As skin adjuvants, LT and CT, appear to give similar enhanced immune responses to coadministered soluble antigens (11). The antibody response induced by LT and CT to the coadministered antigen is predominantly IgG1, characteristic of the Th2 response. It has been reported that LT can induce a stronger Th1 response than CT, and a mixed Th1- and Th2-immune response has been observed. CT, on the other hand, is thought to provoke predominantly a Th2 response, which is characterized by production of IgG1 and IgA antibodies, and $CD4^+$ T-cells producing IL4, IL5, and IL10.

CpG Motifs

TLR agonists have been evaluated as potential adjuvants to enhance the adaptive immune response. For instance, unmethylated bacterial CpG motifs or synthetic oligodeoxynucleotides (ODN) containing CpG motifs (CpG-ODN) are readily recognized as a danger signal by the TLR9 expressed by dendritic cells, macrophages, monocytes, and splenocytes (44). Ligation of CpG to TLR9 triggers the induction of cell-signaling pathways that cause production and release of pro-inflammatory Th-1 driving cytokines such as IL-12, TNFα, IL-1α, and IFN-γ. Thus, the CpG motifs can have a broad adjuvant effect on a coadministered antigen, and skew the immune response to a Th1 phenotype.

In a TCI study, a synthetic CpG-ODN was used in combination with CT to modulate the immune response of a synthetic peptide representing a T-helper epitope of influenza virus HA (6). The CpG-ODN acted synergistically with CT to increase the proliferative T-cell responses to the HA peptide. Thus, the bias toward Th2-type responses stimulated by CT was shifted toward Th1 phenotype because of the presence of CpG in the HA peptide/CT formulation. Interestingly, the CpG administered without CT on the skin exhibited a weak adjuvant effect to the HA peptide, thus highlighting the potent immunomodulatory properties of CpG in combination with ADP-ribosylating exotoxins for TCI application. This and other studies (6,12,45,46) showed that CpG administered with CT or LT can enhance TCI-induced immune responses, and can modulate a Th2 to a Th1 type response.

Imiquimod and Other Adjuvants Used on Skin

Imiquimod, which has been used in topical treatment of genital warts, is a potential adjuvant for TCI (43,47,48). This small molecule has been shown to bind to TLR7 receptors expressed on several dendritic cell (DC) subsets, including skin LCs. The binding gives rise to production of inflammatory cytokines, such as IL-1, IL-6, IL-8, TNF-α, and IFN-α, some of which are critical for antigen uptake by LCs and their migration into the DLNs. Studies have shown that imiquimod can be transcutaneously applied with a peptide antigen to mount a CTL response that is specific for the epitope used for immunization. Interestingly, transcutaneous peptide immunization with imiquimod as the adjuvant does not seem to require disruption of the skin barrier (47).

EXAMPLES OF RECENT TCI APPLICATIONS

TCI has been demonstrated to elicit humoral and cellular immune responses against a wide range of bacterial and viral antigens. Some recent TCI studies illustrating the use of the skin as a noninvasive route for administering antigens are described below.

TCI Applications for Bacterial Diseases

Haemophilus Influenzae

TCI has also been used to deliver a complex bacterial product, such as a semi-synthetic glycoconjugate vaccine. Mawas et al. (49) delivered a *Haemophilus influenzae* type b (Hib) glycoconjugate vaccine coadministered with CT or LT mutants onto rats' skin. The glycoconjugate elicited high antibody titers to the capsular polysaccharide of Hib and to the protein carrier. The anti-Hib polysaccharide antibodies were shown to be passively protective in an infant rat model against a virulent strain of Hib.

Bacillus Anthracis

In a previous TCI study in mice, a recombinant protective antigen (rPA) of *B. anthracis* coadministered with LT on the skin was shown to induce long-lasting neutralizing antibody titers that were superior to those obtained by IM injection of alum-absorbed rPA (50). Moreover, the TCI-induced anti-rPA antibodies completely protected TCI-immunized mice against challenge with *Bacillus* spores from an unencapsulated strain. In a more recent TCI study, Peachman et al. clearly demonstrated the superiority of TCI over the injected alum-adsorbed vaccine in protecting vaccinated mice against intranasally administered *Bacillus spores* from a more virulent encapsulated strain (51). A significant correlation was observed between the TCI-induced toxin-neutralizing antibody titer and mouse survival after the intranasal challenge.

Chlamydia

An ideal vaccine for *Chlamydia trachomatis* should induce (*i*) mucosal IgG and IgA to prevent infection by Chlamydia elementary bodies and (*ii*) a strong cell mediated immune response to limit ascending infection to the uterus and fallopian tubes. Berry et al. (52) have shown in a mouse model that TCI with Chlamydia major outer membrane protein (MOMP) in combination with both CT and CpG can elicit MOMP-specific IgG and IgA in vaginal and uterine lavages, MOMP-specific IgG in serum, and IFN-γ-secreting Th1 cells in the reproductive tract lymph nodes. More importantly, the TCI protocol enhanced clearance of Chlamydia organisms following vaginal challenge.

Cholera

The toxin-coregulated pilin A (TcpA) is a second major virulence factor of *V. cholerae* and is essential for colonization in animal models and humans. Rollenhagen et al. (53) have immunized mice transcutaneously with TcpA with CT, and the immune responses elicited were protective in a mouse cholera challenge model. Interestingly, TCI application of TcpA with CT did not induce anti-TcpA serum IgA, despite induction of prominent anti-TcpA IgG responses. Three TCI applications of TcpA and CT were required to induce the protective anti-TcpA responses in mice, while TCI applications of TcpA without CT did not develop anti-TcpA responses.

Clostridium

Clostridium difficile, the leading cause of nosocomial diarrhea in the industrialized world, causes more than 300,000 cases of diarrhea in the United States and can lead to colitis, toxic megacolon, systemic inflammatory response syndrome, and