Transcutaneous Immunization: An Overview of Advantages, Disease Targets, Vaccines, and Delivery Technologies

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Abstract

Skin is an immunologically active tissue composed of specialized cells and agents that capture and process antigens to confer immune protection. Transcutaneous immunization takes advantage of the skin immune network by inducing a protective immune response against topically applied antigens. This mode of vaccination presents a novel and attractive approach for needle-free immunization that is safe, noninvasive, and overcomes many of the limitations associated with needle-based administrations. In this review we will discuss the developments in the field of transcutaneous immunization in the past decade with special emphasis on disease targets and vaccine delivery technologies. We will also briefly discuss the challenges that need to be overcome to translate early laboratory successes in transcutaneous immunization into the development of effective clinical prophylactics.

INTRODUCTION

Of all the medical interventions in human history, vaccination is arguably the most effective in reducing suffering, disability, and loss of life. Vaccination programs worldwide have been successful in eliminating or significantly reducing the scourge of serious infections such as poliomyelitis and smallpox. The World Bank's *World Development Report* noted that childhood vaccination has been the most cost-effective measure in alleviating the enormous health and economic burdens in developing countries (1). At the launch of World Health Organization's Expanded Program of Immunization in 1974, only 5% of the world's children were immunized against tuberculosis, diphtheria, tetanus, whooping cough, polio, and measles. By 1990, this number had climbed to 80%, saving an estimated 3 million children worldwide each year from death and at least 750,000 from disabilities (2).

In spite of the success achieved with mass-vaccination programs, infectious diseases still account for a third of all fatalities worldwide (3). Antibiotics are the preferred line of therapy for several infectious diseases today. However, the failure of antibiotics through drug resistance has increased the spread of several re-emerging infectious diseases, such as tuberculosis and malaria (4–6). Vaccination seems to be the most optimal and effective deterrent for re-emerging, antibiotic-resistant infectious diseases. Rapid migration of humans, animals, and material around the world by means of express transportation modes has accelerated the rate at which emerging infections, such as severe acute respiratory syndrome (SARS), avian influenza, and the most recent swine influenza, can spread globally and become an epidemic (7–9). A key challenge, therefore, is to design vaccines that can be manufactured, deployed, and administered at a pace that exceeds or at least matches that of the spread of the infection. Another interesting challenge arises, ironically, from the widespread use of vaccines in overcoming infectious diseases. With the success of mass-vaccination programs in mitigating infections, increasing attention is being focused on potential adverse effects and on improved methods to manage and control any harmful consequences of vaccination (10, 11). Current vaccines are administered via intramuscular (IM), subcutaneous (SC), intranasal (IN), intradermal (ID), or oral (OR) routes (12). IM, SC, and ID immunizations are largely performed via needles. Needle-based techniques, although effective in achieving a desired immune response, have several drawbacks related to safety and compliance. Advocates of the next generation of safe and effective vaccines, including prominent public health organizations such as the World Health Organization (WHO), the Global Alliance for Vaccines and Immunization, and the Centers for Disease Control and Prevention, have unequivocally argued in favor of needle-free vaccination strategies for several reasons (13–16).

A primary safety concern with the use of needles for vaccination is the risk of infectious disease transmission between patients owing to reuse of needles, or between patients and healthcare providers owing to accidental needle sticks (17–21). Trim & Elliott aggregated several studies on needle stick injuries and estimated that an average of 4% of needle-based procedures are associated with accidental sticks among healthcare workers (20). The WHO estimates that 12 billion injections are administered worldwide annually, of which approximately 600 million are vaccinations (22). An estimated 20–30 million healthcare workers worldwide are thus potentially exposed to serious infections such as hepatitis B and C or human immunodeficiency virus (HIV) annually (21). The risk from accidental needle sticks can substantially increase during the mass inoculation events that follow a bioterrorism emergency or natural pandemic. Unsafe injection practices also add to the risk of transmission of infectious diseases. A WHO-sponsored review showed that at least 50% of all injections were unsafe in Asia, sub-Saharan Africa, and the former Soviet Republic; these unsafe injections expose patients to the risk of infection from hepatitis, HIV, or other blood-borne pathogens (17, 23). Needle-based inoculations are also associated

MUCOSAL IMMUNIZATION STRATEGIES IN DEVELOPMENT

Mucosal tissues (oral, nasal, vaginal, rectal, ocular, pulmonary, and sublingual) are alternative interfaces for administration of vaccines. Oral delivery of an antigen is perhaps the easiest and most practical mode of immunization. A few vaccines (against polio, typhoid fever, and cholera) based on live attenuated pathogens are currently administered orally. However, oral immunization using nonliving vaccines is challenging due to the poor stability of proteins, peptides, and DNA in the acidic and enzyme-rich environment of the gastrointestinal tract. Nasal immunization using a spray delivered into the nostrils is an attractive practical approach for vaccination. Mucosal as well as systemic immune responses are obtained to antigens exposed to the nasopharynx-associated lymphoid tissue. FluMist, a live influenza-virus vaccine by MedImmune Vaccines, Inc., has already been approved by the U.S. FDA. Vaginal and rectal immunizations using creams are being considered for immunization against sexually transmitted diseases. Pulmonary immunization using aerosolized vaccines has the capability to deliver antigens to deeper levels of the bronchus-associated lymphoid tissue and is under consideration as a possible mode of immunization. The sublingual epithelium contains a dense network of dendritic cells similar to Langerhans cells, and antigen presentation to this tissue has been shown to induce mucosal as well as systemic immune responses that validate sublingual immunization as an attractive approach for vaccination. Ocular immunization using eye drops is yet another attractive mode of vaccination. The success of these different modes of immunization will ultimately depend upon their acceptability, efficacy, cost, and ease of delivery.

with certain physiological risks. For example, incorrect ID immunization by inserting the needle too deep may lead to lymphadenitis in the lymphatic drainage near the site of the injection (24). Also, needles inserted into the buttock to administer vaccines may pass close to the sciatic nerve. Irritant vaccines injected into or close to the nerve have been documented to cause paralysis in some instances (25). Another issue with needle-based administration is that of low compliance, in children and adults alike, owing to perceived and real pain as well as the discomfort arising from injections (26–28). Needle-based immunizations also require some level of expertise and can pose a problem in settings where sufficient clinical supervision is either unavailable, such as in developing countries, or limited, such as during epidemics or after acts of bioterrorism.

Vaccine development has benefited tremendously from advances in pathology, molecular biology, and design of novel drug delivery systems. Several new needle-free immunization devices and approaches are being rapidly developed and tested (13, 14, 16). Significant attention in these efforts is focused on mucosal immunization (see sidebar, Mucosal Immunization Strategies in Development). However, a promising new strategy under study in modern vaccinology aims at immunizations via topical application of vaccines on the skin. This strategy, named transcutaneous immunization (TCI), targets the highly active immunosurveillance agents of the skin to induce potent and functional immune responses (13–16, 29). This review will focus on the developments in the area of TCI in the past decade with special emphasis on vaccine candidates, delivery systems, and disease targets.

THE CUTANEOUS IMMUNE NETWORK

Skin, the largest organ of the human body, serves as a protective barrier in an extremely hostile environment of opportunistic pathogens. It has evolved to keep foreign molecules out by acting as a transport barrier (30) and playing host to a sophisticated immune surveillance network (31). The cutaneous immune network is the most accessible immune compartment of the body, making skin an attractive interface for the administration of vaccines and

TC: transcutaneous

TCI: transcutaneous immunization

immunomodulators (14). Skin is composed of three layers, the stratum corneum, epidermis, and dermis (**Figure 1**). The barrier property of the skin resides in the stratum corneum, which is ~30–50 μm thick and composed of dead cells called corneocytes in a lipid-rich matrix. Directly below the stratum corneum is the epidermis, which is 200–250 μm thick, followed by the dermis, which is 2–3 mm thick. Keratinocytes (KCs) and Langerhans cells (LCs) in the epidermis; fibroblasts (FBs), dendritic cells (DCs), and mast cells (MCs) in the dermis; and T and B lymphocytes (T cells, B cells) in the skin-draining lymph nodes comprise the cutaneous immune network.

Langerhans cells (LCs): key antigen-presenting cells of the cutaneous immune system that reside in the epidermis

DC: dendritic cell

Cytokines: signaling molecules extensively used in communication between the different cells and agents of the immune system

APC: antigen-presenting cell

KCs are the primary constituents of the epidermis and play an active role in innate and adaptive immune responses by secreting a wide variety of cytokines, chemokines, and antimicrobial peptides in response to pathogens and antigens (32–34). Pattern recognition receptors, called Toll-like receptors (TLRs) on KCs play an important role in identifying microbial pathogens or their components. LCs are specialized dendritic cells of the epidermis (31, 35). Immature LCs typically reside in the basal layers of the epidermis, where they survey and sample antigens and microbial pathogens entering the epidermis. Encounters with antigen(s) or other stimuli in the epidermis activate LCs, priming them to capture the antigen(s), process them into immunogenic peptides, and present the peptides as complexes with major histocompatibility complex (MHC) molecules on their surfaces. These cells then migrate via afferent lymphatics to the skin-draining lymph nodes, where they present antigenic peptides to the resident lymphocytes (34, 36, 37). Mesenchyme-derived FBs are the primary constituents of the dermis and are significantly involved in crosstalk with the KCs in maintaining homeostasis of the skin immune system. FBs secrete secondary cytokines in response to secretion of primary cytokines by the KCs. Although KCs can autonomously produce some of the cytokines secreted by the FBs, the recruitment of FBs into the cytokine cascade provides for amplification and release of secondary cytokines (38). $CD1a^{-}CD14^{+}HLA\text{-}DR^{+}$ cells, termed dermal DCs, are believed to specialize in the control of mature B lymphocyte differentiation and, unlike LCs, do not induce $CD4^{+}$ or $CD8^{+}$ T cells. These cells migrate into the outer paracortex, just beneath the B cell follicles, unlike LCs, which migrate to the T cell–rich inner paracortex (35, 39, 40). The dermis is host to many MCs that are known to participate in immunoglobulin E (IgE)-mediated allergic responses. MCs respond to many stimuli and can release a variety of preformed primary mediators and cytokines. Cytokines secreted by MCs are believed to be a significant factor in directing the orientation of T cell responses to certain types of antigens (41, 42). Naïve B cells and T cells are effectors of the adaptive immune response. Residing in the skin-draining lymph nodes, they are activated by antigen-presenting cells (APCs) such as the LCs that arrive via afferent lymphatics. The skin-draining lymph node is a meeting ground for LCs and the resident lymphocytes (31, 35, 37, 40). The cutaneous system is thus a highly complex and functionally rich network of specialized immune cells. Easy access to this immune network by topical application of the antigen or vaccine makes TCI particularly attractive.

BENEFITS OF TRANSCUTANEOUS IMMUNIZATION

TCI offers several advantages over conventional needle-based vaccination methods. A skin patch-based vaccine is painless and therefore patient compliant. Compliance is especially critical for success in immunization programs among children and the elderly, a significant proportion of the target demographic for whom needle-based administrations can be stressful (27, 43). Skin patch-based vaccines offer self-administration capabilities, which reduces the need for a clinical setting or medical supervision. This is especially critical during an epidemic. This mode of immunization also represents a truly patient-centric therapeutic approach that promotes widespread use of vaccines, another prerequisite for success in immunization programs worldwide. TCI using skin patches

has the potential to eliminate or significantly reduce the spread of infections through accidental needle sticks as well as unsafe injection practices.

It is becoming evident that the particular route of vaccine administration can have marked qualitative and quantitative effects on the desired protective immune response. For example, the smallpox virus is known to associate in blood with leukocytes that specifically home to the skin tissue, where subsequent viral replication steps result in the formation of a lesion (31, 44). Protective vaccination with vaccinia virus against smallpox depends strongly on the route of administration. The vaccination is successful only when the virus is administered to the epidermis by a scarification process leading to an epidermal "pox" reaction (45). Vaccination through the skin is most efficient at stimulating skin-homing effector T cells, whereas alternative routes such as oral or intramuscular administration generate effector T cells that are primarily directed toward other tissues (31). Indeed, intramuscular vaccinations with vaccinia virus fail to provoke a pox reaction and are not effective in inducing neutralizing antibodies or virus-specific cytotoxic T cells. Another example of the importance of the route of administration is melanoma. Melanoma is a malignancy of pigment-producing cells, called melanocytes, that are present in the epidermis and hair follicles of the skin. Unfortunately, little attention has been paid to the exact route of vaccination for melanoma therapy (31). Based on prior findings, however, it is anticipated that transdermal administration of the melanoma antigen would generate a skin-homing effector T cell response that should result in superior protection as compared with that obtained from other routes of administration of the same antigen (46–48). Generating skin-homing effector T cells is also critical in providing protection against environmental pathogens that are routinely encountered on the skin surface and may invade through wounds or an otherwise compromised skin barrier.

Administration of vaccines across the skin also provides other advantages. The induction of strong $CD8^+$ T cell (cytotoxic T cell or killer T cell) responses requires a sustained presentation of the antigen to the immune system in a stimulatory context (49). Endo- and exopeptidases present on the cell surface of APCs as well as serum-derived proteases can trim antigens, which results in a decreased presentation of T cell epitopes (50–54). A large number of repeat administrations of the epitopes is therefore necessary to overcome the effects of proteolytic degradation (55, 56). Transdermal immunization via a skin patch presents a rather superior alternative, allowing for a sustained and prolonged dosing that provides robust epitope presentation, thereby compensating for proteolytic loss of the epitope.

The immune response obtained by TCI is quite distinct as compared with the response obtained from ID immunization, even though the apparent site of immunization is the same, owing to the involvement of distinct subsets of APCs (3). On one hand, antigens administered via TCI are captured mainly by LCs resident in the epidermal layer that produce a predominantly T helper type 2 (Th2) response (57–59). On the other hand, antigens administered by ID immunization are captured by DCs that activate both T helper type 1 (Th1) and Th2 immune responses (59, 60). The differential nature of the immune responses is of fundamental consequence and is potentially advantageous for treating Th1-type autoimmune diseases by correcting the Th1/Th2 balance (61). Furthermore, ADP-ribosylating exotoxins such as cholera toxin (CT) and heat-labile enterotoxin (LT) can significantly amplify the desired immune response when coadministered with the antigen (62, 63). These adjuvants can be used safely with TCI and do not exhibit the toxicity associated with their use in OR and IN immunization (64, 65).

Parenteral immunizations can fail to generate a mucosal immune response (66), and immunization by mucosal routes may be necessary to induce optimal protection against mucosal challenge (67). Antigen delivery at mucosal surfaces usually produces somewhat compartmentalized mucosal responses in which antibody responses are greatest at the mucosal surface to which the antigen is administered (66). TCI, however, can generate systemic immune responses (68) as well as mucosal

Cytotoxic T cells: also called cytotoxic T lymphocytes (CTLs), these are a subset of lymphocytes that kill pathogen-infected cells

Th1/Th2 response: immune response by subsets of T helper cells characterized by cytokines produced and type of immunity (humoral or cellular)

Th: T helper

Autoimmune disease: a disease arising from a misdirected immune response against proteins or other constituents naturally present in the body

CT: cholera toxin

LT: heat-labile enterotoxin

Adjuvant: a physical or chemical agent that can amplify the natural immune response to a vaccine when coadministered with the vaccine

responses in the gut, lung, saliva, and female reproductive tract (69) and is thus a unique route by which to deliver antigens to multiple mucosal sites.

ODN: oligonucleotide

DISEASE TARGETS FOR TRANSCUTANEOUS IMMUNIZATION

Infectious Diseases

Infectious diseases continue to be the major causes of illness, disability and death. New infectious diseases and agents are detected frequently. Some of the infectious diseases, which were thought to be under control, are re-emerging owing to resistance to antimicrobial drugs. Vaccination is the only effective strategy against infectious diseases in the long term. Here we discuss a few infectious diseases that are promising targets for the development of TC vaccines.

Bacterial infections. TCI has the potential to prevent many bacterial infections such as those caused by *Chlamydia*, a type of obligate intracellular Gram-negative bacteria that infects epithelial cells in humans. *Chlamydiae pneumoniae* causes a range of respiratory infections in humans including bronchitis, pharyngitis, and pneumonia (65). *Chlamydia* infection has also been implicated in the initiation or exacerbation of coronary artery disease (70), asthma (71), chronic obstructive pulmonary disease (72), and Alzheimer's disease (AD) (73). Another *Chlamydia* strain, *Chlamydia trachomatis*, infects the oral and genital mucosa and is the leading cause of bacterial sexually transmitted disease worldwide. At present no vaccines are available for *C. pneumoniae* or *C. trachomatis*, and antibiotic therapy is the mainstay of treatment. In recent years, however, antibiotic-resistant strains of *Chlamydia* have been identified (74). Further aggravating this situation is the observation that antibiotics promote a chronic or persistent form of infection that potentially can be reactivated at a later stage (75). Berry et al. have successfully demonstrated that TCI with major outer membrane protein (MOMP) of *Chlamydia muridarum* (the equivalent of *C. trachomatis* in humans) in combination with both CT and CpG (cytosine-phosphate-guanine)-oligonucleotide (ODN) as adjuvants elicits MOMP-specific IgG and IgA in vaginal and uterine lavage fluid, MOMP-specific IgG in serum, and interferon-γ (IFN-γ)-secreting T cells in reproductive tract-draining caudal and lumbar lymph nodes. This immunization protocol resulted in enhanced clearance of *C. muridarum* following intravaginal challenge of BALB/c mice (69). In a subsequent study, the same group was also successful in conferring protection against a respiratory challenge with *C. muridarum* in BALB/c mice via TCI using MOMP and a combination of CT and CpG-ODN as adjuvants (65). Ekong et al. (76) used a *Vibrio cholerae* ghost (rVCG) platform coexpressing chlamydial MOMP and CTA2B, a nontoxic derivative of CT, as a carrier and delivery system for transcutaneous (TC) *Chlamydia* vaccines. C57BL/6 mice immunized using this vaccine via the TC route showed increased specific mucosal and systemic antibody and Th1 responses (76). *C. pneumoniae* infection is believed to be the cause of 5–20% of community-acquired pneumonia infections and the cause of 5% of bronchitis and sinusitis cases in adults (65). Approximately $4 billion, 40% of all direct and indirect costs related to non-HIV-related sexually transmitted disease, are spent on treating *C. trachomatis* infections (69). The availability of effective, low cost, patient compliant TC vaccines would help alleviate the escalating global health concern and the socioeconomic burden arising from these infections.

Nontypeable *Haemophilus influenzae* (NTHi) is one of the three main bacterial causes of otitis media (OM), an infection or inflammation of the middle ear. It is estimated that 83% of all children will experience at least one ear infection prior to 3 years of age, which makes OM one of the most significant health problems for children in the United States. Most recently, Novotny et al. have shown that TCI with a chimeric vaccine based on combined epitopes from two distinct NTHi

adhesins [type IV pili and outer membrane protein (OMP) P5] is successful in chinchillas, which upon receiving the vaccine were able to rapidly reduce or completely eliminate NTHi from their nose and ears (77, 78).

Chronic periodontitis threatens oral health by destroying periodontal tissues and thereby causing tooth loss. *Porphyromonas gingivalis*, a Gram-negative bacterium, is putatively responsible for chronic periodontitis. The coaggregation of *P. gingivalis* with Gram-positive and other Gram-negative bacteria is mediated by a 40-kDa outer membrane protein (40k-OMP) found at the cell surface and in extracellular vesicles in many strains of *P. gingivalis*. TCI of BALB/c mice with 40k-OMP elicited 40k-OMP-specific IgG antibody responses in both serum and saliva (79). Use of CT as an adjuvant further amplified the IgG response, and the IgGs were effective in inhibiting the coaggregation of *P. gingivalis* vesicles and *Streptococcus gordonii*, which is one of the first bacteria to colonize newly cleaned teeth and is assumed to promote the colonization of other bacteria.

In another example, enterotoxigenic *Escherichia coli* (ETEC) diarrheal disease is a worldwide problem that potentially can be addressed by TCI with colonization factors (CS6), LT, heat-stable toxin (ST), or CT. Several studies have demonstrated the feasibility of a topical patch for protection against diarrhea (80–83). Enterohemorrhagic *E. coli* (EHEC) are important human food-borne pathogens. EHEC produce potent Shiga toxins (Stx1 and/or Stx2) that are implicated in the development of hemorrhagic colitis and hemolytic-uremic syndrome (84). Zhu et al. have demonstrated the efficacy and practical utility of TCI using Stx1 subunit B in combination with LT for inducing significant systemic immune responses against Stx-induced pathology (84). TCI with CT and toxin-coregulated pilin A (TcpA), which is the main structural subunit of the pilus of *V. cholerae* and required for colonization at the intestinal surface, is potent in inducing anti-TcpA immune responses in a mouse model (85). *Clostridium difficile*, a spore-forming, Gram-positive bacterium, is the leading cause of nosocomial diarrhea and colitis in the industrialized world with more than 300,000 cases of *C. difficile*-associated diarrhea reported each year in the U.S. alone (86). TCI with *C. difficile* toxin A (CDA) and CT in mice is promising because it induces prominent anti-CDA IgG and IgA responses in serum and an anti-CDA IgA response in stool (86). Tetanus, caused by a ubiquitously distributed toxin from *Clostridium tetani*, continues to be a threat to public health with more than 500,000 fatalities each year from the infection. TCI with an adenovirus construct encoding the immunogenic but nontoxic tetanus toxin C fragment has been shown to be effective in protecting 80% of mice challenged with *C. tetani* in a single administration and 100% when two booster applications were administered consecutively using a patch (87).

Viral infections. Respiratory syncytial virus (RSV) is one of the principal causes of bronchiolitis and pneumonia in young children, and currently there is no safe and effective vaccine against RSV. Xu et al. evaluated the ability of a topical vaccine composed of DNA encapsulated in transfersomes to confer protection against RSV challenge in mice. Topical vaccination induced both an RSV-specific mucosal antibody response and IFN-γ-producing cells. Intramuscular vaccination of naked DNA induced a significant anti-RSV IgG antibody response but no remarkable secretory IgA antibody response or virus-specific cellular activity. Lungs from mice receiving topical vaccination also had fewer histopathologic anomalies after RSV challenge as compared with intramuscular vaccination (88).

Adenoviruses usually cause respiratory illness but may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash illness. Using a hydrogel topical patch containing hexon, a major capsid protein from adenovirus, Ishii et al. were successful in immunizing mice against an adenovirus challenge (3). Herpes simplex virus (HSV) affects a large portion of the human population through oral (type 1) and genital (type 2) mucosal infections. In a significant number of cases, both can cause serious complications such as edema, localized lymphadenopathy,

corneal inflammation, and life-threatening encephalitis (89). El-Ghorr et al. have shown that whole inactivated HSV-1 or HSV-1 antigens extracted from infected Vero cells along with CT adjuvant were successful in generating serum and mucosal (fecal) antibodies to HSV in C3H mice. The whole virus vaccine was a more potent stimulator of humoral immunity. The HSV-antigen vaccine, however, was the more potent stimulator of cellular immunity, giving rise to a strong delayed type hypersensitivity response and lymphocyte proliferation in vitro. Upon challenge with epidermal inoculation of HSV, the HSV-antigen vaccine induced a higher level of protection than the whole virus vaccine, which indicated a dependency on cellular immunity rather than humoral immunity for protection (89).

Humoral immunity: immune protection against an antigen or pathogen that is conferred by antibodies that are secreted by B cells

Cellular immunity: immune protection against an antigen or pathogen conferred by cytotoxic T cells, macrophages and natural killer cells

Influenza is a contagious respiratory illness caused by the influenza viruses. It can cause mild to severe illness, and at times can be fatal. Several groups have demonstrated moderate to significant successes in inducing protection with TCI against several strains of influenza using a variety of carrier systems (90–96). Hepatitis B virus (HBV)-related hepatitis is a necro-inflammatory liver condition resulting in chronic liver infection, cirrhosis, and hepatocellular carcinoma. Current therapy involves treatment with systemic IFN-γ, which produces a sustained response in only one-third of patients with chronic hepatitis B. Alternate therapeutic modalities, although available, pose a risk of rapid disease relapse in the short term and the selection of resistant viral variants in the long term. Vaccination is therefore the only measure likely to combat HBV infection (97). Several groups have demonstrated the feasibility of a hepatitis B TC vaccine using a variety of systems (97–102). Japanese encephalitis virus (JEV) is a mosquito-transmitted zoonotic flavivirus that poses a significant threat to public health owing to high rates of mortality (103). No effective treatment is currently available, and vaccination seems to be the most promising modality to counter JEV outbreaks. Cheng et al. have demonstrated that cationic liposomes are effective carriers of a JEV DNA vaccine (103). TCI in C3H/HeN mice with a plasmid expressing whole membrane and envelope protein genes of JEV induced a robust protective antibody response against the infection.

Neurodegenerative Diseases

Neurodegenerative conditions such as Huntington's and Alzheimer's diseases, in which there is an abnormal accumulation of protein aggregates, are promising candidates for vaccination (104). AD immunotherapy accomplished by vaccination with β-amyloid (Aβ) peptide has proved efficacious in AD mouse models. However, "active" Aβ vaccination strategies for the treatment of cerebral amyloidosis without concurrent induction of detrimental side effects are lacking (105). TCI may offer a unique advantage in AD immunotherapy as evidenced by results that have accumulated recently. Seabrook et al. have shown that a dendrimeric vaccine based on Aβ 1–15, when administered along with an adjuvant, LT(R192G), induced a robust humoral immune response resulting in predominantly Th2-biased IgG1 and IgG2b antibodies with low T cell reactivity, suggesting that this immunogen may avoid an autoreactive T cell response, which has been an issue with other modes of immunization (106). Similarly, Nikolic et al. (105) have shown successfully that TCI of C57BL/6 mice with aggregated Aβ1–42 along with CT as an adjuvant results in high-titer IgG1 Aβ antibodies and Aβ1–42-specific splenocyte immune responses. They further evaluated TCI efficacy in reducing cerebral amyloidosis in a transgenic mouse model of AD. Similar to wild-type mice, transgenic mice showed high Aβ antibody titers with significant decrease in cerebral Aβ1–40/42 levels coincident with increased circulating levels of Aβ1–40/42, suggesting brain-to-blood efflux of Aβ1–40/42. More importantly, reduction in cerebral amyloidosis was not associated with deleterious side effects, including brain T cell infiltration or cerebral microhemorrhage.

Autoimmune Diseases

Different tissues appear to have different means of determining the effector class of an immune response (107). This response can also depend strongly on the method of immunization. TCI appears to be particularly well suited to the induction of Th2 immunity as evidenced by high levels of antigen-specific IgG1 and IgE, high levels of IL-4, and low or no IFN-γ or IgG2a (108–110). ID immunization, in contrast, promotes a Th1 response (108). TCI can affect the immune response to a secondary antigen exposure at distant sites such as the gut-associated lymphoid tissue and the lung (61). It not only induces an antigen-specific immune response in the gut, but also specifically enhances a Th2 response following oral ingestion or inhalation (111, 112).

Based on its ability to induce or enhance Th2 immunity, TCI can be used to interfere with the development of Th1 responses or to modify established Th1 responses, which makes it potentially useful in Th1-type autoimmune diseases, such as type 1 diabetes, scleroderma, rheumatoid arthritis, Hashimoto's thyroiditis, and multiple sclerosis (61), that arise from a Th1-driven autoreactive T cell response to self-antigens. Indeed, Bynoe et al. have shown that TCI using a topical patch containing the immunodominant epitope, Ac1–11, of the autoantigenic myelin basic protein (MBP) protected mice that are transgenic for an Ac1–11-specific T cell receptor against the induced as well as the spontaneous form of experimental allergic encephalomyelitis (EAE) (113). The protection was antigen specific and mediated by $CD4^+/CD25^-$ suppressor T cells. These suppressor T cells controlled naïve MBP-specific CD4 T cells by inhibiting both their activation and their capacity to secrete IFN-γ. There was no CD4 T cell infiltration in the brains of protected mice. Also, this method protected two nontransgenic mice models from relapsing-remitting EAE in an antigen-specific and antigen dose-dependent manner. In a subsequent study, Strid et al. showed that TCI with peanut protein induced a potent systemic immune response that was strongly Th2. The induced systemic Th2 response prevented the development of Th1 responses induced through injection of antigen in complete Freund's adjuvant (61). Furthermore, TCI converted an established antigen-specific Th1 response to a Th2 immune response, which established that skin-induced immune responses can modify systemic responses to the same antigen. It is thus plausible that reestablishing the Th1/Th2 balance via TCI can alleviate the symptoms of disease and ultimately reestablish healthy immune regulation and tolerance.

Ghoreishi et al. have shown that skin is an ideal site for tolerance induction. Exposure of skin of C57BL/6 mice to UV light suppresses T cell-mediated immune responses, and TCI through UV-exposed skin results in the generation of antigen-specific regulatory T cells (Treg) (114). Generation of UV-Treg cells provides another opportunity to take advantage of TCI for potentially mitigating autoimmune diseases. In a subsequent study, Ghoreishi et al. demonstrated that antigen-specific Tregs can also be generated via topical application of a vitamin D analog, calcipotriol, before TCI with the antigen and adjuvant (115). $CD4^+CD25^+$ Treg cells generated via this method prevent subsequent antigen-specific $CD8^+$ T cell proliferation and IFN-γ production, and are able to inhibit the induction and the elicitation of protein contact hypersensitivity.

Cancer

Dendritic cells loaded with synthetic tumor peptides have shown significant promise in cancer immunotherapy. Although promising, this method requires generating autologous DCs from the patient, spiking them with the tumor antigen, and reinjecting the cells back into the patient, which makes the process laborious and expensive. Targeting skin DCs by topical immunization seems to be a more practical approach that has demonstrated success. Effective cancer immunotherapy depends on activating $CD4^+$ T cells that can mediate $CD8^+$ T cell effector function and memory

development (116). Protein antigens applied to the skin induce both cell responses (117, 118). In a recent clinical trial, melanoma patients immunized via TCI developed cytotoxic $CD8^+$ T cell responses and exhibited regression of some lesions, which confirmed the targeting of skin DCs as a viable approach to tumor immunotherapy (119). Many additional studies also point to the potential of TCI in cancer immunotherapy. Pitcovski et al. constructed a melanoma multiepitope polypeptide (MEP) from four modified melanoma-associated antigens, gp100 (209–215), gp100 (280–288), MART1 (26–35) and tyrosinase (368–376), fused with a nontoxic mutant of *E. coli* LT (nLT) and delivered it via TCI in BALB/c mice (120). Significant antibody titers were obtained against the MEP in immunized mice. Seo et al. have shown that TCI with synthetic tumor epitope peptides in C57BL/6 mice primed tumor-specific cytotoxic T lymphocytes (CTLs) in the lymph nodes and spleen, protected mice against subsequent challenge with corresponding tumor cells, and suppressed the growth of established tumors (121). Huang et al. have tested the efficacy of TCI using an adenovirus vector expressing the human carcinoembryonic antigen (CEA) gene in BALB/c mice. CEA is a tumor-associated antigen known to be overexpressed in most carcinomas, including gastrointestinal carcinomas, but expressed at lower levels in normal colonic mucosa. TCI with the adenovirus-based vaccine elicited a robust antibody titer to CEA and significantly arrested the early growth of implanted tumor cell lines (122).

Approximately 20 million people in the U.S. are currently infected with genital human papillomavirus (HPV) (123), with half of these infections among adolescents and young adults 15–24 years of age (124). Persistent infection with HPV-16 is responsible for greater than 50% of cervical cancers worldwide (125). Therapeutic HPV-16 vaccination strategies aim at the induction of a cell-mediated immune response directed against the HPV-16 E6 and/or E7 oncoproteins constitutively expressed within tumor cells (126). TCI with a HPV-16 E7 oncoprotein–derived peptide containing multiple (CTL, Th, and B cell) epitopes induced strong E7-specific CTL responses. This peptide was also shown to protect mice against tumor growth after challenge with HPV-16 E7-positive tumor cells. TCI with E7 protein and CT/CpG led to the formation of an E7-specific humoral immune response (126).

Biological Warfare Agents

Use of biological weapons in war has always been a credible threat, but the need to develop curative and prophylactic measures has become more urgent in light of the recent anthrax scare. Biological weapons can be produced from bacteria, viruses, fungi, or toxins produced by these agents (127). Some of these diseases, such as inhalation anthrax and plague, have a high fatality rate. TCI using topical patches presents an especially attractive alternative in the event of a bioterrorism attack because immunization patches can be distributed widely and rapidly with relative ease and administered with little to no medical supervision. Many studies in the recently published literature demonstrate the feasibility of this approach.

Anthrax has recently become a serious tool for bioterrorism. *Bacillus anthracis* can form endospores that are easily transported and highly resistant to inactivation. Although antibiotics, antitoxins, and vaccines are available, concerns over their toxicity and the emergence of resistant strains have driven the development of second-generation products (128, 129). TCI with recombinant protective antigen (rPA) from *B. anthracis* has been shown to induce long-lasting neutralizing antibody titers in mice that are superior to those obtained with IM injection of alum-adsorbed rPA. The antibodies induced through TCI completely protected the immunized mice against challenge with spores of the avirulent strains Sterne and STI (129, 130), as well as the highly virulent Ames strain (131). Cui & Sloat (132) attempted to immunize BALB/c mice using a perflubron-based

microemulsion incorporating a PA-encoding plasmid. TCI with the topical microemulsion induced a weak anthrax-neutralizing immune response. Although not sufficient by itself, the microemulsion vaccine can be potentially combined with existing vaccines to produce a simplified and more convenient dosing schedule (132). γ-irradiated intact *E. coli* particles overexpressing anthrax antigens (PA63, PA83, and LF7) exhibited differential abilities to induce protection against intranasal challenge in A/J mice. PA83 and LF7 particles when coadministered three times at monthly intervals were most effective of all vectors and afforded partial protection (133). Optimized expression of anthrax antigens through codon optimization is expected to improve on preliminary results.

Immunosenescence: a gradual deterioration of the body's natural immune response to infections and pathogens due to age

Yersinia pestis, a Gram-negative bacterium, is the etiologic agent of plague and of significant concern as a possible agent of biological warfare. Several potential subunit vaccines against plague have been evaluated, and the *Y. pestis* proteins F1 and V are most promising in affording protection against flea-borne and aerosol exposure to pneumonic as well as bubonic plague (134, 135). Eyles et al. have investigated the feasibility of TCI with F1 and V in BALB/c mice using CT as an adjuvant and found that two or more immunizations induced significant levels of anti-F1 and anti-V antibodies. Although the antibody levels via TCI were relatively low as compared with SC, ID or IN immunization (136), TCI was very effective in boosting preexisting responses induced by these methods (137).

Immune Boosting and Stimulation

Boosting implies the repetition of an original immunization using a similar or modified dose, adjuvant, vector, site, or mode of immunization. Immune boosters take advantage of the natural immune response to infections or a primary immunization by inducing a stronger (increased concentration of antibodies or T cells), prolonged (increased persistence of antibodies or T cells), and more specific (humoral, mucosal, or a specific subtype of antibody) immune response (138). Heterologous boosting has been shown to produce higher and more sustained immune responses than homologous boosting (139–141). The finding that immunization by one route can prime for a secondary response by another route is important. This is especially true in the case of an epidemic or bioterrorism event in which a TCI patch can boost a parenteral priming dose, which will significantly reduce the burden on already stretched healthcare networks (142). TCI boosting of SC primed animals with F1-V vaccines against *Y. pestis* yielded higher concentrations of F1-V antibodies as compared with SC boosting (142). A prominent mucosal-humoral immune response was observed in a dual immunization approach that included oral priming with the B subunit of CT (CtxB) followed by TCI with CT boosters, which suggests that oral immunization may help target mucosal-humoral immune responses to subsequent TCI (143).

Immunosenescence in the elderly can mitigate the benefits of vaccination by inhibiting the induction of a robust immune response (144). TCI with strong adjuvants such as LT induces potent immune responses owing to the LT-induced migration of activated APCs from the skin to the proximal draining lymph nodes (68). An immunostimulant patch containing only LT (LT-IS) placed on the skin at the site of vaccine administration can significantly amplify the immune response to vaccines such as influenza with minimal adverse reactions at the site of immunization (145–148). Addition of an LT-IS patch can thus compensate for reduced immune function in the aged while providing a safe, cost-effective, and simple immunization strategy.

Given the preliminary success obtained with TCI in targeting various diseases, this approach potentially can be extended to other targets such as contraception (149), addiction, and fetal immunization (138).

VACCINES FOR TRANSCUTANEOUS IMMUNIZATION

A wide variety of agents have been studied for TCI as active vaccine candidates. Here we will review the developments in the design of TC vaccines within the past decade.

Subunit vaccine: a minimally sufficient immunogenic region of an antigen that can elicit an immune response similar to the whole antigen

DNA Vaccines

DNA immunization involves the introduction of plasmid DNA encoding the antigenic protein. Upon delivery of the plasmid to the target cells of the organism, the protein antigen is expressed within the cells and results in induction of an immune response to the antigen. Because the antigen is expressed in vivo, similar to natural viral infections, the post-translational and intracellular processing of the protein is considered to be authentic and both protective immune responses, cellular and humoral, are induced (98, 150). DNA vaccines are stable and less expensive as compared with traditional vaccines, easy to modify in response to pathogenic mutations, and safer than subunit or viral-based vaccines (147). Also, DNA vaccines can be designed to target the antigen to specific cellular compartments to enhance the desired immune response (151, 152). Several studies have successfully demonstrated the induction of a protective immune response via TCI of DNA vaccines (92, 99, 102, 103, 152–154).

Peptide Subunit Vaccines

B and T cells, key components of the immune response, identify antigens in different ways. Ig receptors on B cells bind to specific regions on the antigen called B cell epitopes. Receptors on T cells, in contrast, recognize the peptide sequences of the antigen presented by APCs as class I (cytotoxic T cell epitopes) or class II (T helper epitopes) MHC molecules (64). B and T cell epitopes can potentially be used instead of the entire antigen or pathogen as subunit vaccines. Advances in peptide chemistry have facilitated the rapid and inexpensive identification and synthesis of immunodominant epitopes for use as subunit vaccines (64, 155, 156). Specific peptide sequences from the antigen can be used to induce a $CD4^+$ T helper response (64, 93, 157) or a $CD8^+$ cytotoxic T cell response (121, 156, 158). Mixtures (159) or fusions of class I and II peptide epitopes (126), regulatory polypeptides (54), and peptide epitopes fused to carrier proteins for improved immunogenicity (160) have also been tested for TCI. Subunit vaccines are especially advantageous for TCI because short peptide sequences can be delivered across the skin with relative ease and do not pose toxicity issues owing to crossing the blood-brain barrier when administered systemically (30).

Recombinant Proteins

Human recombinant proteins are perhaps the most widely studied immunogen candidates for vaccination. Advances in recombinant DNA technology and the use of bacterial and mammalian cell culture systems make it possible to produce full-length protein antigenic signatures from pathogens or a fusion of polypeptide sequences that represent multiple antigenic signatures in a single protein. Several studies have demonstrated that full-length proteins can be delivered across the skin by topical application for desired immune responses (3, 84, 161–163). Limiting factors in using recombinant human proteins are the high costs associated with the use of cell-based expression systems that have low yields, the purification of the desired protein to remove any bacterial or other potentially immunogenic components, and maintaining the vaccines in a cold chain to preserve their stability and efficacy (13).

Glycoconjugates

Capsular polysaccharides (CPSs) are predominantly expressed on the surface of some pathogens and are a major target for the immune system. CPSs are considered T cell–independent antigens, and their use in vaccines has been optimal only with protein–carrier chemical conjugation. Successful glycoconjugate vaccines have been developed against *Haemophilus influenzae* type b (Hib) and several serogroups of *Streptococcus pneumoniae* and *Neisseria meningitides* (164). The main protective antigen of Hib is the capsule polysaccharide, polyribosyl ribitol phosphate (PRP). TCI with a commercial vaccine containing PRP conjugated to cross-reacting material (CRM197) has been shown to be successful in eliciting high antibody responses to PRP (94). TCI with a *V. cholerae* neoglycoconjugate (CHO-BSA) composed of a synthetic terminal hexasaccharide of the O-specific polysaccharide of *V. cholerae* O1 (Ogawa) conjugated with bovine serum albumin (BSA) was safe and immunogenic but predominantly induced systemic lipopolysaccharide responses of the IgG isotype (165). Glycoprotein and glycolipid constructs are overexpressed on the cell surfaces of malignant cells and can be used as tumor-associated antigens for the development of anticancer vaccines for use with TCI (166, 167).

VACCINE DELIVERY TECHNOLOGIES

The intact skin barrier is either impermeable or offers very low permeability to molecules greater than 500 Da (168). This has been a constraint in delivering vaccines across the skin. However, this challenge can be overcome through the use of innovative technologies. In this section we will review the various delivery systems (summarized in **Figure 2**) and adjuvants that have been used successfully for delivering vaccines by topical application on the skin.

Vaccine Carriers

A wide variety of carrier systems have been studied for development of TC vaccines. Vaccine carriers can stabilize the active vaccine, protect the vaccine from being degraded by proteolysis, target the vaccine to specific compartments in the skin, or act synergistically as a vaccine adjuvant. Here we review commonly studied vaccine carriers in TCI.

Nanoparticles. Nanoparticles have been shown to aggregate in follicular openings and penetrate along the follicular duct when applied topically on barrier-disrupted skin (169–171). In particular, 40 nm particles have been reported to penetrate not only into the hair follicle but also through the epithelium (172). This is of particular consequence because LCs reside at particularly high densities in this region. Targeting LCs in the hair follicle using nanoparticles therefore provides a potentially useful strategy for TCI (154, 173).

Viral vectors. TCI with whole inactivated viruses such as influenza and herpes simplex virus can induce humoral as well as cellular immune responses (89, 90). Adenovirus vectors that contain genetic material encoding antigens have been shown to be successful in patch-based TCI (29, 87, 122). Virus-like particles (VLP) are inert, empty capsids of viruses that contain no DNA/RNA from the virus itself but retain the structure of a virus. VLPs can be engineered to have antigens attached to them and thus to be used for TCI (174).

***E. coli* vectors.** *E. coli* particles overexpressing antigens can be used effectively to induce a robust immune response by TCI (133). Use of intact *E. coli* eliminates the need for

downstream purification processes to isolate the antigen or DNA from the bacterial components. Furthermore, *E. coli* ligands are capable of activating APCs and T cells, thereby acting as natural adjuvants during TCI (175, 176). A potential synergy can be expected between TCI with *E. coli*-vectored vaccines and preexisting immunity against *E. coli* owing to prior exposure in the gastrointestinal tract (177).

Vesicular systems. Vesicular systems have gained much attention recently as carriers of DNA and recombinant proteins as immunogens for TCI (178). Commonly employed vesicular systems for TCI include liposomes (97, 99, 103), niosomes (102, 179), transfersomes (88, 179), and vesosomes (180). It has been proposed that much like nanoparticles, vesicular systems gain access to epidermal APCs through hair follicles or pilosebaceous routes. Vesicles not only promote antigen transport across the skin but also offer protection for DNA vaccines from hydrolysis by deoxyribonuclease enzymes and act as depots for the sustained release of antigen.

Microemulsions. Microemulsions offer great potential for topical DNA vaccination (153). These systems are relatively easy to design, can easily accommodate various antigens and adjuvants, provide high entrapment efficiencies, and have high stability. A few studies have demonstrated the potential of microemulsions as carriers for TCI (132, 181).

Permeation enhancers. Permeation enhancers have been studied extensively for transdermal drug delivery (182–186). Synergistic mixtures of chemicals can potentially facilitate the passage of macromolecules across the skin (187). A few studies have demonstrated that carefully designed mixtures of commonly used chemicals can deliver protein antigens across the skin for a desired immune response (161).

Adjuvants

A wide variety of adjuvants have been studied for TCI. Selection of the optimal adjuvant is based largely on the desired type of response and a balance between attaining a high degree of directed immune response and reducing nonspecific adverse inflammatory consequences. Newer vaccines based on purified recombinant proteins, synthetic peptides, and plasmid DNA tend to stimulate a poorer immunogenic response, so effective adjuvants become more important (138). **Figure 3** summarizes the various adjuvants used in TCI.

ADP-ribosylating toxins. ADP-ribosylating bacterial toxins, such as CT, LT, and their derivatives, have received considerable attention owing to their notable success in TCI (54, 65, 157, 188–192). Although exact mechanisms have not been completely elucidated, recent observations may shed light on the potent adjuvanticity of these toxins. When applied to hydrated skin, CT diffuses rapidly throughout the epidermis and binds to epidermal KCs, which results in an increase in cyclic adenosine monophosphate (cAMP) levels (160). This may stimulate the secretion of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), which in turn act on LCs to trigger their maturation and migration to regional lymph nodes. Alternatively, CT and LT may act directly on LCs to promote their activation and migration to regional lymph nodes (146).

CpG motifs. CpG motifs are unmethylated CpG dinucleotides flanked by certain bases and abundant in microbial genomes but rare or absent from vertebrate genomes. Synthetic ODN-bearing CpG motifs have been studied for TCI because they are strong activators of innate

immunity. CpG motifs can stimulate LCs to secrete IL-12 and enhance the expression of the costimulatory molecules MHC-II and CD86 (65). They can also activate B cells, macrophages, natural killer cells (through IL-12), and T cells, acting as effective adjuvants for cellular and humoral immunity (116, 193–196). Generally, when placed on bare skin, CpG-ODNs exert an immunomodulatory effect that shifts immune responses to a Th1-type response (93, 197–200). CpG-ODNs can act synergistically with other adjuvants such as CT (69, 160, 197). Their effect is especially more pronounced with lower doses of CT, which could be advantageous given that high doses of CT can facilitate autoimmune reactions (197, 201).

TLR ligands. Imiquimod is a synthetic immunomodulatory ligand for TLR7 (Toll-like receptor 7) (202). It can induce maturation of DCs along with the release of inflammatory cytokines, thus enhancing antigen presentation (203–205). Imiquimod has shown a proven efficacy by topical administration in patients with HPV-associated or malignant skin diseases (204, 206, 207). TCI with imiquimod is effective in inducing potent CTL responses in vivo (158). TCI with peptide and imiquimod resulted in the generation of strong CTL responses that led to efficient antitumor immunity in a mouse model (208).

Cytokines. Cytokines are important mediators of cutaneous immune responses. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an essential cytokine for growth, viability, and maturation of DCs (209). The incorporation of GM-CSF with TCI results in increased infiltration of epidermal DCs in the skin at the site of antigen exposure (210). This strategy greatly increases cytokine gene expression in the lymph nodes and spleen, and enhances both antibody and cell-based immune responses to the antigen (209). Topical application of chemokine C-C motif ligand 20 (CCL-20) also increases the numbers of MHC-II positive cells in the epidermis (210). Several cytokines are involved in the growth, recruitment, and activation of DCs and are thus potential adjuvants for TCI (211). It is postulated that topical application of fms-related tyrosine kinase 3 ligand (Flt3L) alone or together with IL-3 can be used to initiate a strong Th1 response (209).

Chemicals. Several chemicals such as surfactants, when topically applied on the skin, are known to induce an irritation response similar to immunostimulatory responses (161). Topical formulations of chemicals can thus potentially be used as adjuvants for TCI. Pretreatment of skin with an anionic surfactant, sodium lauryl sulfate, greatly enhanced the antibody response to a topically applied antigen (212). Combinations of commonly used chemicals have also been shown to exhibit adjuvanticity when applied topically without adverse effects (161). Combinatorial and rational design strategies can be used to discover synergistic chemical mixtures that enhance antigen transport across the skin as well as enhance immune response against the antigen (161).

Barrier disruption. Skin barrier disruption leads to the secretion of proinflammatory cytokines such as TNF-α, IL-1α, IL-1β, and GM-CSF by KCs. TNF-α and IL-1β facilitate the migration of LCs by promoting disassociation from KCs and their maturation by upregulating the expression of a6 integrins, intercellular adhesion molecule 1 (ICAM-1), CD86, and MHC class II molecules. Disruption of the skin barrier also elicits the induction of antibacterial peptides involved in innate immune defense (201). These observations indicate that, by itself, skin disruption is highly immunostimulatory and can enhance antigen-specific immune responses (68, 95, 121, 201, 213–215). Other methods of skin barrier disruption, such as ultrasound (216), microneedles (217), electroporation (152), and gene gun delivery (152), also have the potential to act as effective adjuvants for immunization. These agents also enhance the delivery of vaccines into the skin, which contributes as much, if not more, toward inducing an immune response against topically applied vaccines.

Several reviews have focused on the ability of these methods to enhance molecular delivery into skin, so this aspect is not explicitly discussed here (218, 219).

Hyperthermia. Mild hyperthermia activates the immune system and produces conditions similar to adjuvants but with the potential for less toxicity (162). Hyperthermia can induce migration (220) and maturation of LCs by increase in the expression of costimulatory molecules such as CD80 and CD86 (162). TCI with diphtheria toxoid using local hyperthermia as an adjuvant results in an antibody response against the toxin. Hyperthermia-adjuvanted TCI is likely to have higher compliance as it does not cause any pain or visible damage to the skin.

CHALLENGES AND FUTURE OUTLOOK

In the 10 years since the early successful demonstration of TCI (192), many studies collectively have pointed to the potential and promise of needle-free immunization using a skin patch. Continued advances in the development of transdermal delivery systems, improved understanding of skin immunobiology, and technological innovations in vaccine design should further accelerate the translation of early laboratory successes with TCI into clinical prophylactics. There are, however, several issues and constraints that need adequate attention. The majority of studies related to TCI have demonstrated success in small animals. However, many small laboratory animal models possess a skin barrier that is more permeable than human skin (26). There are also other important structural differences. For example, human skin has 7–10 KC layers in the epidermis, whereas mouse skin has only 2–4 layers (153). As a result, extrapolation of results from animal studies to humans is not entirely straightforward. Furthermore, many TCI studies employ some sort of skin pretreatment, such as tape-stripping, ethanol swab, shaving, depilation, or abrasion, that may cause moderate to significant barrier disruption. These treatments can potentially reduce the skin transport barrier and cause artifacts such as amplified or larger than actual responses to immunization. As discussed before, even modest barrier disruption is immunostimulatory and may confound the effects of the antigen and the adjuvant. An important challenge in development of TCI strategies is therefore to design safe, effective, and quantifiable approaches to increase skin permeability to vaccine formulations.

Another important consideration is the use of mouse models in TCI studies and the similarity between human and mouse immune systems. For example, Aβ immunization in transgenic mice does not induce meningoencephalitis as it does in humans (106). It is therefore critical to follow up on small animal studies with human primate studies in which immunological equivalence has been established. Another concern arises from the use of CD34-derived DCs and LCs in in vitro studies related to TCI. Although these cells are generally similar to their in vivo counterparts, significant differences exist. Specifically, in vitro–derived LCs and DCs have distinct patterns of TLR expression and show marked differences in their ability to secrete cytokines upon binding to TLR ligands (221). These differences should be considered when translating in vitro findings to in vivo studies.

Bacterial toxins such as LT and CT have become quite popular as adjuvants in TCI studies. However, some studies indicate that application of CT to intact mouse skin induces and enhances autoimmune diseases affecting organs at distant anatomic sites, whereas its administration by the mucosal route has been reported to have the opposite effect (222). Repeated topical application of a central nervous system autoantigen along with CT on the intact skin of healthy mice induced relapsing paralysis with demyelinating immunopathologic features similar to multiple sclerosis. CT also exacerbated the severity of conventional experimental autoimmune encephalomyelitis induced by the autoantigen. Similarly, application of CT onto the intact skin of diabetes mouse

models, with or without insulin B peptide 9–23, exacerbated insulitis and T lymphocyte-derived IFN-γ and IL-4 production in the islets of Langerhans, resulting in an increased incidence and rate of onset of autoimmune diabetes (222). Such effects need to be further investigated.

TCI provides the benefit of prolonged exposure of an antigen to the skin immune system via a patch. Indeed, some studies have shown that merely prolonging the duration of antigen on the skin induces a potent antigen-specific antibody response even in the absence of an adjuvant (223). However, previous studies have shown that multiple vaccinations with the same peptide can result in reduced numbers of functional peptide-specific T cells (224). TCI protocols should therefore be developed so that the outcome is protective immunity and not tolerance to the antigen.

In summary, TCI offers an exciting avenue for the development of patient-compliant, needle-free, safe, and effective prophylactics. The ultimate success of this method will depend on effectively addressing the practical and scientific challenges discussed above.

SUMMARY POINTS

1. Transcutaneous immunization can induce humoral as well as cellular immune responses. The immune protection conferred by TCI is not restricted to the site of immunization but can be observed at distant sites such as the lung and gut-associated mucosa.
2. TCI can be used to target a wide range of diseases such as bacterial and viral infections, neurodegenerative diseases, cancer, and autoimmune diseases. It can be employed to confer protection against agents of biological warfare or to augment immune responses in people with disease- or age-compromised immune systems.
3. A variety of vaccine delivery systems, such as viral particles, *E. coli*, nanoparticles, vesicular systems, microemulsions, and chemical mixtures, can be utilized for TCI (**Figure 2**).
4. Bacterial toxins, cytokines, CpG motifs, hyperthermia, TLR ligands, and chemicals can be used as effective adjuvants for TCI.
5. Adjuvants that exhibit high toxicity via OR or IN routes are relatively safe when used with TCI.
6. Skin barrier disruption not only allows for effective vaccine delivery but acts as an adjuvant by initiating inflammatory processes similar to the innate immune response.

FUTURE ISSUES

1. How well do TCI results in animal models translate to humans?
2. What is a good in vitro and in vivo model of the human cutaneous immune system?
3. How does TCI compare with other mucosal immunization techniques (nasal, oral, vaginal and rectal) in terms of efficacy, cost, and ease of use?
4. Can TCI approaches be extended to design vaccines against novel targets such as substance abuse?

DISCLOSURE STATEMENT

P.K. and S.M. are inventors of technologies that have applications for transcutaneous immunization. S.M. is a consultant to fqubed Inc.

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RELATED RESOURCES

Centers for Disease Control and Prevention (CDC). Information on vaccine safety: **http://www.cdc.gov/vaccinesafety/index.htm**

National Institute of Allergy and Infectious Diseases (NIAID). Information on vaccines: **http://www3.niaid.nih.gov/topics/vaccines/**

World Health Organization (WHO). Information on immunization and vaccine development: **http://www.searo.who.int/EN/Section1226.asp**

Global Alliance for Vaccines and Immunization (GAVI): **http://www.gavialliance.org/**

Grand Challenges in Global Health Initiative on improving vaccines: **http://www.grandchallenges.org/ImproveVaccines/Pages/default.aspx**

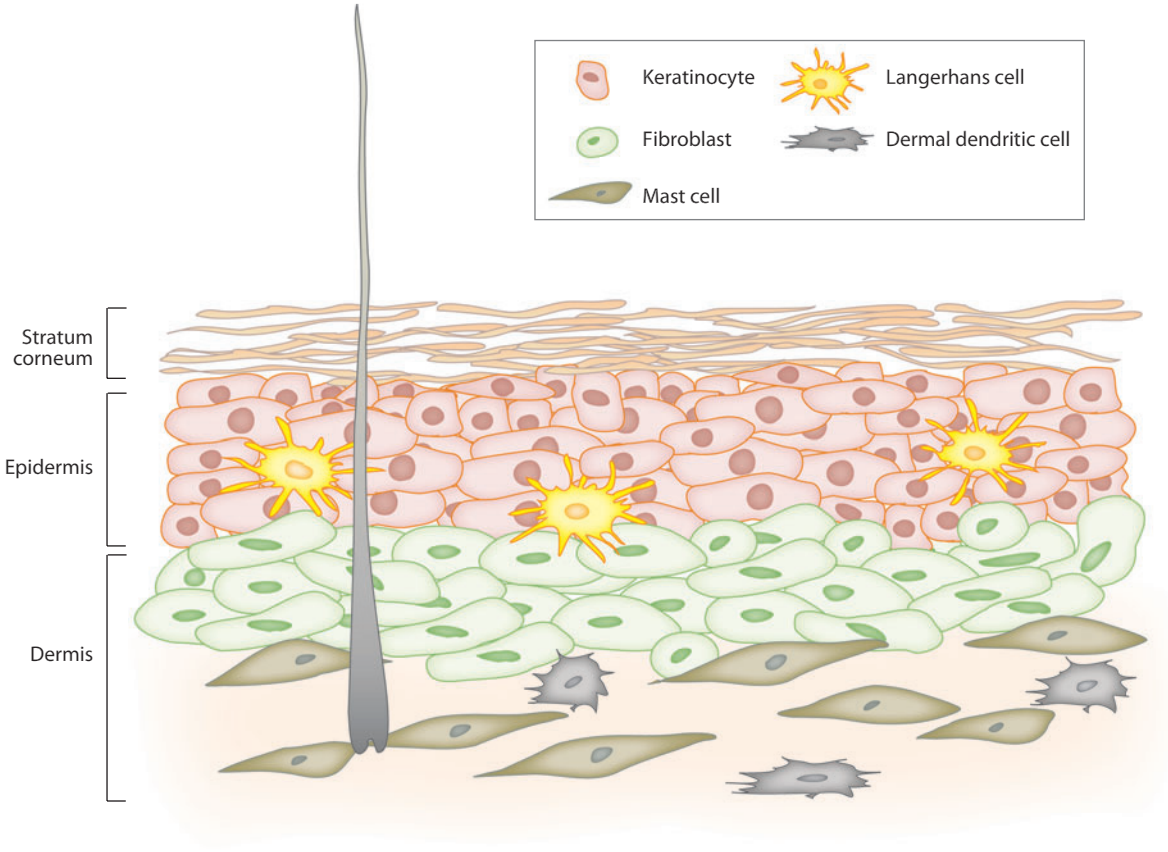

Figure 1

Schematic showing the structure of skin and the various components of the cutaneous immune network. Skin is composed of three layers: stratum corneum, epidermis, and dermis. Keratinocytes (KCs) and Langerhans cells (LCs) in the epidermis; fibroblasts (FBs), dendritic cells (DCs), and mast cells (MCs) in the dermis; and T and B lymphocytes (T and B cells) in the skin-draining lymph nodes (not shown) comprise the cutaneous immune network.

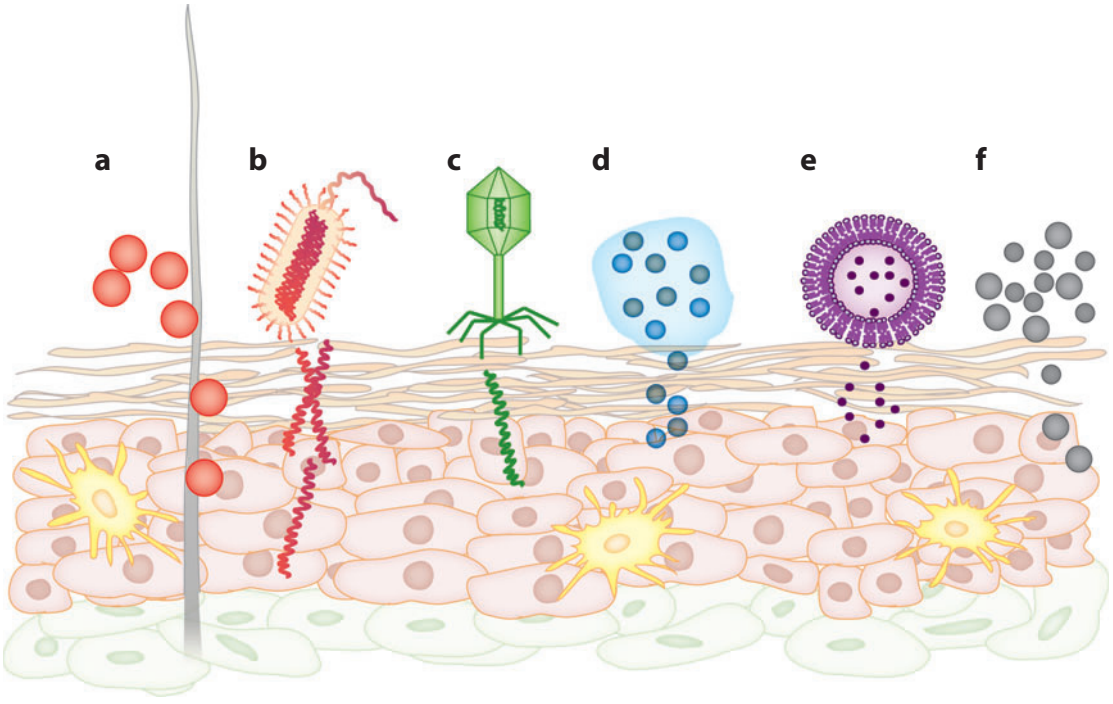

Figure 2

Vaccine delivery technologies used for transcutaneous immunization (TCI). (*a*) Nanoparticles: Topical application of nanoparticles leads to their aggregation in the hair follicles of the skin, which contain a high density of Langerhans cells. (*b*) *E. coli* vectors: Intact or irradiated *E. coli* particles overexpressing an antigen induce a strong immune response. (*c*) Viral vectors: Whole inactivated viruses, adenoviruses, and virus-like particles can all induce a cutaneous immune response. (*d*) Permeation enhancers: Synergistic mixtures of chemicals can facilitate the passage of antigens across the skin by disrupting the stratum corneum. (*e*) Vesicular systems: Liposomes, niosomes, transfersomes, and vesosomes encapsulate DNA and recombinant protein immunogens and deliver them across the stratum corneum. Vesicles can protect DNA vaccines from hydrolysis by enzymes and act as depots for the sustained release of the antigen. (*f*) Microemulsions: Microemulsions are well suited for transcutaneous vaccination because they are relatively easy to design, can easily accommodate various antigens and adjuvants, provide high entrapment efficiencies, and are highly stable.

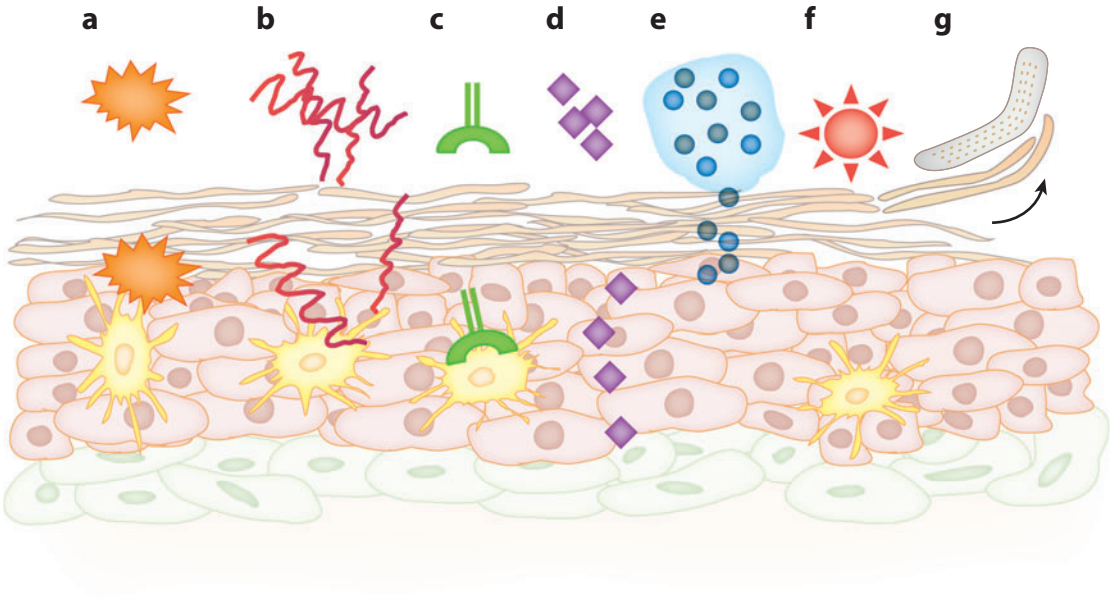

Figure 3

Adjuvants used for transcutaneous immunization (TCI). (*a*) Bacterial toxins: ADP-ribosylating toxins bind to Langerhans cells (LCs), which causes their migration to lymph nodes. (*b*) Cytosine-phosphate-guanine (CpG) motifs: Poly-dinucleotide CpG motifs stimulate LCs and enhance the expression of the costimulatory molecules major histocompatibility complex (MHC)-II and CD86. They can also activate B cells, macrophages, natural killer cells, and T cells. (*c*) Toll-like receptor (TLR) ligands: These bind to Toll-like receptors and induce maturation of LCs along with the release of inflammatory cytokines, thus enhancing antigen presentation. (*d*) Cytokines: Cytokines are important mediators of cutaneous immune responses and essential for the growth, viability, and maturation of dendritic cells. Topically applied cytokines can act as potent adjuvants. (*e*) Chemicals: Topical mixtures of chemicals can induce an inflammatory response similar to an innate immune response and enhance the presentation and processing of antigens by LCs. (*f*) Hyperthermia: Mild hyperthermia activates the immune system and produces conditions similar to adjuvants but with the potential for less toxicity. Hyperthermia can induce migration and maturation of LCs by increasing the expression of costimulatory molecules such as CD80 and CD86. (*g*) Barrier disruption: Skin barrier disruption leads to the secretion of proinflammatory cytokines and facilitates the migration of LCs by promoting disassociation from keratinocytes. Disruption of the skin barrier also elicits the induction of antibacterial peptides involved in innate immune defense. Barrier disruption methods also enhance molecular delivery and can be potentially included under delivery technologies.

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Contents

Errata

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