The B subunit of Shiga toxin coupled to full-size antigenic protein elicits humoral and cell-mediated immune responses associated with a Th1-dominant polarization

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Abstract

A number of studies in animal models and humans have shown that both humoral and cell-mediated immune responses play an important role in the control of viral infection and tumor development. In most cases, vaccination with non-vectorized peptides or proteins induces low antibody responses and fails to elicit specific cytotoxic T lymphocytes (CTL). In order to make vaccination more efficient, we chemically coupled the non-toxic B subunit of Shiga toxin (STxB) to a full-size antigenic model protein, ovalbumin (OVA), yielding STxB-OVA. We found that STxB-OVA delivers OVA-derived peptides into both the MHC class I- and II-restricted presentation pathways in mouse dendritic cells. Accordingly, the study of STxB trafficking in these cells revealed that, after internalization, a fraction of STxB followed the retrograde transport pathway to the endoplasmic reticulum, while another fraction was targeted to late endosomes/lysosomes. Vaccination of mice with STxB-OVA primed a specific anti-OVA CTL response without the use of adjuvants. Splenocytes and, particularly, CD4+ T cells from mice immunized with STxB-OVA also produced higher amounts of the Th1 cytokine IFN-γ and IgG2a-type antibodies than mice immunized with non-vectorized ovalbumin. In conclusion, this study identifies a unique non-live vaccine delivery system for polyepitopic antigens that elicits antigen-specific CTL, a humoral immune response and a Th1-type polarization without the use of adjuvant.

Introduction

In recent years, various strategies have been used for the development of vaccines against viruses and tumors. In contrast to current anti-bacterial vaccines whose efficacy mainly depends on their ability to elicit protective antibodies, vaccines designed to induce anti-tumor or anti-viral immunity must also boost the cell-mediated immune response, especially specific cytolytic T lymphocytes (CTL) (1–2). Since CD8+ and CD4+ T lymphocytes recognize peptides associated with MHC class I or II molecules respectively, peptide reagents were first used as immunogens for these vaccines. Synthetic peptide vaccines have led to tumor protection or virus clearance in murine models (3,4). However, in most cases, peptides failed to elicit efficient immune and clinical responses (5). Furthermore, in some instances, peptides have been found to induce specific T lymphocyte tolerance (6–7). Various groups have claimed that an improvement of the immunogenicity of peptides was observed when they were associated with adjuvants, coupled to vectors, pulsed to dendritic cells or engineered to enhance their affinity for MHC molecules. Nevertheless, human trials conducted with
peptides in these different formulations gave rise to modest clinical responses (8–10). When CTL were elicited after high-dose peptide vaccination, multiple in vitro immunizations were required to detect these CTL which often presented a low avidity (11–14).

The relative failure of peptide-based vaccines is likely related to recently discovered escape mechanisms developed by viruses and tumors.

Indeed, a mono-epitopic CTL response against retroviruses has frequently led to the emergence of virus escape mutants and subsequent disease progression (15–16). Cumulative evidence also suggests that tumor cells could escape immune attacks induced by peptides by means of partial or total loss of expression of tumor antigen and MHC class I molecules (17).

The use of peptide vaccines also raises a number of concerns, as immunodominant peptides have not been identified for most antigenic proteins. When a candidate immunodominant epitope has been selected, it varies from one individual to another, which may explain why, even in subjects with the same HLA class I haplotype, the detectable CTL response to a given pathogen is not necessarily identical (18). The use of peptides is also restricted to patients with a particular type of HLA and is not applicable to large outbred populations.

All these considerations argue in favor of the use of full-size proteins as model antigens to overcome many of the disadvantages of peptide vaccines. Unfortunately, administration of soluble proteins alone generally does not induce CTL responses, presumably because the antigen is not introduced into the MHC class I antigen presentation pathway. Its efficiency to elicit antibody is also often poor, requiring the use of adjuvants (19).

Attenuated recombinant live vectors, such as virus or bacteria, and naked plasmid DNA encoding large antigenic proteins have been shown to induce humoral and cytotoxic T cell responses (20–22). However, a number of safety issues have not been conclusively resolved (23). For example, reversion of attenuated live vectors to virulent strains by genetic recombination cannot be excluded. Furthermore, even attenuated viral or bacterial strains are associated with health risks for immunodeficient recipients. With respect to DNA vaccines, the consequences of stable integration of exogenous DNA into the host genome have not been clearly established. Synthetic vectors or adjuvants with the ability to efficiently target peptides into the MHC class I pathway and mediate the delivery and presentation of peptides derived from an antigenic model protein by both MHC class I and II molecules. In addition, STxB elicits a humoral and cytotoxic T cell response associated with a Th1-dominant polarization in mice.

**Methods**

**Mice**

Female C57BL/6 (H-2b) and BALB/c mice from Iffa Credo (L’Arbresle, France) at were used 6–8 weeks of age.

**Recombinant proteins and peptides**

To allow for chemical coupling of antigenic peptides or proteins to a defined acceptor site in STxB, a Cys was added to the C-terminus of the wild-type protein, yielding STxB-Cys. The recombinant mutant STxB-Cys protein was produced as previously described (25). Endotoxin concentrations determined by the Limulus assay test (Biowhittaker, Walkersville, MD) were <0.5 EU/ml.

Purified chicken ovalbumin (OVA) (grade V) was obtained from Sigma (St Quentin Fallavier, France).

OVA was linked to STxB-Cys by chemical coupling. Briefly, OVA was first activated via amino groups on lysine side chains using the hetero-bifunctional cross-linker MBS (Pierce, Rockford, IL). Activated OVA was then reacted with STxB-Cys, and the reaction product was purified by gel filtration and immunoaffinity chromatography.

The synthetic OVA-derived peptides OVA\textsubscript{257-264} (SL8) and OVA\textsubscript{323-339} encompassing the 257–264 and 323–339 residues of OVA respectively were obtained from Altegron (Schiltigheim, France) and stored in PBS.

**Cell lines**

The D1 dendritic cell line, obtained from P. Ricciardi-Castagnoli, was cultured in IMDM (Sigma) supplemented with 10% heat-inactivated FCS, 2 mM glutamine (Sigma), 5 mM sodium pyruvate and 50 μM 2-mercaptoethanol with 30% conditioned medium from granulocyte macrophage colony stimulating factor-producing NIH-3T3 (R1 medium), as previously described (26). The mouse thymoma cell line EL4 (H-2b) was kindly provided by K. Rock (University of Massachusetts Medical School, Worcester, MA), and maintained in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM glutamine (Sigma), 5 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin. B3Z is a CD8+ T cell hybridoma specific for the OVA\textsubscript{257-264} peptide in the context of K\textsuperscript{b}, which carries a LacZ construct driven by NF-AT elements from the IL-2 promoter. It was a generous gift from N. Shastry (University of California, Berkeley, CA).

BO97.10.5, a CD4+ T cell hybridoma specific for the OVA\textsubscript{323-339} in the context of I-A\textsuperscript{b}, was provided by L. Adorini (Roche Milano Ricerche, Italy).

Bone marrow-derived dendritic cells were prepared as previously described (25).

**Inhibition of Gb\textsubscript{3} expression**

D1 dendritic cells were cultured for 6 days with 5 μM 1-phenyl-2-hexa-decanoylamino-3-morpholino-1-propanol (PPMP; Calbiochem, La Jolla, CA). Gb\textsubscript{3} and K\textsuperscript{b} expression were monitored by cytometry.

**Antigen-presentation assay**

For OVA-derived SL8 K\textsuperscript{b}-restricted peptide presentation, dendritic cells (10\textsuperscript{5} cells/well) were first pulsed with antigen
for 5 h and washed twice, before being co-cultured overnight with B3Z hybridoma cells (2 × 10⁵ cells/well). A colorimetric assay with ONPG (o-nitrophenyl β-D-galactopyranoside) (Sigma) as substrate was used to detect β-galactosidase activity in B3Z lysates.

For the analysis of OVA<sub>323-339</sub> I-A<sup>β</sup>-restricted peptide presentation, dendritic cells (10<sup>5</sup> cells/well), B097.10.5 hybridoma cells (10<sup>5</sup> cells/well) and antigen were co-cultured for 18 h.

Activation of the hybridoma cells was determined by IL-2 production in the supernatants harvested 18 h after co-culture and measured by a commercial ELISA (PharMingen, San Diego, CA).

In some experiments, lactacystin (Biomol, Plymouth Meeting, PA) was used for inhibition studies.

### Cytotoxicity assay

Cytotoxicity was assessed on <sup>51</sup>Cr-labeled target cells, as previously described (27).

### Serological analysis

Mice were immunized i.p. 3 times at 2-week intervals with the various immunogens. Blood was collected 1 week after the last immunization by retro-orbital puncture using heparinized glass pipettes.

Anti-OVA IgG1 and IgG2a antibodies were measured by ELISA. Briefly, 96-well break-away, flat-bottomed microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 µl of OVA (50 µg/ml) diluted in carbonate buffer (0.1 M Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>, pH 9.6) overnight at 4°C. The plates were then saturated with 200 µl of PBS/1% BSA for 1 h at room temperature. After washes with PBS/0.05% Tween 20 (Merck, Schuchardt, Germany), 100 µl of diluted mouse serum was added and incubated for 2 h at 37°C. After washes, the plates were incubated with 100 µl of alkaline phosphatase-labeled goat anti-mouse IgG1 or IgG2a antibodies (Southern Biotechnologies, Birmingham, AL) for 2 h at room temperature. The wells were then rinsed 3 times and 50 µl of phosphatase substrate (Sigma) was added. The absorbance was read at a wavelength of 405 nm.

### Cytokine assay

Splenocytes, previously primed in vivo, were plated on 96-well plates at 8 × 10⁵ cells/well with culture medium alone or with various concentrations of OVA. Culture supernatants were collected 48 h later, and IFN-γ and IL-4 were measured by ELISA using commercial kits (PharMingen).

### Immunofluorescence study

Immunofluorescence was performed as previously described (28). Briefly, Cy3-labeled STxB was bound to D1 cells on ice, which were then incubated for 45 min at 37°C. The cells were fixed with 3% paraformaldehyde, quenched with ammonium chloride, permeabilized and stained using the CTR433 mAb recognizing a medial-Golgi marker (gift of Michel Bornens) or an anti-Lamp-1 antibody (PharMingen).

### STxB degradation experiment

To measure the proteolytic degradation of STxB in D1 cells, iodinated STxB (5000 c.p.m./ng) was bound to D1 cells on ice. These cells were then shifted to 37°C and the amount of TCA-soluble material in the culture medium was determined as a percentage of total cell-associated radioactivity, at the indicated times.

### Glycosylation experiment

The glycosylation of the STxB variant STxB-Glyc-KDEL was performed as previously described (28). In brief, 50 nM recombinant iodinated STxB carrying an N-glycosylation site was incubated with D1 cells for 45 min on ice (time 0). After washing, cells were incubated at 37°C for various lengths of time. At the end of each incubation period, cells were solubilized in sample buffer and lysates were analyzed on 10-20% SDS-PAGE gradient gels, followed by autoradiography.

### Results

**Receptor-dependent delivery of exogenous whole protein into the MHC class I and II pathways via STxB**

Our previous data showed that peptide-fused STxB allowed MHC class I-restricted presentation of exogenous peptides (25). In this study, we assessed the delivery of peptides derived from full-size protein into the MHC class I and II pathways, using STxB as a vector.

D1 dendritic cells (H-2<sup>b</sup>), sensitized with full-size OVA chemically coupled to STxB (STxB-OVA), were able to present the immunodominant OVA-derived SL8 peptide to B3Z, an anti-SL8-specific CD8<sup>+</sup> T cell hybridoma (Fig. 1A). To control the specificity of T cell activation, we demonstrated that an irrelevant anti-Cw3 T cell hybridoma was not activated under the same conditions (data not shown). As low as 5 nM STxB-OVA was sufficient to sensitize the D1 cell line for SL8 presentation (Fig. 1A). In contrast, dendritic cells incubated with as much as 500 nM of non-vectorized OVA protein failed to allow presentation of the SL8 peptide (Fig. 1A and data not shown).

STxB could also target the SL8 peptide derived from OVA in the MHC class I-restricted presentation pathway in bone marrow-derived dendritic cells (Fig 1B).

Since STxB specifically binds to the glycosphingolipid globotriaosyl ceramide, Gb3 (CD77), we tested whether this receptor was involved in STxB-dependent MHC class I-restricted presentation.

When dendritic D1 cells were cultured for 6 days with PPMP, an inhibitor of Gb3 synthesis, marked down-regulation of Gb3 expression at the plasma membrane was observed, while the expression of K<sup>b</sup> class I molecules at the plasma membrane was not changed [(25) and data not shown]. PPMP-treated and STxB-OVA-sensitized D1 cells lost the capacity to present the SL8 peptide, whereas exogenous synthetic SL8 peptides incubated with PPMP-treated cells activated the specific T cell hybridoma (Fig. 1C). The role of Gb3 was also supported by the inefficiency of STxB to deliver exogenous antigen into the MHC class I pathway of Gb3<sup>-/-</sup> cells such as T lymphocytes and some epithelial or tumor cells (data not shown).

Since CD4<sup>+</sup> T cell responses play a critical role in the initiation of a humoral immune response, and the priming and
differentiation of CD8+ T cells (29), we also examined the ability of STxB-OVA to target peptides onto MHC class II molecules. When D1 cells were pulsed with soluble OVA or STxB-coupled OVA (STxB-OVA), they presented the immunodominant OVA323-339 CD4 peptide to a specific I-Ab T cell hybridoma (Fig. 2). However, compared to soluble OVA, STxB-dependent MHC class II-restricted presentation was at least 50-fold more efficient (Fig. 2A and B).

Similarly to antigen delivery into the MHC class I pathway, STxB targeting of peptides into the MHC class II pathway occurred in a receptor-dependent manner. In contrast, the presentation of peptides derived from non-vectorial soluble OVA was not inhibited by the presence of Gb3 inhibitor (Fig 2). When a high concentration of STxB-OVA was used in the presence of PPMP, MHC class II presentation could occur, which corresponds to fluid phase internalization, as in the case of non-vectorized OVA (Fig. 2A).

STxB therefore allows both MHC class I- and II-restricted presentation of peptides derived from full-size exogenous protein.

**STxB-OVA processing is dependent on proteasome**

The presence of lactacystin, a specific proteasome inhibitor, during incubation of the bone marrow-derived dendritic cells with STxB-OVA inhibited the presentation of the SL8 epitope to CD8+ T cells, whereas direct presentation of the synthetic exogenous peptide SL8 was not affected by the presence of this drug (Fig. 3).

**Analysis of the intracellular transport pathway of STxB in the D1 dendritic cells**

We have previously reported that STxB has different intracellular transport itineraries in different cell types (28). Immunofluorescence and biochemical studies were carried out in order to analyze STxB trafficking in D1 cells. In a first experiment, STxB was covalently linked to a fluorescent probe (Cy3) and internalized for 45 min at 37°C into D1 cells, after binding to the plasma membrane. Accumulation of STxB in various cytoplasmic structures was observed (Fig. 4A). In a double labeling immunofluorescence experiment, an overlap was revealed between STxB and a medial-Golgi marker (Fig. 4A), suggesting that STxB enters the retrograde route in D1 cells. These results were confirmed by a biochemical assay, in which glycosylation of a STxB variant (carrying an N-glycosylation site) by endoplasmic reticulum-located oligosaccharyl transferase was observed (Fig. 4C). These results therefore show that after internalization into D1 cells, a fraction of STxB follows the retrograde transport pathway to the endoplasmic reticulum. Whether this targeting results in retrotranslocation of antigen into the cytosol (30,31) remains to be established (see Discussion).

The fluorescent studies also indicated that another fraction of STxB accumulated in the punctuate cytoplasmic structure that partially overlapped with markers of late endosomes/lysosomes (Fig. 4B). To directly test whether STxB could be degraded in D1 lysosomes, we monitored the appearance of TCA-soluble counts in the external medium. We observed that...
up to 14% of cell-associated STxB was degraded after 16 h (Fig. 4D). These results are consistent with partial targeting of STxB into the endosomal/lysosomal pathway, in which peptides resulting from proteolytic processing could associate with MHC class II molecules.

**STxB-OVA primes a specific cytotoxic T cell response**

The ability of STxB to target full-size exogenous proteins in both MHC class I- and II-restricted presentation pathways led us to test whether this special feature was associated with an increased immunogenicity of proteins coupled to this vector.

C57BL/6 mice were immunized on days 0 and 21 with STxB-OVA or non-vectorized OVA. On day 28, CTL activity was determined after a secondary in vitro stimulation with the immunodominant OVA-derived peptide, SL8.

As shown in Fig. 5, spleen cells from mice immunized with STxB-OVA (1 nmol) efficiently lysed EL4 target cells loaded with the SL8 peptide, whereas no cytotoxicity was observed against EL4 alone. In contrast, no cytotoxic activity could be demonstrated when mice were immunized with soluble OVA alone (Fig. 5).

As a control we showed that STxB alone did not induce anti-SL8 CTL (data not shown).

When mice were immunized with lower doses of STxB-OVA, the induction of CTL was weaker (data not shown).

**STxB polarizes a Th1-dominant immune response**

Spleen cells from immunized mice were also tested in vitro for their capacity to produce cytokines in response to various doses of soluble OVA.

Splenocytes of mice vaccinated with STxB-OVA secreted significantly more IFN-γ in response to OVA than splenocytes of mice immunized with OVA alone or OVA associated with adjuvants (Fig. 6). Depletion of CD4+ T cells suppressed IFN-γ secretion, confirming that the observed Th1 response was mainly mediated by CD4+ T cells (data not shown).

We did not detect any IL-4 production by these splenocytes after vaccination with STxB-OVA or soluble OVA, although
they secrete IL-4 after non-specific mitogenic stimulation (data not shown).

To assess the in vivo relevance of these results, we analyzed the antibody isotype profiles in serum of mice immunized with STxB-OVA, as a high IgG2a:IgG1 antibody ratio has been correlated with Th1 polarization of the immune response (32). Mice from two different genetic backgrounds (BALB/c and C57BL/6) were immunized 3 times at 2-week intervals with STxB-OVA, OVA alone or OVA successively mixed with complete (CFA) and incomplete Freund’s (IFA) adjuvants. Sera were obtained 10 days after the last immunization. Mice primed with OVA emulsified in CFA and boosted with IFA-associated antigen, followed by a third immunization with antigen alone, showed high levels of anti-OVA IgG1 antibodies, whereas mice immunized with STxB-OVA or soluble OVA alone produced lower levels of these antibodies (Fig. 7A and B). In contrast, anti-OVA IgG2a antibodies were higher in STxB-OVA-immunized mice than in those vaccinated with OVA alone or mixed with adjuvants (Fig. 7C and D). These results therefore support the Th1-dominant polarization induced by STxB.

Interestingly, STxB did not simply act as an adjuvant, like some other protein toxins, but rather exerted its activity as a vector, as no increase in IgG2a anti-OVA titers was observed when STxB and OVA were injected into mice as a simple mixture, without being physically linked (data not shown).
Discussion

In the present study, we have shown that STxB induces both MHC class I- and II-restricted presentation of peptides derived from STxB-coupled full-size exogenous protein. We have also shown that, inside D1 dendritic cells, STxB traffics through two pathways and its targeting to the late endosomal/lysosomal pathway may explain its ability to target exogenous protein into the MHC class II pathway.

The mechanisms underlying STxB-mediated MHC class I-restricted antigen presentation have yet to be fully established. STxB follows the retrograde pathway in D1 cells (Fig. 4), as demonstrated for other cell types (33). In toxin-sensitive cells, this transport pathway allows for retrotranslocation of the catalytic toxin A subunit from the lumen of the endoplasmic reticulum to the cytosol (30–31). It therefore appears possible that STxB targets exogenous protein into the cytosol via this pathway. However, it cannot be excluded that, at least in some cell types, STxB escapes to the cytosol by a membrane translocation mechanism operating at the level of endosomes or lysosomes, as suggested for other toxins (34).

When we previously analyzed STxB trafficking in human monocyte-derived dendritic cells, no indication for STxB targeting to the retrograde route could be obtained (28). Further work is required to establish at what level STxB-vectorized antigenic peptides and proteins cross the endomembranes to reach the cytosol.

Vaccination of mice with STxB-coupled full-size protein enhanced both the cell-mediated and humoral immune responses against this antigen. In mice, STxB primed CTL without the use of adjuvant (Fig. 5), which confirms and extends our previous results on CTL induction using peptide-coupled STxB (25).

The improvement of strategies to prime CTL represents a major goal of anti-tumor and anti-viral vaccines for various reasons. In mice, a correlation has generally been found between the ability to elicit anti-tumor CTL and clinical responses (5). In immunotherapy protocols based on the use of ex vivo antigen-pulsed dendritic cells, the detection of T cell immunity against a defined antigen after vaccination coincides with clinical responses (35–36). Similarly, CD8+ T cells mediate immunity to a broad range of viral, intracellular bacteria and protozoal pathogens (37).

Other synthetic vectors, such as pseudomonas exotoxin or Tat, efficiently introduce exogenous peptides into the MHC class I pathway, but failed to elicit CTL in mice (38–40). The fact that STxB, like other toxins, preferentially targets dendritic cells (25,41), the most powerful professional antigen-presenting cells (APC), may be a clue to explain its efficiency at inducing CTL. However, a recent study has shown that the delivery of antigen to dendritic cells without induction of their maturation failed to produce sustained antigen-specific immunity (42). Some non-live vectors that are efficient in triggering T cell immunity, such as the outer membrane protein A from Klebsiella pneumoniae (OmpA) or some heat shock proteins, target professional APC and induce their maturation (43–44). STxB does not appear to directly favor the maturation of DC, but it has been shown that STxB stimulates the production of tumor necrosis factor-α, a cytokine which acts as a maturation factor (45).
In contrast, the use of vectors that lead to rapid dissemination of antigens through the body and internalization into non-professional APC may lead to tolerance.

Splenocytes, and particularly CD4+ T cells, from mice immunized with STxB-OVA also produced higher amounts of the Th1 cytokine IFN-γ than mice immunized with non-vectorized OVA (Fig. 6). Since Th1 responses play an important role in protection against intracellular pathogens and tumors, the induction of such Th1 polarization is highly desirable for vaccines designed to target these diseases (46,47).

Although cytotoxic CD8+ T cells are considered to be the principal effectors of most anti-tumor and anti-viral immune responses, CD4+ T cells provide crucial helper functions required to prime and sustain the CD8 response, and activate NK and B cells. Recent studies have indicated that the licensing signal required for efficient priming of CTL and provided by CD4+ T cells to dendritic cells is mediated via CD154 signaling on CD40 of host APC (48–50). Efficient CTL induction also requires that the formation of peptide-class II complexes which are recognized by CD4+ T cells occurs as a result of intracellular processing and not following direct loading of free peptide onto class II molecule (51). The use of polypeptides or full-size proteins for the design of vaccines that efficiently induce cell-mediated responses therefore appears preferable over CD8 peptides alone or mixtures of CD4 and CD8 peptides.

Finally, immunization of mice with STxB coupled to OVA enhanced the production of IgG2a anti-OVA antibodies when compared to vaccination with OVA alone or OVA associated with CFA and IFA (Fig. 6C and D). IgG2a production might be secondary to STxB-induced IFN-γ secretion, a cytokine that favors the switch from IgM to IgG2a (32). In this context, although methods to induce antibody production are less actively pursued in the development of anti-tumor or anti-viral vaccines, convincing data nevertheless demonstrated that antibodies may participate in the host defense in these clinical situations. For example, tumor protection induced after vaccination with idioype-pulsed dendritic cells was associated with the induction of high titers of anti-Id antibodies of the IgG2a subclass (52). Similarly, a number of studies in animal and human models have shown a protective role of antibodies against acute infection with HIV-1 and simian HIV strains (53–55). It is noteworthy that protective antibodies are also elicited in the course of natural HIV infection (56).

When live vectors such as recombinant viruses are used for vaccine development, the immune response to virus proteins is a significant limitation to successful immunization in animals and humans. Even when viruses whose natural hosts are non-mammalian are used, such as avian poxvirus, a remaining problem is that immunity to antigenically complex vaccine vectors may diminish the reactivity to the selected antigen via the poorly understood mechanism of immunodominance (57). Immunogenicity concerns appear less relevant in the case of synthetic vectors, as it has been shown that preimmunization of mice with these non-live vectors did not compromise the subsequent immune response (27,58,59).

Conclusions

There is a great need for immunological adjuvants or vectors that are capable of stimulating both antibody and CTL
responses to vaccine antigens. STxB is a powerful vaccine delivery system for polyepitopic antigens that can elicit antigen-specific CTL, humoral immune responses and Th1 polarization without the use of adjuvant.

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**Abbreviations**

- APC: antigen-presenting cell
- CFA: complete Freund’s adjuvant
- CTL: cytotoxic T lymphocyte
- IFA: incomplete Freund’s adjuvant
- ONPG: o-nitrophenyl-β-D-galactopyranoside
- OVA: ovalbumin
- PPMP: 1-phenyl-2-hexa-decanoylamino-3-morpholino-1-propanol
- STxB: B subunit of *Shigella dysenteriae* toxin
The B subunit of Shiga toxin: an efficient non-live vector for vaccine

References


