



Figure 1 Intercell's skin preparation device and dry patch system. (A). In this overhead photo, Intercell's single-use disposable skin preparation device is shown relative to the size of a U.S. quarter. (B). shows a schematic diagram of the patch components.

was placed at the pretreatment site. A separate group was also pretreated on the thigh by clinicians using the prototype SPS followed by placement of the dry LT patch. Two doses of 50 μg of LT were used and anti-LT antibody responses were then measured. No difference was seen in the physician versus self-pretreated group (24.5- vs. 26.8-fold rise in the serum IgG), and 95% seroconversion was achieved in both groups (15,41). The SPS has been specifically designed for simple use, including self-application, and these data suggest that a self-applied vaccine patch system can be developed.

ADJUVANTS FOR SKIN IMMUNIZATION

The presence of an adjuvant is critical to induce a strong systemic and mucosal immune response to the coadministered antigen by TCI. Various adjuvants have been used for skin immunization, and since they can bias immunity toward a Th1 or Th2 immune response, their selection should be based on whether a Th1 or Th2, or mixed Th1/Th2, immune response is preferred. Furthermore, skin immunization allows the use of potent adjuvants, whereas their use would be prohibited for parenteral or mucosal administration because of the toxicity they exert.

Bacterial ADP-Ribosylating Exotoxins

Besides being strong immunogens, the bacterial ADP-ribosylating exotoxins (bAREs), such as *E. coli* LT and CT, are potent adjuvants in the context of the skin. Numerous preclinical and clinical studies (3,11,15,24) have documented the safe use of bAREs on the skin. Our laboratory has safely applied the skin patch containing LT in over 3,000 human subjects without any vaccine-related serious adverse events (SAEs), and the LT patch is currently under U.S. IND for phase II clinical trials.

As potent immunogens with strong adjuvant properties, LT and CT are commonly used in TCI protocols to induce systemic and mucosal antibodies against the toxins themselves, as well as to various types of coadministered vaccine antigens including proteins, peptide antigens, and whole and split viruses. Importantly, preexisting antibodies to LT or CT do not adversely affect the adjuvanticity of these adjuvants on repeated TCI administrations. In this respect, TCI is akin to intranasal (IN) or oral mucosal immunization with adjuvants, as the simple admixture of LT with a coadministered antigen such as tetanus toxoid (TTx) or influenza hemagglutinin (HA) on the skin results in markedly higher systemic and mucosal antibody responses compared to the administration of antigens alone.

The basis for the adjuvanticity of bAREs has been described to some degree. Observations that the enzymatically active holotoxins are more potent immunoadjuvants in inducing systemic antibody responses, while their purified B-subunits are marginally effective, support the notion that the strong adjuvant action is associated with their ADP-ribosylating enzymatic activity (11,15). In support of this hypothesis, a number of molecular events have been proposed to explain the strong adjuvant action of bAREs on the skin. The LT (or CT) holotoxin consists of homopentamers of B subunits (LTB or CTB) associated with a single A subunit (LTA or CTA). The LTB binds specifically to GM1 ganglioside receptors that are expressed on skin epithelial cells, that is, the LCs and keratinocytes. The LTB/GM1 binding facilitates the entry of LTA into the cells. The LTA enzymatically ADP-ribosylates the G_s protein of adenylyl cyclase, which results in increased levels of cAMP. The rise in cAMP signals the skin epidermal cells (keratinocytes and LCs) to stimulate the secretion of proinflammatory cytokines such as IL-1 and $\text{TNF}\alpha$, which in turn act on LCs to trigger their maturation and migration to regional lymph nodes, as well as to other distal mucosal sites, where they present peptide fragments to naïve T-cells.

Some have suggested that binding to the GM1 receptors is more critical than ADP-ribosylating activity in explaining the adjuvant effect of bAREs. For instance, the binding of LT or CT through their B subunit to ganglioside receptors on skin epithelial cells can induce changes in membrane channel potential that results in altered skin permeability and enhanced penetration and uptake of coadministered antigens in mice (42). However, this explanation is inconsistent with human skin biopsies, which had no microscopic changes in spite of the induction of robust immune response via TCI (5). Another group has suggested that the adjuvant effect is due to the bAREs' ability to disrupt the skin barrier function by inducing apoptosis of the skin keratinocytes. This apoptosis of the skin keratinocytes, in turn, creates intercellular spaces or voids in the skin that allow more efficient and rapid diffusion of adjuvant and antigen molecules to the underlying epidermis, where they are taken up and processed by the LCs (17,43).