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Advances in Transcutaneous Vaccine Delivery

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INTRODUCTION

Transcutaneous immunization (TCI) is a noninvasive, pain-free, and easy-to-use vaccination technique that introduces antigens and/or adjuvants topically onto the skin in the form of a patch, a liquid solution, or ointment. The skin is one of the largest immune organs and forms an integral part of an immune system network known as the skin-associated lymphoid tissue (SALT) (1). The skin epidermis is naturally populated with numerous resident professional antigen-presenting cells (APCs) known as Langerhans cells (LCs). These APCs form an immune network to efficiently capture microbial pathogens or antigens that have penetrated through the skin's outer barrier, the stratum corneum (SC), and have entered into the epidermal tissues. LCs play an important role in the induction of immune responses. APCs sample and process pathogen-derived antigens and traffic to nearby regional skin, draining lymph nodes (DLN) to present the antigen fragments to naïve resting T and B cells to induce production of antigen-specific $CD4^+$ and $CD8^+$ T cells as well as systemic and mucosal immunity (2–5).

Because the SALT system is capable of mounting effective immune responses to pathogen-derived antigens (1), the skin has become an attractive, noninvasive route for vaccine delivery. TCI targets delivery of antigens through the SC and into the epidermis to exploit the skin's immune system. Within the last decade, remarkable progress, as evidenced by >200 publications, has been made in developing TCI as an alternative vaccine delivery route. Based on many of these published studies, some general principles regarding the TCI approach have emerged. First, there appears to be no restriction to the type of antigens or their molecular size that can be used for TCI. Successful skin immunizations have been conducted with a variety of protein and DNA antigens, including those derived from bacterial and viral microorganisms. Molecular sizes for TCI have ranged from small to medium-sized peptides carrying T-cell and cytotoxic T lymphocyte (CTL) epitopes (6–9), to large molecular weight subunit protein antigens (10–13), to split and whole-inactivated viruses (14–16). Second, minor disruption of the SC at the site of vaccine application enhances efficiency of antigen penetration and skin immunity. SC disruption can be achieved by physical or chemical means and is further discussed in this chapter. Third, adjuvants can be used to generate a robust immune response to coadministered antigens by TCI. Adjuvants commonly used are cholera toxin (CT) from *Vibrio cholerae* and the heat-labile enterotoxin (LT) of enterotoxigenic *Escherichia coli* (ETEC). These potent adjuvants are too toxic to be administered orally or intranasally, but can be safely used on the skin (3,4,15).

In this chapter, we present recent insights and strategies in using TCI as a new vaccine delivery paradigm. Topics discussed include (*i*) skin structure as related to immunology; (*ii*) methods for SC disruption, including Intercell's skin preparation system (SPS) device; (*iii*) types of skin adjuvants used; (*iv*) recently published TCI applications; and (*v*) Intercell's dry patch formulation technology for preparing thermostable vaccine patches for LT and influenza. Finally, we describe Intercell's recent clinical studies using a dry LT-patch for the prevention and reduction of travelers' diarrheal illness, and as an immunostimulant (IS) patch for a pandemic influenza vaccine candidate.

SKIN STRUCTURE AND IMMUNE FUNCTION

The skin consists of three layers—the SC, epidermis, and dermis. In humans, the SC is approximately 10 to 20 μm thick and is composed of dead keratinocytes cells surrounded by a lipid mortar. The epidermis, which underlies the SC, is a continuously growing layer of epithelium (50–100 μm) that consists of about 90% to 95% of keratinocytes at various progressive stages of differentiation. The remaining 2% to 8% of the epidermal cell population consists of immature dendritic cells, known as LCs. Because of their dense population and long dendritic protrusions, LCs form a network that covers about 20% of the entire surface area of the skin. The dermis (1–3 mm thick) supports the epidermis with connective tissue and contains blood vessels, lymphatics, nerve endings, hair follicles, and sweat glands. The dermis also contains dendritic cells and mature LCs in transit, but the density of these APCs in the dermis does not match that of the epidermis (2,3,15).

Vaccine delivery by TCI is targeted to the superficial layer of the epidermis due to the presence of the immature LCs, which are sufficiently networked to sample and process microbial antigens. In their normal resting state, the immature LCs express low levels of cell surface histocompatibility complex (MHC) molecules and costimulatory molecules. This resting state is influenced by constitutive secretion of IL-10 and transforming growth factor TGF-β cytokines by the neighboring keratinocytes (17). Upon encountering a microbial ''danger signal,'' the LCs become activated, triggering uptake and processing of the antigens that results in LC migration from the skin epidermis via afferent lymphatics to the DLNs, where they present the processed antigens to both $CD4^+$ and $CD8^+$ T lymphocytes to initiate antibody and cellular immune responses. During their migration to the DLN, the LCs undergo a maturation process that includes upregulation of membrane-associated co-stimulatory and adhesion molecules (i.e., CD80,