of antigens fused to the amino [Laloi et al., 1996; Song et al., 2004] or carboxyl [Kim et al., 2004; Wang et al., 2010] terminus of CTB, being GM1-binding much more efficient in the latter case [Liljeqvist et al., 1997], but also protein epitopes have been introduced at internal positions in CTB

[Dertzbaugh and Elson, 1993]. Some examples of genetic incorporation of epitopes to CTB include triple glutamic acid decarboxylase [Gong et al., 2009], dodecapeptide repeat of the serine-rich *Entamoeba histolytica* protein [Zhang et al., 1995] and human insulin B-chain [Sadeghi et al., 2002]. There are many studies showing the induction of immune responses through immunization of mice with CTB fused to soluble antigens expressed both in bacteria [Larsson et al., 2004; Lee et al., 2003; Sun et al., 1999; Tsuji et al., 2003] and in transgenic plants [Jani et al., 2004; Matsumoto et al., 2009]. In all cases there was generation of IgG and IgA antigen-specific antibodies and, in some cases, protection. Some examples of the adjuvant action of CTB are shown in Table 1.

One of the strategies for using CTB as an adjuvant genetically fused to antigens has been described by Arêas *et al.* and is based on the expression vector called pAEctxB (Fig. 3.). In the generation of the vector, the gene *ctxB* was modified to ensure that the codons were those most frequently used by *E. coli*, *L. casei* and *S. typhimurium* [Areas et al., 2002]. The genetically engineered ORF was then cloned into the expression vector pAE [Ramos et al., 2004] and includes two consecutive restriction sites *MluI* and *HindIII*. The resulting vector allows expression, under the control of a T7 promoter, of proteins fused to the C-terminus of CTB with 6 histidine residues at the N terminus, which facilitate protein purification by immobilized metal ion affinity chromatography.

The pAE-ctxB plasmid was used to clone the pneumococcal surface adhesin A (PspA) [Areas et al., 2004], the *Leptospira interrogans* protein LipL32 [Habarta et al., 2010], the fatty-acid binding protein from *Schistosoma mansoni* S14 [Henrique Roman Ramos, 2010], and the *Bordetella pertussis* type III secretion system effector protein Bsp22 (Olivera et al., unpublished results). Intradermal immunization with CTB-PspA induced high titers of anti-PspA IgG and partially protected mice after challenge with *S. pneumonia* [Areas et al., 2005]. Moreover, intranasal immunization with CTB-PsaA protected mice against colonization with *S. pneumoniae* without alteration of the natural oral or nasopharyngeal microbiota of mice [Pimenta et al., 2006]. CTB-Sm14 itself was not able to reduce *Schistosoma mansoni* worm burden on intranasally immunized BALB/c mice, but reduced the hepatic granulomas around trapped eggs. CTB-LipL32 generated higher specific titers in mice immunized without external adjuvant than co-administration of CTB with LipL32, supporting CTB-LipL32 as a promising antigen for use in the control and study of leptospirosis.

## 8. CTB for mucosal immunotherapy

Mucosal administration by the oral, sublingual or nasal routes of many antigens can induce peripheral tolerance. Mucosal-induced tolerance has been recognized for a long time as a promising approach to prevent or treat allergic or autoimmune disorders and is characterized by a decreased immune response to systemic immunization with the same antigen [Sun et al., 2009; Sun et al., 1994]. In this regard, promising results have been obtained with auto-antigen coupled to CTB in order to induce oral tolerance. Although not known the mechanism by which CTB conjugated to antigens has the ability to potentiate the induction of oral tolerance, it is believed that in addition to the processes already mentioned before for CT, it may result in selected DC subsets with increased ability to induce different types of TGF- $\beta$ -expressing suppressor T cells including CD4<sup>+</sup> CD25<sup>+</sup> Tr cells [Holmgren et al., 2005] and a direct depletion of effector T cells since CTB induces CD4<sup>+</sup> and CD8<sup>+</sup> T cell apoptosis [Christelle Basset, 2010].

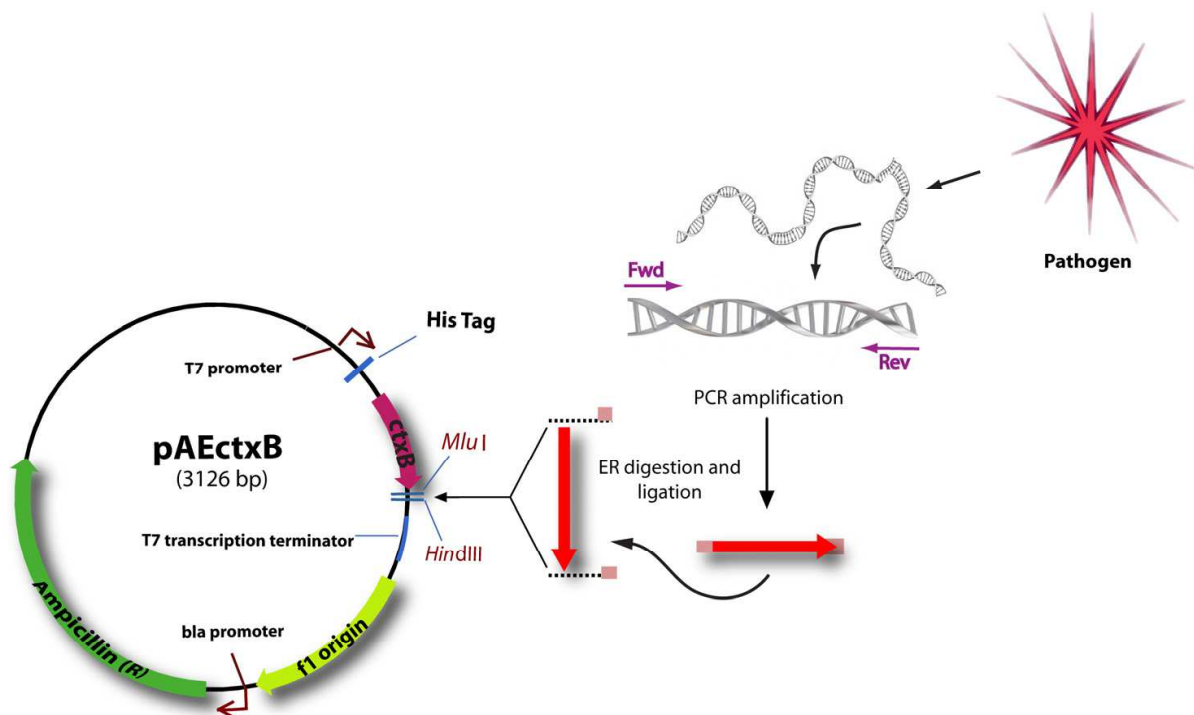


Fig. 3. Cloning strategy into pAEctxB plasmid

Oral delivery of CTB conjugated to myelin basic protein protected mice [Sun et al., 1996; Yuki et al., 2001] and rats [Sun et al., 2000b] against the development of experimental autoimmune encephalomyelitis. It was proposed that the inhibitory effect was a result of both the induction of TGF- $\beta$ -producing Tr cells and down-regulation of IFN $\gamma$ , IL-12, TNF $\alpha$ , MCP-1 and RANTES in the CNS [Wang et al., 2009].

Oral administration of a CTB-insulin conjugate prevented diabetes in non-obese diabetic (NOD) mice [Arakawa et al., 1998; Bergerot et al., 1997; Gong et al., 2007; Petersen et al., 2003; Ploix et al., 1999], which was associated with a reduction in IFN $\gamma$  production and Tr cell migration into pancreatic islets [Aspard et al., 2002; Sobel et al., 1998]. On the other hand, oral administration of CTB-proinsulin fusion protein showed an increased expression of IL-4 and IL-10 in the pancreas of NOD-treated mice, suggesting that Th2 lymphocyte-mediated oral tolerance is a likely mechanism for the prevention of pancreatic insulinitis [Ruhlman et al., 2007].

Oral delivery of CTB conjugated to a 60 kDa heat-shock protein derived peptide prevented mucosal induced uveitis in rats, an effect that was associated with enhanced IL-10 and TGF- $\beta$ , and reduced IL-12 and IFN- $\gamma$  production [Phipps et al., 2003]. Furthermore, a I/II phase clinical trial of the same peptide conjugated to CTB administered orally to 8 patients allowed the withdrawal of all immunosuppressive drugs in 5 of the 8 patients without a relapse of uveitis [Stanford et al., 2004].

In addition, oral administration of CTB in mice inhibits the induction of trinitrobenzene sulfonic acid-induced colitis and reverses such colitis after it has been established. This inhibition is associated with suppression of IL-12 and IFN- $\gamma$  production [Boirivant et al., 2001; Coccia et al., 2005]. In a recent clinical trial, 40% of patients with active Crohn's disease responded to treatment with CTB [Stal et al., 2010].

CTB conjugates were also effective in the induction of tolerance to type II collagen, leading to a suppression of chondritis in a model of autoimmune ear disease [Kim et al., 2001]. Oral administration of allogeneic antigen linked to CTB induced immunological tolerance against allograft rejection [Sun et al., 2000a]. Finally, transconjunctival immunotherapy using CTB could suppress clinical effects for experimental allergic conjunctivitis in guinea pigs [Oikawa et al., 2011].

## 9. Conclusion

CT has been studied for over 40 years. Both CT and its non-toxic derivatives or its B subunit, have shown to be excellent mucosal adjuvants. The possibility to use them as biotechnological tools in the development of new vaccines is being intensively studied in the present. In recent years, the prospect to use CTB fused to different protein antigens became relevant because these proteins can be expressed in high levels in a soluble form and directly purified in their active form, requiring only one fermentation step. In addition, several reports have shown that CTB can generate oral tolerance to different conjugated antigens, opening ways for the treatment of autoimmune diseases. Hopefully, future studies will focus on the use of CTB in such important issues.

## 10. Acknowledgements

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT 07-00642 and PICT 07-00028 (RMG).

## 11. References

- Abraham E, Robinson A. 1991. Oral immunization with bacterial polysaccharide and adjuvant enhances antigen-specific pulmonary secretory antibody response and resistance to pneumonia. *Vaccine* 9(10):757-764.
- Adel-Patient K, Bernard H, Ah-Leung S, Creminon C, Wal JM. 2005. Peanut- and cow's milk-specific IgE, Th2 cells and local anaphylactic reaction are induced in Balb/c mice orally sensitized with cholera toxin. *Allergy* 60(5):658-664.
- Agren LC, Ekman L, Lowenadler B, Lycke NY. 1997. Genetically engineered nontoxic vaccine adjuvant that combines B cell targeting with immunomodulation by cholera toxin A1 subunit. *J Immunol* 158(8):3936-3946.
- Akhiani AA, Nilsson LA, Ouchterlony O. 1997. Intranasal administration of *Schistosoma mansoni* adult worm antigen in combination with cholera toxin induces a Th2 cell response. *Parasite Immunol* 19(4):183-190.
- Andersen CS, Dietrich J, Agger EM, Lycke NY, Lovgren K, Andersen P. 2007. The combined CTA1-DD/ISCOMs vector is an effective intranasal adjuvant for boosting prior *Mycobacterium bovis* BCG immunity to *Mycobacterium tuberculosis*. *Infect Immun* 75(1):408-416.
- Arakawa T, Chong DK, Merritt JL, Langridge WH. 1997. Expression of cholera toxin B subunit oligomers in transgenic potato plants. *Transgenic Res* 6(6):403-413.

- Arakawa T, Yu J, Chong DK, Hough J, Engen PC, Langridge WH. 1998. A plant-based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes. *Nat Biotechnol* 16(10):934-938.
- Areas AP, Oliveira ML, Miyaji EN, Leite LC, Aires KA, Dias WO, Ho PL. 2004. Expression and characterization of cholera toxin B-pneumococcal surface adhesin A fusion protein in *Escherichia coli*: ability of CTB-PsaA to induce humoral immune response in mice. *Biochem Biophys Res Commun* 321(1):192-196.
- Areas AP, Oliveira ML, Miyaji EN, Leite LC, Ho PL. 2005. Intradermal immunization of mice with cholera toxin B-pneumococcal surface protein A fusion protein is protective against intraperitoneal challenge with *Streptococcus pneumoniae*. *Infect Immun* 73(6):3810-3813.
- Areas AP, Oliveira ML, Ramos CR, Sbrogio-Almeida ME, Raw I, Ho PL. 2002. Synthesis of cholera toxin B subunit gene: cloning and expression of a functional 6XHis-tagged protein in *Escherichia coli*. *Protein Expr Purif* 25(3):481-487.
- Arimitsu H, Tsukamoto K, Ochi S, Sasaki K, Kato M, Taniguchi K, Oguma K, Tsuji T. 2009. Lincomycin-induced over-expression of mature recombinant cholera toxin B subunit and the holotoxin in *Escherichia coli*. *Protein Expr Purif* 67(2):96-103.
- Aspord C, Czerkinsky C, Durand A, Stefanutti A, Thivolet C. 2002. alpha4 integrins and L-selectin differently orchestrate T-cell activity during diabetes prevention following oral administration of CTB-insulin. *J Autoimmun* 19(4):223-232.
- 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(6):922-930.
- Bergerot I, Ploix C, Petersen J, Moulin V, Rask C, Fabien N, Lindblad M, Mayer A, Czerkinsky C, Holmgren J, Thivolet C. 1997. A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc Natl Acad Sci U S A* 94(9):4610-4614.
- Bergquist C, Johansson EL, Lagergard T, Holmgren J, Rudin A. 1997. Intranasal vaccination of humans with recombinant cholera toxin B subunit induces systemic and local antibody responses in the upper respiratory tract and the vagina. *Infect Immun* 65(7):2676-2684.
- Bergquist C, Lagergard T, Lindblad M, Holmgren J. 1995. Local and systemic antibody responses to dextran-cholera toxin B subunit conjugates. *Infect Immun* 63(5):2021-2025.
- Bessen D, Fischetti VA. 1990. Synthetic peptide vaccine against mucosal colonization by group A streptococci. I. Protection against a heterologous M serotype with shared C repeat region epitopes. *J Immunol* 145(4):1251-1256.
- Bianchi AT, Zwart RJ, Van der Heijden PJ. 1990. Induction of an enteric Ig-response against ovalbumin and stimulation of the response by cholera toxin and its B-subunit in mice. *Reg Immunol* 3(3):131-138.
- Boirivant M, Fuss IJ, Ferroni L, De Pascale M, Strober W. 2001. Oral administration of recombinant cholera toxin subunit B inhibits IL-12-mediated murine experimental (trinitrobenzene sulfonic acid) colitis. *J Immunol* 166(5):3522-3532.

- Bourguin I, Chardes T, Mevelec MN, Woodman JP, Bout D. 1991. Amplification of the secretory IgA response to *Toxoplasma gondii* using cholera toxin. *FEMS Microbiol Lett* 65(3):265-271.
- Braun MC, He J, Wu CY, Kelsall BL. 1999. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor beta1 and beta2 chain expression. *J Exp Med* 189(3):541-552.
- Bromander A, Holmgren J, Lycke N. 1991. Cholera toxin stimulates IL-1 production and enhances antigen presentation by macrophages in vitro. *J Immunol* 146(9):2908-2914.
- Clapp B, Golden S, Maddaloni M, Staats HF, Pascual DW. 2010. Adenovirus F protein as a delivery vehicle for botulinum B. *BMC Immunol* 11:36.
- Coccia EM, Remoli ME, Di Giacinto C, Del Zotto B, Giacomini E, Monteleone G, Boirivant M. 2005. Cholera toxin subunit B inhibits IL-12 and IFN- $\gamma$  production and signaling in experimental colitis and Crohn's disease. *Gut* 54(11):1558-1564.
- Cong Y, Oliver AO, Elson CO. 2001. Effects of cholera toxin on macrophage production of co-stimulatory cytokines. *Eur J Immunol* 31(1):64-71.
- Cong Y, Weaver CT, Elson CO. 1997. The mucosal adjuvanticity of cholera toxin involves enhancement of costimulatory activity by selective up-regulation of B7.2 expression. *J Immunol* 159(11):5301-5308.
- Cuburu N, Kweon MN, Song JH, Hervouet C, Luci C, Sun JB, Hofman P, Holmgren J, Anjuere F, Czerkinsky C. 2007. Sublingual immunization induces broad-based systemic and mucosal immune responses in mice. *Vaccine* 25(51):8598-8610.
- Chen KS, Strober W. 1990. Cholera holotoxin and its B subunit enhance Peyer's patch B cell responses induced by orally administered influenza virus: disproportionate cholera toxin enhancement of the IgA B cell response. *Eur J Immunol* 20(2):433-436.
- Chinnapen DJ, Chinnapen H, Saslowsky D, Lencer WI. 2007. Rafting with cholera toxin: endocytosis and trafficking from plasma membrane to ER. *FEMS Microbiol Lett* 266(2):129-137.
- Christelle Basset FT, Cyrille Di Martino, John Holton, John D. Clements and Evelyne Kohli. 2010. Cholera-Like Enterotoxins and Regulatory T cells. *Toxins* 2:1774-1795.
- Datta SK, Sabet M, Nguyen KP, Valdez PA, Gonzalez-Navajas JM, Islam S, Mihajlov I, Fierer J, Insel PA, Webster NJ, Guiney DG, Raz E. Mucosal adjuvant activity of cholera toxin requires Th17 cells and protects against inhalation anthrax. *Proc Natl Acad Sci U S A* 107(23):10638-10643.
- De SN. 1959. Enterotoxicity of bacteria-free culture-filtrate of *Vibrio cholerae*. *Nature* 183(4674):1533-1534.
- Dertzbaugh MT, Elson CO. 1993. Reduction in oral immunogenicity of cholera toxin B subunit by N-terminal peptide addition. *Infect Immun* 61(2):384-390.
- Douce G, Fontana M, Pizza M, Rappuoli R, Dougan G. 1997. Intranasal immunogenicity and adjuvanticity of site-directed mutant derivatives of cholera toxin. *Infect Immun* 65(7):2821-2828.
- Drew MD, Estrada-Correa A, Underdown BJ, McDermott MR. 1992. Vaccination by cholera toxin conjugated to a herpes simplex virus type 2 glycoprotein D peptide. *J Gen Virol* 73 ( Pt 9):2357-2366.

- Elson CO, Ealding W. 1984. Cholera toxin feeding did not induce oral tolerance in mice and abrogated oral tolerance to an unrelated protein antigen. *J Immunol* 133(6):2892-2897.
- Eriksson AM, Schon KM, Lycke NY. 2004. The cholera toxin-derived CTA1-DD vaccine adjuvant administered intranasally does not cause inflammation or accumulate in the nervous tissues. *J Immunol* 173(5):3310-3319.
- Fecek RJ, Marcondes Rezende M, Busch R, Hassing I, Pieters R, Cuff CF. 2010. Enteric reovirus infection stimulates peanut-specific IgG2a responses in a mouse food allergy model. *Immunobiology* 215(12):941-948.
- Fischer R, McGhee JR, Vu HL, Atkinson TP, Jackson RJ, Tome D, Boyaka PN. 2005. Oral and nasal sensitization promote distinct immune responses and lung reactivity in a mouse model of peanut allergy. *Am J Pathol* 167(6):1621-1630.
- Flach CF, Lange S, Jennische E, Lonroth I, Holmgren J. 2005. Cholera toxin induces a transient depletion of CD8<sup>+</sup> intraepithelial lymphocytes in the rat small intestine as detected by microarray and immunohistochemistry. *Infect Immun* 73(9):5595-5602.
- Freytag LC, Clements JD. 2005. Mucosal adjuvants. *Vaccine* 23(15):1804-1813.
- Fromantin C, Jamot B, Cohen J, Piroth L, Pothier P, Kohli E. 2001. Rotavirus 2/6 virus-like particles administered intranasally in mice, with or without the mucosal adjuvants cholera toxin and Escherichia coli heat-labile toxin, induce a Th1/Th2-like immune response. *J Virol* 75(22):11010-11016.
- Fujinaga Y, Wolf AA, Rodighiero C, Wheeler H, Tsai B, Allen L, Jobling MG, Rapoport T, Holmes RK, Lencer WI. 2003. Gangliosides that associate with lipid rafts mediate transport of cholera and related toxins from the plasma membrane to endoplasmic reticulum. *Mol Biol Cell* 14(12):4783-4793.
- Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT. 2000. Cholera toxin induces maturation of human dendritic cells and licenses them for Th2 priming. *Eur J Immunol* 30(8):2394-2403.
- George-Chandy A, Eriksson K, Lebens M, Nordstrom I, Schon E, Holmgren J. 2001. Cholera toxin B subunit as a carrier molecule promotes antigen presentation and increases CD40 and CD86 expression on antigen-presenting cells. *Infect Immun* 69(9):5716-5725.
- Glenn GM, Scharton-Kersten T, Vassell R, Mallett CP, Hale TL, Alving CR. 1998. Transcutaneous immunization with cholera toxin protects mice against lethal mucosal toxin challenge. *J Immunol* 161(7):3211-3214.
- Gong Z, Jin Y, Zhang Y. 2007. Suppression of diabetes in non-obese diabetic (NOD) mice by oral administration of a cholera toxin B subunit-insulin B chain fusion protein vaccine produced in silkworm. *Vaccine* 25(8):1444-1451.
- Gong Z, Long X, Pan L, Le Y, Liu Q, Wang S, Guo J, Xiao B, Zhou M, Mei D. 2009. Cloning, expression, purification and characterization of the cholera toxin B subunit and triple glutamic acid decarboxylase epitopes fusion protein in Escherichia coli. *Protein Expr Purif* 66(2):191-197.
- Gong ZH, Jin HQ, Jin YF, Zhang YZ. 2005. Expression of cholera toxin B subunit and assembly as functional oligomers in silkworm. *J Biochem Mol Biol* 38(6):717-724.

- Goto N, Maeyama J, Yasuda Y, Isaka M, Matano K, Kozuka S, Taniguchi T, Miura Y, Ohkuma K, Tochikubo K. 2000. Safety evaluation of recombinant cholera toxin B subunit produced by *Bacillus brevis* as a mucosal adjuvant. *Vaccine* 18(20):2164-2171.
- Guo L, Zheng M, Ding Y, Li D, Yang Z, Wang H, Chen Q, Sui Z, Fang F, Chen Z. 2010. Protection against multiple influenza A virus subtypes by intranasal administration of recombinant nucleoprotein. *Arch Virol* 155(11):1765-1775.
- Gupta RK, Taylor DN, Bryla DA, Robbins JB, Szu SC. 1998. Phase 1 evaluation of *Vibrio cholerae* O1, serotype Inaba, polysaccharide-cholera toxin conjugates in adult volunteers. *Infect Immun* 66(7):3095-3099.
- Habarta A, Abreu PA, Olivera N, Hauk P, Cedola MT, Ferrer MF, Ho PL, Gomez RM. 2010. Increased immunogenicity to LipL32 of *Leptospira interrogans* when expressed as a fusion protein with the cholera toxin B subunit. *Curr Microbiol* 62(2):526-531.
- Hagiwara Y, Kawamura YI, Kataoka K, Rahima B, Jackson RJ, Komase K, Dohi T, Boyaka PN, Takeda Y, Kiyono H, McGhee JR, Fujihashi K. 2006. A second generation of double mutant cholera toxin adjuvants: enhanced immunity without intracellular trafficking. *J Immunol* 177(5):3045-3054.
- Harokopakis E, Hajishengallis G, Michalek SM. 1998. Effectiveness of liposomes possessing surface-linked recombinant B subunit of cholera toxin as an oral antigen delivery system. *Infect Immun* 66(9):4299-4304.
- Hein MB, Yeo TC, Wang F, Sturtevant A. 1996. Expression of cholera toxin subunits in plants. *Ann N Y Acad Sci* 792:50-56.
- Henrique Roman Ramos PAM, Celso Raul Romero Ramos, Ana Paula de Mattos Arêas, Toshie Kawano and Paulo Lee Ho. 2010. A Genetic Fusion between Sm14 and CTB does not Reduce *Schistosoma mansoni* Worm Burden on Intranasally Immunized BALB/c Mice. *J Vaccin Vaccinat* 1(3):doi: 10.4172/2157-7560.1000111.
- Hervouet C, Luci C, Cuburu N, Cremel M, Bekri S, Vimeux L, Maranon C, Czerkinsky C, Hosmalin A, Anjuere F. 2010. Sublingual immunization with an HIV subunit vaccine induces antibodies and cytotoxic T cells in the mouse female genital tract. *Vaccine* 28(34):5582-5590.
- Holmgren J, Adamsson J, Anjuere F, Clemens J, Czerkinsky C, Eriksson K, Flach CF, George-Chandy A, Harandi AM, Lebens M, Lehner T, Lindblad M, Nygren E, Raghavan S, Sanchez J, Stanford M, Sun JB, Svennerholm AM, Tengvall S. 2005. Mucosal adjuvants and anti-infection and anti-immunopathology vaccines based on cholera toxin, cholera toxin B subunit and CpG DNA. *Immunol Lett* 97(2):181-188.
- Holmgren J, Lonnroth I, Svennerholm L. 1973. Tissue receptor for cholera exotoxin: postulated structure from studies with GM1 ganglioside and related glycolipids. *Infect Immun* 8(2):208-214.
- Imaoka K, Miller CJ, Kubota M, McChesney MB, Lohman B, Yamamoto M, Fujihashi K, Someya K, Honda M, McGhee JR, Kiyono H. 1998. Nasal immunization of nonhuman primates with simian immunodeficiency virus p55gag and cholera toxin adjuvant induces Th1/Th2 help for virus-specific immune responses in reproductive tissues. *J Immunol* 161(11):5952-5958.



- Isaka M, Yasuda Y, Mizokami M, Kozuka S, Taniguchi T, Matano K, Maeyama J, Mizuno K, Morokuma K, Ohkuma K, Goto N, Tochikubo K. 2001. Mucosal immunization against hepatitis B virus by intranasal co-administration of recombinant hepatitis B surface antigen and recombinant cholera toxin B subunit as an adjuvant. *Vaccine* 19(11-12):1460-1466.
- Jackson RJ, Fujihashi K, Xu-Amano J, Kiyono H, Elson CO, McGhee JR. 1993. Optimizing oral vaccines: induction of systemic and mucosal B-cell and antibody responses to tetanus toxoid by use of cholera toxin as an adjuvant. *Infect Immun* 61(10):4272-4279.
- Jani D, Meena LS, Rizwan-ul-Haq QM, Singh Y, Sharma AK, Tyagi AK. 2002. Expression of cholera toxin B subunit in transgenic tomato plants. *Transgenic Res* 11(5):447-454.
- Jani D, Singh NK, Bhattacharya S, Meena LS, Singh Y, Upadhyay SN, Sharma AK, Tyagi AK. 2004. Studies on the immunogenic potential of plant-expressed cholera toxin B subunit. *Plant Cell Rep* 22(7):471-477.
- Jhon Carlos Castaño Osorio JSP. 2002. Inmunización intranasal de ratones con la proteína Sag2 de *Toxoplasma gondii* asociada con la toxina colérica. *Rev Cubana Invest Biomed* 1:35-45.
- Jiang W, Baker HJ, Smith BF. 2003. Mucosal immunization with helicobacter, CpG DNA, and cholera toxin is protective. *Infect Immun* 71(1):40-46.
- Jiang XL, He ZM, Peng ZQ, Qi Y, Chen Q, Yu SY. 2007. Cholera toxin B protein in transgenic tomato fruit induces systemic immune response in mice. *Transgenic Res* 16(2):169-175.
- Johansson EL, Rask C, Fredriksson M, Eriksson K, Czerkinsky C, Holmgren J. 1998. Antibodies and antibody-secreting cells in the female genital tract after vaginal or intranasal immunization with cholera toxin B subunit or conjugates. *Infect Immun* 66(2):514-520.
- Kang SM, Yao Q, Guo L, Compans RW. 2003. Mucosal immunization with virus-like particles of simian immunodeficiency virus conjugated with cholera toxin subunit B. *J Virol* 77(18):9823-9830.
- Kim N, Cheng KC, Kwon SS, Mora R, Barbieri M, Yoo TJ. 2001. Oral administration of collagen conjugated with cholera toxin induces tolerance to type II collagen and suppresses chondritis in an animal model of autoimmune ear disease. *Ann Otol Rhinol Laryngol* 110(7 Pt 1):646-654.
- Kim TG, Gruber A, Langridge WH. 2004. HIV-1 gp120 V3 cholera toxin B subunit fusion gene expression in transgenic potato. *Protein Expr Purif* 37(1):196-202.
- Kim YS, Kim MY, Kim TG, Yang MS. 2009. Expression and assembly of cholera toxin B subunit (CTB) in transgenic carrot (*Daucus carota* L.). *Mol Biotechnol* 41(1):8-14.
- Klimpel GR, Asuncion M, Haithcoat J, Niesel DW. 1995. Cholera toxin and *Salmonella typhimurium* induce different cytokine profiles in the gastrointestinal tract. *Infect Immun* 63(3):1134-1137.
- Kohama H, Harakuni T, Kikuchi M, Nara T, Takemura Y, Miyata T, Sato Y, Hirayama K, Arakawa T. 2010. Intranasal administration of *Schistosoma japonicum* paramyosin induced robust long-lasting systemic and local antibody as well as delayed-type

- hypersensitivity responses, but failed to confer protection in a mouse infection model. *Jpn J Infect Dis* 63(3):166-172.
- Laloi P, Munro CL, Jones KR, Macrina FL. 1996. Immunologic characteristics of a *Streptococcus mutans* glucosyltransferase B sucrose-binding site peptide-cholera toxin B-subunit chimeric protein. *Infect Immun* 64(1):28-36.
- Larsson C, Holmgren J, Lindahl G, Bergquist C. 2004. Intranasal immunization of mice with group B streptococcal protein rib and cholera toxin B subunit confers protection against lethal infection. *Infect Immun* 72(2):1184-1187.
- Lavelle EC, Jarnicki A, McNeela E, Armstrong ME, Higgins SC, Leavy O, Mills KH. 2004. Effects of cholera toxin on innate and adaptive immunity and its application as an immunomodulatory agent. *J Leukoc Biol* 75(5):756-763.
- Lavelle EC, McNeela E, Armstrong ME, Leavy O, Higgins SC, Mills KH. 2003. Cholera toxin promotes the induction of regulatory T cells specific for bystander antigens by modulating dendritic cell activation. *J Immunol* 171(5):2384-2392.
- Leal-Berumen I, Snider DP, Barajas-Lopez C, Marshall JS. 1996. Cholera toxin increases IL-6 synthesis and decreases TNF-alpha production by rat peritoneal mast cells. *J Immunol* 156(1):316-321.
- Lebens M, Sun JB, Sadeghi H, Backstrom M, Olsson I, Mielcarek N, Li BL, Capron A, Czerkinsky C, Holmgren J. 2003. A mucosally administered recombinant fusion protein vaccine against schistosomiasis protecting against immunopathology and infection. *Vaccine* 21(5-6):514-520.
- Lee JB, Jang JE, Song MK, Chang J. 2009. Intranasal delivery of cholera toxin induces th17-dominated T-cell response to bystander antigens. *PLoS One* 4(4):e5190.
- Lee SF, Halperin SA, Salloum DF, MacMillan A, Morris A. 2003. Mucosal immunization with a genetically engineered pertussis toxin S1 fragment-cholera toxin subunit B chimeric protein. *Infect Immun* 71(4):2272-2275.
- Lencer WI, Tsai B. 2003. The intracellular voyage of cholera toxin: going retro. *Trends Biochem Sci* 28(12):639-645.
- Levine MM, Kaper JB, Black RE, Clements ML. 1983. New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. *Microbiol Rev* 47(4):510-550.
- Lian T, Bui T, Ho RJ. 1999. Formulation of HIV-envelope protein with lipid vesicles expressing ganglioside GM1 associated to cholera toxin B enhances mucosal immune responses. *Vaccine* 18(7-8):604-611.
- Liang XP, Lamm ME, Nedrud JG. 1988. Oral administration of cholera toxin-Sendai virus conjugate potentiates gut and respiratory immunity against Sendai virus. *J Immunol* 141(5):1495-1501.
- Liljeqvist S, Stahl S, Andreoni C, Binz H, Uhlen M, Murby M. 1997. Fusions to the cholera toxin B subunit: influence on pentamerization and GM1 binding. *J Immunol Methods* 210(2):125-135.
- Lonnroth I, Holmgren J. 1973. Subunit structure of cholera toxin. *J Gen Microbiol* 76(2):417-427.

- Lopes LM, Maroof A, Dougan G, Chain BM. 2000. Inhibition of T-cell response by *Escherichia coli* heat-labile enterotoxin-treated epithelial cells. *Infect Immun* 68(12):6891-6895.
- Lycke N, Severinson E, Strober W. 1990. Cholera toxin acts synergistically with IL-4 to promote IgG1 switch differentiation. *J Immunol* 145(10):3316-3324.
- Lycke N, Tsuji T, Holmgren J. 1992. The adjuvant effect of *Vibrio cholerae* and *Escherichia coli* heat-labile enterotoxins is linked to their ADP-ribosyltransferase activity. *Eur J Immunol* 22(9):2277-2281.
- Malley R, Morse SC, Leite LC, Areas AP, Ho PL, Kubrusly FS, Almeida IC, Anderson P. 2004. Multiserotype protection of mice against pneumococcal colonization of the nasopharynx and middle ear by killed nonencapsulated cells given intranasally with a nontoxic adjuvant. *Infect Immun* 72(7):4290-4292.
- Marinaro M, Staats HF, Hiroi T, Jackson RJ, Coste M, Boyaka PN, Okahashi N, Yamamoto M, Kiyono H, Bluethmann H, Fujihashi K, McGhee JR. 1995. Mucosal adjuvant effect of cholera toxin in mice results from induction of T helper 2 (Th2) cells and IL-4. *J Immunol* 155(10):4621-4629.
- Massey S, Banerjee T, Pande AH, Taylor M, Tatulian SA, Teter K. 2009. Stabilization of the tertiary structure of the cholera toxin A1 subunit inhibits toxin dislocation and cellular intoxication. *J Mol Biol* 393(5):1083-1096.
- Matsumoto Y, Suzuki S, Nozoye T, Yamakawa T, Takashima Y, Arakawa T, Tsuji N, Takaiwa F, Hayashi Y. 2009. Oral immunogenicity and protective efficacy in mice of transgenic rice plants producing a vaccine candidate antigen (As16) of *Ascaris suum* fused with cholera toxin B subunit. *Transgenic Res* 18(2):185-192.
- McKenzie SJ, Halsey JF. 1984. Cholera toxin B subunit as a carrier protein to stimulate a mucosal immune response. *J Immunol* 133(4):1818-1824.
- Mekalanos JJ, Collier RJ, Romig WR. 1979. Enzymic activity of cholera toxin. II. Relationships to proteolytic processing, disulfide bond reduction, and subunit composition. *J Biol Chem* 254(13):5855-5861.
- Merritt EA, Sarfaty S, van den Akker F, L'Hoir C, Martial JA, Hol WG. 1994. Crystal structure of cholera toxin B-pentamer bound to receptor GM1 pentasaccharide. *Protein Sci* 3(2):166-175.
- Mishra S, Yadav DK, Tuli R. 2006. Ubiquitin fusion enhances cholera toxin B subunit expression in transgenic plants and the plant-expressed protein binds GM1 receptors more efficiently. *J Biotechnol* 127(1):95-108.
- Miyata T, Harakuni T, Tsuboi T, Sattabongkot J, Kohama H, Tachibana M, Matsuzaki G, Torii M, Arakawa T. 2010. *Plasmodium vivax* ookinete surface protein Pvs25 linked to cholera toxin B subunit induces potent transmission-blocking immunity by intranasal as well as subcutaneous immunization. *Infect Immun* 78(9):3773-3782.
- Mohsen Arzanlou AR, Nader Shahrokhi, Ahmad Zavarani Hossini, Yoko Yasuda, Kunio Tochikubo, Rezaee MA. 2005. Expression of cholera toxin B subunit in *Saccharomyces cerevisiae*. *Annals of Microbiology*(2):145-150.
- Muller CP, Beauverger P, Schneider F, Jung G, Brons NH. 1995. Cholera toxin B stimulates systemic neutralizing antibodies after intranasal co-immunization with measles virus. *J Gen Virol* 76 ( Pt 6):1371-1380.

- Nishibuchi M, Seidler RJ. 1983. Medium-dependent production of extracellular enterotoxins by non-O-1 *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio fluvialis*. *Appl Environ Microbiol* 45(1):228-231.
- Northrup RS, Fauci AS. 1972. Adjuvant effect of cholera enterotoxin on the immune response of the mouse to sheep red blood cells. *J Infect Dis* 125(6):672-673.
- Nurkkala M, Wassen L, Nordstrom I, Gustavsson I, Slavica L, Josefsson A, Eriksson K. Conjugation of HPV16 E7 to cholera toxin enhances the HPV-specific T-cell recall responses to pulsed dendritic cells in vitro in women with cervical dysplasia. *Vaccine* 28(36):5828-5836.
- Oikawa A, Shoji J, Inada N, Sawa M. 2011. Transconjunctival immunotherapy using cholera toxin B to treat experimental allergic conjunctivitis in a mouse model. *Jpn J Ophthalmol* 55(5):534-540.
- Okahashi N, Yamamoto M, Vancott JL, Chatfield SN, Roberts M, Bluethmann H, Hiroi T, Kiyono H, McGhee JR. 1996. Oral immunization of interleukin-4 (IL-4) knockout mice with a recombinant *Salmonella* strain or cholera toxin reveals that CD4+ Th2 cells producing IL-6 and IL-10 are associated with mucosal immunoglobulin A responses. *Infect Immun* 64(5):1516-1525.
- Oszvald M, Kang TJ, Tomoskozi S, Jenes B, Kim TG, Cha YS, Tamas L, Yang MS. 2008. Expression of cholera toxin B subunit in transgenic rice endosperm. *Mol Biotechnol* 40(3):261-268.
- Pascale JM, Shaw MM, Durant PJ, Amador AA, Bartlett MS, Smith JW, Gregory RL, McLaughlin GL. 1999. Intranasal immunization confers protection against murine *Pneumocystis carinii* lung infection. *Infect Immun* 67(2):805-809.
- Peltola H, Siitonen A, Kyronseppa H, Simula I, Mattila L, Oksanen P, Kataja MJ, Cadoz M. 1991. Prevention of travellers' diarrhoea by oral B-subunit/whole-cell cholera vaccine. *Lancet* 338(8778):1285-1289.
- Petersen JS, Bregenholt S, Apostolopolous V, Homann D, Wolfe T, Hughes A, De Jongh K, Wang M, Dyrberg T, Von Herrath MG. 2003. Coupling of oral human or porcine insulin to the B subunit of cholera toxin (CTB) overcomes critical antigenic differences for prevention of type I diabetes. *Clin Exp Immunol* 134(1):38-45.
- Phipps PA, Stanford MR, Sun JB, Xiao BG, Holmgren J, Shinnick T, Hasan A, Mizushima Y, Lehner T. 2003. Prevention of mucosally induced uveitis with a HSP60-derived peptide linked to cholera toxin B subunit. *Eur J Immunol* 33(1):224-232.
- Pierce NF, Cray WC, Jr., Sacci JB, Jr. 1982. Oral immunization of dogs with purified cholera toxin, crude cholera toxin, or B subunit: evidence for synergistic protection by antitoxic and antibacterial mechanisms. *Infect Immun* 37(2):687-694.
- Pimenta FC, Miyaji EN, Areas AP, Oliveira ML, de Andrade AL, Ho PL, Hollingshead SK, Leite LC. 2006. Intranasal immunization with the cholera toxin B subunit-pneumococcal surface antigen A fusion protein induces protection against colonization with *Streptococcus pneumoniae* and has negligible impact on the nasopharyngeal and oral microbiota of mice. *Infect Immun* 74(8):4939-4944.
- Ploix C, Bergerot I, Durand A, Czerkinsky C, Holmgren J, Thivolet C. 1999. Oral administration of cholera toxin B-insulin conjugates protects NOD mice from

- autoimmune diabetes by inducing CD4<sup>+</sup> regulatory T-cells. *Diabetes* 48(11):2150-2156.
- Price GA, Russell MW, Cornelissen CN. 2005. Intranasal administration of recombinant *Neisseria gonorrhoeae* transferrin binding proteins A and B conjugated to the cholera toxin B subunit induces systemic and vaginal antibodies in mice. *Infect Immun* 73(7):3945-3953.
- Qadri F, Wenneras C, Ahmed F, Asaduzzaman M, Saha D, Albert MJ, Sack RB, Svennerholm A. 2000. Safety and immunogenicity of an oral, inactivated enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine in Bangladeshi adults and children. *Vaccine* 18(24):2704-2712.
- Qu D, Zheng B, Yao X, Guan Y, Yuan ZH, Zhong NS, Lu LW, Xie JP, Wen YM. 2005. Intranasal immunization with inactivated SARS-CoV (SARS-associated coronavirus) induced local and serum antibodies in mice. *Vaccine* 23(7):924-931.
- Quiding M, Nordstrom I, Kilander A, Andersson G, Hanson LA, Holmgren J, Czerkinsky C. 1991. Intestinal immune responses in humans. Oral cholera vaccination induces strong intestinal antibody responses and interferon-gamma production and evokes local immunological memory. *J Clin Invest* 88(1):143-148.
- Raghavan S, Ostberg AK, Flach CF, Ekman A, Blomquist M, Czerkinsky C, Holmgren J. 2010. Sublingual immunization protects against *Helicobacter pylori* infection and induces T and B cell responses in the stomach. *Infect Immun* 78(10):4251-4260.
- Raghavan S, Svennerholm AM, Holmgren J. 2002. Effects of oral vaccination and immunomodulation by cholera toxin on experimental *Helicobacter pylori* infection, reinfection, and gastritis. *Infect Immun* 70(8):4621-4627.
- Ramos CR, Abreu PA, Nascimento AL, Ho PL. 2004. A high-copy T7 *Escherichia coli* expression vector for the production of recombinant proteins with a minimal N-terminal His-tagged fusion peptide. *Braz J Med Biol Res* 37(8):1103-1109.
- Renuga G, Saravanan\* R, Babu Thandapani A, Arumugam KR. 2010. Expression of Cholera toxin B subunit in Banana callus culture. *JPharm Sci & Res* 2:26-33.
- Reuman PD, Keely SP, Schiff GM. 1991. Similar subclass antibody responses after intranasal immunization with UV-inactivated RSV mixed with cholera toxin or live RSV. *J Med Virol* 35(3):192-197.
- Rolland-Turner M, Farre G, Muller D, Rouet N, Boue F. 2004. Immunological tools for the assessment of both humoral and cellular immune responses in Foxes (*Vulpes vulpes*) using ovalbumin and cholera toxin B as an antigenic model. *Vaccine* 22(31-32):4163-4172.
- Ruhlman T, Ahangari R, Devine A, Samsam M, Daniell H. 2007. Expression of cholera toxin B-proinsulin fusion protein in lettuce and tobacco chloroplasts--oral administration protects against development of insulinitis in non-obese diabetic mice. *Plant Biotechnol J* 5(4):495-510.
- Russell MW, Wu HY. 1991. Distribution, persistence, and recall of serum and salivary antibody responses to peroral immunization with protein antigen I/II of *Streptococcus mutans* coupled to the cholera toxin B subunit. *Infect Immun* 59(11):4061-4070.

- Sadeghi H, Bregenholt S, Wegmann D, Petersen JS, Holmgren J, Lebens M. 2002. Genetic fusion of human insulin B-chain to the B-subunit of cholera toxin enhances in vitro antigen presentation and induction of bystander suppression in vivo. *Immunology* 106(2):237-245.
- Salmond RJ, Luross JA, Williams NA. 2002. Immune modulation by the cholera-like enterotoxins. *Expert Rev Mol Med* 4(21):1-16.
- Sanchez J, Holmgren J. 1989. Recombinant system for overexpression of cholera toxin B subunit in *Vibrio cholerae* as a basis for vaccine development. *Proc Natl Acad Sci U S A* 86(2):481-485.
- Sanchez J, Holmgren J. 2008. Cholera toxin structure, gene regulation and pathophysiological and immunological aspects. *Cell Mol Life Sci* 65(9):1347-1360.
- Sanchez J, Holmgren J. 2011. Cholera toxin - a foe & a friend. *Indian J Med Res* 133(2):153-163.
- Sasaki K, Kato M, Takahashi T, Ochi S, Ichinose Y, Shiraki K, Asano Y, Iwanaga M, Tsuji T. 2003. Live varicella vaccine polarizes the mucosal adjuvant action of cholera toxin or its B subunit on specific Th1-type helper T cells with a single nasal coadministration in mice. *J Med Virol* 70(2):329-335.
- Seo JY, Seong SY, Ahn BY, Kwon IC, Chung H, Jeong SY. 2002. Cross-protective immunity of mice induced by oral immunization with pneumococcal surface adhesin a encapsulated in microspheres. *Infect Immun* 70(3):1143-1149.
- Shen X, Lagergard T, Yang Y, Lindblad M, Fredriksson M, Holmgren J. 2000. Systemic and mucosal immune responses in mice after mucosal immunization with group B streptococcus type III capsular polysaccharide-cholera toxin B subunit conjugate vaccine. *Infect Immun* 68(10):5749-5755.
- Shin JS, Abraham SN. 2001. Cell biology. Caveolae--not just craters in the cellular landscape. *Science* 293(5534):1447-1448.
- Simmons CP, Mastroeni P, Fowler R, Ghaem-maghami M, Lycke N, Pizza M, Rappuoli R, Dougan G. 1999. MHC class I-restricted cytotoxic lymphocyte responses induced by enterotoxin-based mucosal adjuvants. *J Immunol* 163(12):6502-6510.
- Sjoblom-Hallen A, Marklund U, Nerstedt A, Schon K, Ekman L, Bergqvist P, Lowenadler B, (2010) Lycke NY. Gene expression profiling identifies STAT3 as a novel pathway for immunomodulation by cholera toxin adjuvant. *Mucosal Immunol* 3(4):374-386.
- Slos P, Dutot P, Reymund J, Kleinpeter P, Prozzi D, Kieny MP, Delcour J, Mercenier A, Hols P. 1998. Production of cholera toxin B subunit in *Lactobacillus*. *FEMS Microbiol Lett* 169(1):29-36.
- Sobel DO, Yankelevich B, Goyal D, Nelson D, Mazumder A. 1998. The B-subunit of cholera toxin induces immunoregulatory cells and prevents diabetes in the NOD mouse. *Diabetes* 47(2):186-191.
- Soenawan E, Srivastava I, Gupta S, Kan E, Janani R, Kazzaz J, Singh M, Shreedhar V, Vajdy M. 2004. Maintenance of long-term immunological memory by low avidity IgM-secreting cells in bone marrow after mucosal immunizations with cholera toxin adjuvant. *Vaccine* 22(11-12):1553-1563.
- Song H, Zhou L, Fang W, Li Y, Wang X, Fang H, Li X, Wu M, Qiu B. 2004. High-level expression of codon optimized foot-and-mouth disease virus complex epitopes and

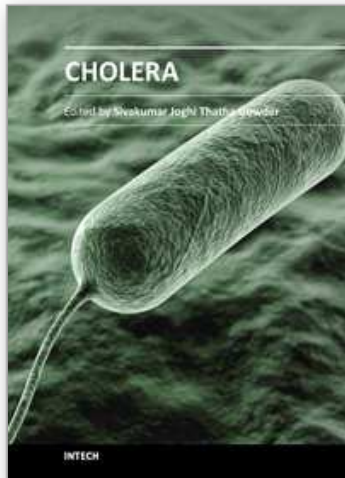
- cholera toxin B subunit chimera in *Hansenula polymorpha*. *Biochem Biophys Res Commun* 315(1):235-239.
- Soriani M, Bailey L, Hirst TR. 2002. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. *Microbiology* 148(Pt 3):667-676.
- Spangler BD. 1992. Structure and function of cholera toxin and the related *Escherichia coli* heat-labile enterotoxin. *Microbiol Rev* 56(4):622-647.
- Spira WM, Fedorka-Cray PJ. 1984. Purification of enterotoxins from *Vibrio mimicus* that appear to be identical to cholera toxin. *Infect Immun* 45(3):679-684.
- Staats HF, Ennis FA, Jr. 1999. IL-1 is an effective adjuvant for mucosal and systemic immune responses when coadministered with protein immunogens. *J Immunol* 162(10):6141-6147.
- Stal P, Befrits R, Ronnblom A, Danielsson A, Suhr O, Stahlberg D, Brinkberg Lapidus A, Lofberg R. 2010. Clinical trial: the safety and short-term efficacy of recombinant cholera toxin B subunit in the treatment of active Crohn's disease. *Aliment Pharmacol Ther* 31(3):387-395.
- Stanford M, Whittall T, Bergmeier LA, Lindblad M, Lundin S, Shinnick T, Mizushima Y, Holmgren J, Lehner T. 2004. Oral tolerization with peptide 336-351 linked to cholera toxin B subunit in preventing relapses of uveitis in Behcet's disease. *Clin Exp Immunol* 137(1):201-208.
- Sun JB, Czerkinsky C, Holmgren J. 2009. Mucosally induced immunological tolerance, regulatory T cells and the adjuvant effect by cholera toxin B subunit. *Scand J Immunol* 71(1):1-11.
- Sun JB, Holmgren J, Czerkinsky C. 1994. Cholera toxin B subunit: an efficient transmucosal carrier-delivery system for induction of peripheral immunological tolerance. *Proc Natl Acad Sci U S A* 91(23):10795-10799.
- Sun JB, Li BL, Czerkinsky C, Holmgren J. 2000a. Enhanced immunological tolerance against allograft rejection by oral administration of allogeneic antigen linked to cholera toxin B subunit. *Clin Immunol* 97(2):130-139.
- Sun JB, Mielcarek N, Lakew M, Grzych JM, Capron A, Holmgren J, Czerkinsky C. 1999. Intranasal administration of a *Schistosoma mansoni* glutathione S-transferase-cholera toxoid conjugate vaccine evokes antiparasitic and antipathological immunity in mice. *J Immunol* 163(2):1045-1052.
- Sun JB, Rask C, Olsson T, Holmgren J, Czerkinsky C. 1996. Treatment of experimental autoimmune encephalomyelitis by feeding myelin basic protein conjugated to cholera toxin B subunit. *Proc Natl Acad Sci U S A* 93(14):7196-7201.
- Sun JB, Xiao BG, Lindblad M, Li BL, Link H, Czerkinsky C, Holmgren J. 2000b. Oral administration of cholera toxin B subunit conjugated to myelin basic protein protects against experimental autoimmune encephalomyelitis by inducing transforming growth factor-beta-secreting cells and suppressing chemokine expression. *Int Immunol* 12(10):1449-1457.
- Taniguchi T, Harada T, Hayashi T, Tanikawa T, Kurohane K, Miyake M, Imai Y. 2008. Elevated production of Legionella-specific immunoglobulin A in A/J mice is accompanied by T-helper 1-type polarization. *Immunol Lett* 121(2):123-126.

- Teter K, Jobling MG, Sentz D, Holmes RK. 2006. The cholera toxin A1(3) subdomain is essential for interaction with ADP-ribosylation factor 6 and full toxic activity but is not required for translocation from the endoplasmic reticulum to the cytosol. *Infect Immun* 74(4):2259-2267.
- Thomas S, Preda-Pais A, Casares S, Brumeanu TD. 2004. Analysis of lipid rafts in T cells. *Mol Immunol* 41(4):399-409.
- Tsai B, Rodighiero C, Lencer WI, Rapoport TA. 2001. Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. *Cell* 104(6):937-948.
- Tsuji N, Suzuki K, Kasuga-Aoki H, Isobe T, Arakawa T, Matsumoto Y. 2003. Mice intranasally immunized with a recombinant 16-kilodalton antigen from roundworm *Ascaris* parasites are protected against larval migration of *Ascaris suum*. *Infect Immun* 71(9):5314-5323.
- Vajdy M, Lycke NY. 1992. Cholera toxin adjuvant promotes long-term immunological memory in the gut mucosa to unrelated immunogens after oral immunization. *Immunology* 75(3):488-492.
- Vanden Broeck D, Horvath C, De Wolf MJ. 2007. *Vibrio cholerae*: cholera toxin. *Int J Biochem Cell Biol* 39(10):1771-1775.
- Wang BN, Yang Y, Kuang Y, Cao K, Li MY, Li WY. 2010. [Construction eukaryotic plasmids of the ureI and ctB-ureI and profile its' expression in cell]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 26(8):764-766.
- Wang J, Lu ZH, Gabius HJ, Rohowsky-Kochan C, Ledeen RW, Wu G. 2009. Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. *J Immunol* 182(7):4036-4045.
- Wang L, Kedzierski L, Wesselingh SL, Coppel RL. 2003. Oral immunization with a recombinant malaria protein induces conformational antibodies and protects mice against lethal malaria. *Infect Immun* 71(5):2356-2364.
- Wilson AD, Bailey M, Williams NA, Stokes CR. 1991. The in vitro production of cytokines by mucosal lymphocytes immunized by oral administration of keyhole limpet hemocyanin using cholera toxin as an adjuvant. *Eur J Immunol* 21(10):2333-2339.
- 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(1):314-322.
- Wu HY, Russell MW. 1998. Induction of mucosal and systemic immune responses by intranasal immunization using recombinant cholera toxin B subunit as an adjuvant. *Vaccine* 16(2-3):286-292.
- Yamamoto S, Takeda Y, Yamamoto M, Kurazono H, Imaoka K, Fujihashi K, Noda M, Kiyono H, McGhee JR. 1997. Mutants in the ADP-ribosyltransferase cleft of cholera toxin lack diarrheagenicity but retain adjuvant activity. *J Exp Med* 185(7):1203-1210.
- Yang P, Tang C, Luo D, Zhan Z, Xing L, Duan Y, Jia W, Peng D, Liu X, Wang X. Cross-clade protection against HPAI H5N1 influenza virus challenge in BALB/c mice intranasally administered adjuvant-combined influenza vaccine. *Vet Microbiol* 146(1-2):17-23.



- Young-Sook Kim B-GK, Tae-Geum Kim, Tae-Jin Kang, Moon-Sik Yang. 2006. Expression of a cholera toxin B subunit in transgenic lettuce (*Lactuca sativa* L.) using *Agrobacterium*-mediated transformation system. *Plant Cell Tiss Organ Cult* 87:203-210.
- Yuki Y, Byun Y, Fujita M, Izutani W, Suzuki T, Udaka S, Fujihashi K, McGhee JR, Kiyono H. 2001. Production of a recombinant hybrid molecule of cholera toxin-B-subunit and proteolipid-protein-peptide for the treatment of experimental encephalomyelitis. *Biotechnol Bioeng* 74(1):62-69.
- Zhang T, Li E, Stanley SL, Jr. 1995. Oral immunization with the dodecapeptide repeat of the serine-rich *Entamoeba histolytica* protein (SREHP) fused to the cholera toxin B subunit induces a mucosal and systemic anti-SREHP antibody response. *Infect Immun* 63(4):1349-1355.

IntechOpen



## **Cholera**

Edited by Dr. Sivakumar Gowder

ISBN 978-953-51-0415-5

Hard cover, 218 pages

**Publisher** InTech

**Published online** 28, March, 2012

**Published in print edition** March, 2012

Cholera, a problem in Third World countries, is a complicated diarrheal disease caused by the bacterium *Vibrio cholerae*. The latest outbreak in Haiti and surrounding areas in 2010 illustrated that cholera remains a serious threat to public health and safety. With advancements in research, cholera can be prevented and effectively treated. Irrespective of "Military" or "Monetary" power, with one's "Own Power", we can defeat this disease. The book "Cholera" is a valuable resource of power (knowledge) not only for cholera researchers but for anyone interested in promoting the health of people. Experts from different parts of the world have contributed to this important work thereby generating this power. Key features include the history of cholera, geographical distribution of the disease, mode of transmission, *Vibrio cholerae* activities, characterization of cholera toxin, cholera antagonists and preventive measures.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Olivera Noelia, Maia Cédola and Ricardo M Gómez (2012). The Cholera Toxin as a Biotechnological Tool, Cholera, Dr. Sivakumar Gowder (Ed.), ISBN: 978-953-51-0415-5, InTech, Available from: <http://www.intechopen.com/books/cholera/the-cholera-toxin-as-a-biotechnological-tool->

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen