

Review

Mucosal immunisation and adjuvants: a brief overview of recent advances and challenges

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Abstract

Mucosal immunisation may be used both to prevent mucosal infections through the activation of anti-microbial immunity and to treat selected autoimmune, allergic or infectious-immunopathological disorders through the induction of antigen-specific tolerance. The development of mucosal vaccines, whether for prevention of infectious diseases or for immunotherapy, requires antigen delivery and adjuvant systems that can efficiently help to present vaccine or immunotherapy antigens to the mucosal immune system. Promising advances have recently been made in the design of more efficient mucosal adjuvants based on detoxified bacterial toxin derivatives or CpG motif-containing DNA, and perhaps even more striking progress has been done in the use of virus-like particles as mucosal delivery systems for vaccines and of cholera toxin B subunit as antigen vector for immunotherapeutic tolerance induction. However, it is a memento that two recently developed mucosal vaccines for human use against rotavirus diarrhoea and influenza were withdrawn after a short period in the market because of adverse reactions among the vaccinees, thus emphasising the difficult and challenging task also for mucosal immunisation of combining vaccine and adjuvant efficacy with safety and acceptability.

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1. A need for mucosal vaccines and adjuvants

Mucosal immunisation, especially by the oral route, has recently attracted much interest both as a means for eliciting protective immunity against infectious diseases and also as a possible approach for immunological treatment of various diseases caused by an aberrant immune response associated with tissue-damaging inflammation (e.g. rheumatoid arthritis, inflammatory bowel diseases, Bechet's disease, lupus erythematosus).

There are several important reasons for using a mucosal route of vaccination instead of a parenteral route. The first and most important one is that the vast majority of infections occur at or take their departure from a mucosal surface and in these infections topical application of a vaccine is usually required to induce a protective immune response. Examples of such infections include: in the gastrointestinal tract, infections caused by *Helicobacter pylori*, *Vibrio cholerae*, enterotoxigenic *Escherichia coli*

(ETEC), *Shigella* spp., *Clostridium difficile*, rotaviruses and calici viruses; in the respiratory tract, infections caused by *Mycoplasma pneumoniae*, influenza virus and respiratory syncytial virus; and in the urogenital tract, infections associated with sexually transmitted diseases such as those caused mainly by HIV, *Chlamydia*, *Neisseria gonorrhoeae* and herpes simplex virus, and urinary tract infections caused by selected strains of *E. coli*. These infections still represent an enormous challenge for development of vaccines targeted to induce immunity that can prevent the infectious agent from attaching and colonising at the mucosal epithelium (non-invasive bacteria), or from penetrating and replicating in the mucosa (viruses and invasive bacteria), and/or can block the binding and action of microbial toxins. In many cases, the main protective effector function elicited by immunisation is the stimulation of a secretory local immunoglobulin A (IgA) antibody response and an associated mucosal immunologic memory. In other instances, although still less extensively studied, there is evidence for an important role also for the cellular arm of the immune response including mucosal CD8⁺ cytotoxic T lymphocytes (CTL), CD4⁺ T helper cells as well as natural killer (NK) cells [1].

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There are also important practical–logistic reasons to try to even replace some of the existing injectable vaccines used to prevent systemic infections with orally administered vaccines. Oral vaccines would be easier to administer than parenteral vaccines. They would also carry less risk of transmitting the type of infections still associated with the use of injectable vaccines in several parts of the world, such as hepatitis B virus and HIV infections. Oral vaccines can also be expected to have much greater acceptability than injectable vaccines by causing no sore arms, etc. Oral vaccine administration could also lead to simplified manufacturing vaccine, thereby increasing the potential for local vaccine production in developing countries.

Two additional indications for mucosal vaccination relate to the specific potential of mucosal vaccines to overcome the known barriers of parenteral vaccination caused by either pre-existing systemic immunity from previous vaccination or in young children from maternal antibodies, or selective immunodepression such as that caused by HIV infection. Thus, it was recently shown that vaginal mucosal vaccination can overcome the barrier to recombinant HIV vaccine immunisation caused by pre-existing poxvirus immunity [2]. It has also been shown that it is possible to induce a practically intact mucosal antibody responses to oral cholera vaccination in AIDS patients even after they had completely lost their ability to respond to an injectable vaccine (tetanus toxoid) [3].

Mucosal immunisation may also induce peripheral systemic tolerance especially against those T-cell-mediated immune reactions that are associated with the development of delayed type hypersensitive (DTH) reactions. This phenomenon, often referred to as “oral tolerance” because it was initially documented by the effect of oral administration of antigen, is characterised by the fact that animals which have been fed with or have inhaled an antigen may become refractory or have diminished capability to develop an immune response when re-exposed to the same antigen introduced by a parenteral injection. Oral tolerance is an important natural physiological mechanism to avoid development of DTH and other allergic reactions to many ingested food proteins and other antigens. Oral tolerance and induction of mucosal immunity in response to a mucosal immunisation are, however, not mutually exclusive aspects of an immune response. Although they usually involve different types of antigens, oral tolerance (or ignorance) being the default response to ingested food proteins or auto-antigens but difficult or impossible to induce with strong immunogens (whilst the converse is true for a mucosal immune response), sometimes the same mucosal immunisation may concomitantly give rise both to a local significant IgA antibody formation and to tolerance or suppressed immune responsiveness peripherally. Indeed, certain cytokines, such as transforming growth factor (TGF)- β and interleukin (IL)-4, are known to be involved both in the development of the mucosal immune response associated with local production of secretory IgA at the mucosal surface and in the induction

of oral tolerance peripherally. Since oral tolerance, at variance with many other forms of immunotherapy, is specific for the antigen initially ingested or inhaled and does not influence the development of systemic immune responses against other antigens, the induction of oral tolerance has become an attractive strategy for prevention and potential treatment of illnesses that are caused by immunopathological reactions against specific antigens encountered or expressed in preferentially non-mucosal tissues including both foreign antigens and auto-antigens [4].

Therefore, mucosal immunisation and oral tolerance induction represent promising approaches to protect an individual against, respectively, mucosal infectious agents and against systemic inflammatory “immunopathologies” due to selected chronic infections, autoimmune disorders and allergies.

2. Why so few mucosal vaccines?

However, despite these many attractive features it has often proved difficult in practice to stimulate strong mucosal IgA immune responses and protection by oral–mucosal administration of antigens, and the results to date of mucosal vaccination efforts using soluble protein antigens have been, with a few notable exceptions, rather disappointing. Indeed, only half a dozen of the current vaccines that are approved for human use are administered mucosally: the oral polio vaccine, oral killed whole-cell B subunit and live-attenuated cholera vaccines, an oral live-attenuated typhoid vaccine, an oral BCG live vaccine (used in Brazil for vaccination against tuberculosis) and an oral adenovirus vaccine (the latter vaccine being restricted to military personnel). Two recent additional mucosal vaccines, an oral live-attenuated vaccine against rotavirus diarrhoea and a nasal enterotoxin-adjuvanted inactivated influenza vaccine, were withdrawn after a short time on the market because of potential serious adverse reactions (intussusception and facial paresis, respectively), thus illustrating the complexity of mucosal vaccine development [5,6]. Likewise, in the area of oral tolerance, although promising results were obtained in initial clinical trials that used this principle for immunotherapy of autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, larger randomised placebo-controlled multicenter trials failed to show any significant therapeutic benefits of these agents over those achieved by the placebo [7,8].

As it will be discussed in this brief overview, current efforts to overcome obstacles to the development of effective mucosal vaccines and/or immunotherapies are mainly directed towards finding more efficient means of delivering appropriate antigens to the mucosal immune system, and towards developing effective, safe mucosal adjuvants or immunoregulatory agents that, depending on the goal and the systems used, provide protective immunity against infectious agents or induce the suppression of peripheral immunopathological disorders, respectively.

3. Bacterial toxins and derivatives as mucosal adjuvants

The most widely experimentally used mucosal adjuvants in animals are cholera toxin (CT) and the closely related *E. coli* heat labile enterotoxin (LT). CT and LT consist of a homo-pentamer of cell-binding B subunits associated with a single toxic-active A subunit. The A subunit enzymatically ADP-ribosylates the Gs protein of adenylate cyclase leading to increased cAMP production in the affected cells [9]. Both CT and LT are powerful mucosal adjuvants. They strongly potentiate the immunogenicity of most other antigens, whether these are linked to or simply admixed with the toxins, provided that the other antigen is given at the same time and at the same mucosal surface as the toxins. However, both CT and LT are normally considered to be much too toxic for human use. Great efforts have recently been made to define whether it would be possible to separate the adjuvant activity from their toxic activity as a basis for development of mucosal adjuvants effective for human use.

3.1. Adjuvant action of CT and LT

CT and LT have been found to affect several steps in the induction of a mucosal immune response, which alone or in combination might explain their strong adjuvant action after oral immunisation. These effects include: (a) increased permeability of the intestinal epithelium leading to enhanced uptake of co-administered antigen; (b) enhanced antigen presentation by a variety of cell types; (c) promotion of isotype differentiation in B cells leading to increased IgA formation; and (d) complex stimulatory as well as inhibitory effects on T-cell proliferation and cytokine production. In addition, CT and LT have been shown to not only fail to induce oral tolerance but even to abrogate otherwise efficient regimens for tolerance induction by oral antigen administration [10,11].

Among these many effects, those leading to enhanced antigen presentation by various APC are probably of the greatest importance for the potent adjuvant activity of CT and LT. CT has been shown to increase antigen presentation by dendritic cells (DC), macrophages and B cells and has also been found, at least in vitro, to make intestinal epithelial cells to become effective APC. Consistent with this activity, CT has been shown on both murine and human DC and other APC to up-regulate the expression of MHC/HLA-DR molecules, CD80/B7.1 and CD86/B7.2 co-stimulatory molecules as well as chemokine receptors such as CCR7 and CXCR4 [12,13]. Furthermore, CT also induces the secretion of IL-1 β from DC [13], thus supporting similar observations made earlier for macrophages. IL-1 not only induces the maturation of DC, but is also by itself an efficient mucosal adjuvant when co-administered with protein antigens and might be responsible for a substantial part of CT adjuvant activity [14].

It has been claimed that CT primarily induces Th2 type immune responses characterised by CD4⁺ T cells produc-

ing IL-4, IL-5, IL-6 and IL-10 and by the production of IgA, IgG1 and IgE antibodies. LT, on the other hand, has been reported to induce a mixed Th1 and Th2 type immune response. However, our own work indicates that also CT induces mixed Th1 and Th2 types of immune responses, in contrast to CT B unit (CTB), which appears to induce a more restricted Th2 type of immune response [13]. Thus, DC, which had been pre-treated in vitro with a protein antigen (ovalbumin, OVA) linked to or admixed with CT and then injected into mice, did indeed induce in an antigen-specific manner both Th1 and CTL responses in addition to a Th2 response. In contrast, DC pulsed in vitro with OVA linked to CTB only gave rise to a Th2 type of immune response.

3.2. Development of non-toxic derivatives as mucosal adjuvants

To avoid toxicity, isolated B units of CT and LT (CTB and LTB) have been explored for their ability to augment immune responses against co-administered antigens. However, their capacity as mucosal adjuvants has proven to be much less than that of the holotoxins. Indeed, both CTB and LTB are poor adjuvants, if at all active, when given to animals together with non-coupled antigens by the oral route, although they display a more significant adjuvant activity when administered via the nasal route. Mice vaccinated intranasally with the influenza virus HA vaccine PR8 H1N1 together with LTB had higher levels of anti-viral IgA and IgG both in sera and in nasal and lung secretions compared to mice given the subunit vaccine alone, and were also protected against an intranasal viral challenge [15]. Adjuvanticity of CTB or LTB is much improved when coupled to antigens, possibly because of efficient presentation of the coupled antigen not only by DC and macrophages but also by naïve B cells [16].

Recently, site-directed mutagenesis has permitted the generation of LT and CT mutants that have reduced toxicity, but which retain significant adjuvanticity when given to animals by the nasal–mucosal route or, even though they then perform less well, by the oral–mucosal route [17]. Another approach that is used to circumvent the harmful drawbacks of CT or LT adjuvants is to link the enzymatically active A subunit domain of the toxin to a cell-binding moiety other than the natural B subunit, such as the cell-binding domain of *Staphylococcus aureus* protein A (CTA1-DD). CTA1-DD, like most other toxin derivatives, functions when applied nasally but not when given orally. This limitation has recently been overcome by the incorporation of CTA1-DD fused to a short peptide into immune stimulating complexes (ISCOM). Oral vaccination with the ISCOM–CTA1-DD complex induced systemic and mucosal responses with both Th1 and Th2 characteristics [18].

To achieve detoxification of CT, yet another type of mutants was recently described in which peptides are added to the CTA1 amino end. The added peptides seem to reduce both enterotoxicity and ADP-ribosylating activity by

sterical hindrance with the CTA1 active site [19]. In general, in these (like in all other detoxified) constructs, adjuvanticity decreased with decreasing enterotoxicity/ADP-ribosylation. However, a mutant (eCT6), with 10–20-fold lower enterotoxicity, displayed a level of adjuvanticity comparable to that of the wild type CT. Another mutant with a longer peptide linked to CTA1 (eCT23) and no detectable toxic activity, although being also much less potent in adjuvant activity than either CT or eCT6, was superior to CTB as both mucosal immunogen and as adjuvant for a co-administered antigen.

4. Mucosal stimulation of innate and adaptive immunity by CpG DNA

The innate immune system senses infectious/harmful agents through pattern recognition Toll-like receptors (TLR) that specifically bind several pathogen-associated molecular patterns (so-called PAMP), such as lipopolysaccharide, peptidoglycan, flagellin, and bacterial CpG DNA [20]. Recognition of PAMP by TLR triggers a signalling pathway that leads to activation of NF κ B transcription factors and members of the MAP kinase family. This results in production of pro-inflammatory cytokines and up-regulation in expression of co-stimulatory molecules, thereby activating not only innate immunity but ultimately also acquired immune responses. Thus, activation of the innate immune system through TLR by using TLR ligands may represent an attractive strategy to enhance immune responses against pathogens.

Bacterial DNA containing cytosine–phosphate–guanine (CpG) motifs, in which the cytosine is unmethylated, as well as synthetic oligodeoxynucleotides containing immunostimulatory CpG motifs (CpG ODN), have well-characterised adjuvant properties when administered systemically together with an antigen [21]. The adjuvanticity of CpG DNA, which results from the binding of the CpG-rich DNA to TLR-9, is associated with the induction of both pro-inflammatory and Th1-inducing cytokines and chemokines, and the induction of MHC and co-stimulatory molecules on APC. The resulting immune responses in mice are Th1 dominated with high levels of cytotoxic T lymphocytes, IFN- γ production, and IgG2a antibody production.

Recent studies show that CpG ODN are also effective as mucosal adjuvants. Intranasal administration of CpG ODN together with purified protein antigens promotes a mucosal Th2 response with IgA antibody formation and a systemic Th1 response [22]. The concomitant appearance of mucosal IgA antibodies that can prevent the uptake of mucosally transmitted pathogens in conjunction with systemic complement-activating antibodies and cytotoxic T cells should be of great benefit in combating many infections that take their departure from a mucosal surface. The applicability of this approach for inducing protective immunity was recently documented in a murine model of genital herpes infection [23]. Nasal vaccination with a purified envelope

glycoprotein (gB) from HSV-1 induced strong vaginal IgA and systemic IgG2a responses together with the induction of both systemic and mucosal CTL. Importantly, the immune response obtained was cross-protective against the related HSV-2. Thus, approximately 50% of the animals were protected against a lethal vaginal challenge with HSV-2.

Recent work from our laboratory has documented the ability of vaginal–mucosal and oral–mucosal administration of immunostimulatory CpG ODN to stimulate innate immune responses in the murine female genital tract mucosa and the gastrointestinal tract mucosa, respectively [24,25]. Thus, a single mucosal–vaginal administration of immunostimulatory CpG ODN induced rapid production of the Th1 associated cytokines IFN- γ , IL-12, and IL-18 as well as CC chemokines RANTES, MIP-1 α , and MIP-1 β (unpublished data) in the murine female genital tract mucosa. Remarkably, a single vaginal dose of CpG ODN, in the absence of any viral antigen, also elicited protective immunity against a normally lethal genital herpes infection [24]. The treatment led to the development of a memory response conferring protection against re-infection. We could also show that immunostimulatory CpG DNA enhances innate immunity in the gastrointestinal tract mucosa. Thus, intragastric administration with CpG ODN was found to elicit local production of the CC chemokines RANTES, MIP-1 α and MIP-1 β and of the CXC chemokine IP-10 in the stomach and/or intestine. Importantly, intragastric administration of CpG ODN, in the absence of any exogenous bacterial antigen, also suppressed bacterial colonisation in the stomach of mice with an already established *H. pylori* infection (“Orally administered CpG oligodeoxynucleotide induces production of CXC and CC chemokines in the gastric mucosa and suppresses bacterial colonisation in a mouse model of *Helicobacter pylori* infection”; Raghavan et al., manuscript in preparation).

5. Mucosal adjuvant action of cytokines and chemokines

Most adjuvants exert at least some of their adjuvant activity through the induction of inflammatory or Th1-inducing cytokines and chemokines. In general, the more potent the adjuvant, the more inappropriate it is for human use. One approach to circumvent the problem with overtly toxic adjuvants is to mimic the signals they induce in vivo by simply adding these signalling molecules either directly as proteins, or indirectly as coding DNA. As discussed above, the most powerful mucosal adjuvants known to date are CT and LT, which promote strong mucosal IgA responses, systemic IgG responses and CTL to co-administered antigens. Several combinations of cytokines have been shown capable of replacing CT (or LT) as a nasal adjuvant. The most important of these is IL-1, which in combination with Th1-inducing cytokines such as IL-12, IL-18 and GM-CSF, can bring about as strong mucosal and systemic responses as CT [25]. The combination of IL-1 and IL-12/IL-18/GM-CSF gives

rise to a combined Th1 (CTL and IFN- γ)/Th2 (mucosal IgA) profile also against weak antigens such as synthetic peptides.

The addition of genes coding for specific chemokines, e.g. the CCR7 ligands which are involved in directing DC to the T-cell areas of secondary lymphoid organs, to a HSV-2 DNA plasmid vaccine has stimulated the immune responses in mice following both nasal and intragastric vaccination [26]. The use of RANTES (a chemoattractant for monocytes, T cells and NK cells and a potent inducer of Th1/CTL responses) as a mucosal adjuvant has also given promising initial results. Nasal co-administration of RANTES and a protein antigen was shown to enhance Th1 and Th2 responses both at local and remote mucosal tissues as well as systemically [27]. The protective efficacy of the different chemokines, however, remains to be determined.

6. Virus-like particles as mucosal antigen delivery and adjuvant systems

A variety of mucosal antigen delivery systems have been developed, including various inert systems as well as different live-attenuated bacterial or viral vector systems.

Among the inert vaccine delivery systems tested extensively in the last 10-year period are various lipid-based structures such as liposomes, ISCOMs and so-called cochlates; different types of biodegradable particles of different composition such as starch or co-polymers of poly(lactide-co-glycolide); and different mucosa-binding proteins, including both classical plant lectins and bacterial proteins such as CTB or LTB, to which antigens have been linked either chemically or as gene fusion proteins.

Among the many live bacterial vectors developed two main categories can be distinguished: those based on attenuated pathogens such as *Salmonella*, BCG or *Bordetella*; and those that use commensal bacteria, such as lactobacilli or certain streptococci and staphylococci. Among viral vectors developed, the initial use of vaccinia as the primary vector candidate has progressively been replaced by other poxviruses, such as canary poxvirus, and by adenoviruses. Several of the live vectors of both bacterial and viral origins have also been used to provide various cytokines to further stimulate or modulate the immune responses induced.

However, although many of these systems have shown promise there is still no such either inert or live vector approved for human use. It would go beyond the scope of this overview to discuss each of these systems and their potentials and limitations (which are partly covered by other articles in this volume). Here, we will instead briefly focus on the promising results reported recently from the use of so-called pseudoviruses, or virus-like particles (VLP). These are self-assembling non-replicating viral core structures, often from non-enveloped viruses, that are produced recombinantly in vitro. VLP are cheap and easy to make as well as highly immunogenic and are therefore of commercial interest as viral vaccines in their own right. VLP can, however,

also be used as carriers/adjuvants both for foreign antigens expressed recombinantly on the surface of the pseudovirus, and for DNA vaccines carried within VLP. VLP are especially interesting from a mucosal vaccine point of view as they offer opportunity to use the natural route of transmission of the present virus for vaccine delivery. Successful use of this principle has been reported from studies both in animals and in humans with VLP from several mucosal viral pathogens including papillomavirus, calicivirus (Norwalk agent) and hepatitis E virus. Thus, oral delivery of hepatitis E VLP was shown to be a strong inducer of both systemic and mucosal IgA responses both to hepatitis E virus and to foreign epitopes expressed as chimeric proteins on the VLP surface, and did not require any external adjuvants [28]. Intranasal delivery of VLP seems more efficient and requires lower doses of antigen than oral delivery, at least in the case of Norwalk VLP [29]. Furthermore, vaccination with human papillomavirus VLP coding for the papillomavirus onco-gene E7 protected mice against a mucosal challenge with an E7-expressing bovine papillomavirus VLP [30], proving the feasibility of this approach.

Another interesting VLP delivery system is the Sindbis virus replicon vector that specifically targets immature DC [31]. Both vaginal and rectal delivery of Sindbis virus replicon vectors expressing HIV gag proteins induced gag-specific IFN-secreting T cells in the vaginal mucosa and also protected mice against a vaginal challenge with a gag-expressing vaccinia virus [32].

7. Immune deviation and tolerization immunotherapy through mucosal antigen delivery

Mucosal tolerance is a mechanism whereby the immune system refrains from responding in a deleterious manner to harmless antigens that are contacted through mucosal surfaces. This permits us to co-exist with our normal flora and to eat large amounts of foreign food proteins without inducing harmful systemic immune responses. The immunological basis of this phenomenon is not fully understood, but at least three different mechanisms have been described: (a) ignorance of the antigen by the immune system; (b) anergization or deletion of T cells that respond to the ingested antigen; and/or (c) the generation of regulatory T cells that control and down-modulate the inflammatory response [4]. Thus, various mucosally delivered “vaccines” that mimic the natural induction of oral tolerance could potentially be useful as prophylactic or therapeutic treatments for inflammatory diseases. However, the application of this concept in patients with autoimmune diseases has largely failed to show any effect over that of placebo in large multi-center trials [7,8]. It appears clear that, just as adjuvants are required for effective induction of mucosal immunity, there is a need for delivery systems and/or adjuvants that enhance mucosal tolerance for effective immunotherapeutic application.

7.1. Adjuvant action of CTB for mucosal tolerization

Currently, the most promising vehicle for inducing mucosal tolerance is CTB. Recombinantly produced CTB, which was linked chemically or fused genetically to various auto-antigens, has been shown to dramatically enhance the tolerogenic effect over that of mucosal administration of auto-antigen alone, and to suppress the development of various autoimmune diseases in animal models. Thus, in rat and/or mouse models of experimental autoimmune encephalitis (EAE), type I diabetes and collagen-induced arthritis, the development of disease was totally or markedly suppressed by the oral or nasal administration of a few low-dose treatments with chemical conjugates or gene fusion proteins between CTB and, respectively, myelin basic protein, insulin or collagen II and/or selected peptides derived from these antigens [33–35]. Further, prolonged oral treatment with low doses of allergen conjugated to CTB markedly suppressed IgE antibody responses and allergic reactions in sensitised mice [36].

The specific actions in the strong enhancement of oral tolerance by CTB–antigen conjugates are probably complex and include: (a) increased uptake of tolerizing antigen across the mucosal surface; (b) increased high affinity delivery of both specific antigen and CTB to the relevant mucosal APC; and (c) a direct immunomodulatory effect of bound CTB on these APC. Effective treatment with different CTB–antigen conjugates or fusion proteins are associated with the development of actively tolerogenic APC as well as of TGF- β -secreting suppressive–regulatory T cells in mucosal tissues and draining lymph nodes. Furthermore, tolerization also results in a local down-regulation in the target tissue of certain chemokines, such as MCP-1 and RANTES, known to selectively promote both the attraction and differentiation of inflammatory–pathogenic Th1 cells [35]. CTB has also been shown to be anti-inflammatory per se. Oral feeding of CTB both prevents and cures Th1-driven experimental colitis in mice, through an unidentified mechanism involving a reduced production of IL-12 within the large bowel [37].

It is now clear that mucosal administration of CTB conjugates can also prevent infection-induced pathologic changes. Conjugates of CTB and *Schistosoma mansoni* egg and/or worm antigen, or selected peptides from such antigens genetically fused to CTB, given nasally to infected mice suppressed hepatic granuloma formation and reduced mortality following *S. mansoni* infection, in a process that involves the appearance of TGF- β -producing T cells [38,39]. Likewise, nasal administration of a CTB–LACK conjugate to mice selectively induced tolerization of Th1-like cells and delayed the onset of lesion development in mice infected with *Leishmania major*, whereas oral or nasal administration of recombinant LACK antigen alone did not change the outcome of infection [40].

A particularly interesting recent development in the field of oral tolerance induction with CTB–antigen conjugates

relates to work indicating that oral treatment with a disease-specific human heat-shock protein 60 (HSP60) derived peptide linked to CTB suppresses uveitis in patients with Bechet's disease. Bechet's disease patients have T cells reacting with a specific peptide (p336–351) within HSP60. Systemic or mucosal immunisation with this peptide can induce uveitis in rats, and recent work showed that such uveitis could be prevented with a specific efficacy of 75% by oral tolerization with the peptide linked to CTB [41]. This led us to test this strategy for treatment of Bechet's disease in humans in a phase I–II clinical trial (Stanford, manuscript in preparation). Oral administration of p336–351–CTB conjugate, three times weekly, had no adverse effects and enabled gradual withdrawal of existing treatment with immunosuppressive drugs in five out of eight unselected patients with Bechet's disease, without any relapse of uveitis. Indeed, relapse of uveitis was prevented after complete withdrawal of existing treatment in four of five patients, in whom pre-existing treatment had maintained the patient in clinical remission prior to initiating the tolerizing regime. Most strikingly, remission has been maintained up to 1 year following withdrawal of the peptide–CTB treatment, without a need of any systemic immunosuppression. Associated with the control of uveitis in the Bechet's disease patients was a lack of peptide-specific CD4⁺ T-cell proliferation and various other immunological parameters. This study is the first instance where mucosal tolerization with a CTB-specific antigen conjugate has been tested in humans. The promising results of this study clearly need to be confirmed by a placebo-controlled phase III trial. However, if the efficacy of the peptide–CTB oral tolerance treatment observed here were to be confirmed, this novel treatment strategy may be applicable generally to autoimmune diseases in which oral tolerization with specific antigen alone has not been effective.

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