Vesicular Carriers for Transcutaneous Immunization

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21.1 Introduction

Immunization or vaccination is an approach of providing defensive obstruction to the body, which helps the body to fight against incoming pathogenic attack/invasion, which the body is immunized against. Thus, immunization creates the ability in the body to fight against various microorganisms and their products (Singh et al. 2002). The role of the skin as a barrier to the external environment depends on the dynamic role of the skin-associated immune-responsive cells, which have been explored and exploited for their active participation in intradermal vaccination (Streilin 1985). Being immunologically rich, the skin offers an attractive route for vaccination (Chen et al. 2001). Vaccination seems to be the most promising strategy for the prevention of many diseases as it is able to promote protective immune response both systemically and at mucosal surfaces. Dermal and transdermal delivery of proteinaceous bioactives encounter massive challenges (Foldvari et al. 1999). Efforts are continuously being done to develop an efficient technique which could deliver peptides and proteins through the skin into the dermal layers. Dermal delivery is having advantages over other routes of administration of peptides, proteins, and antigens as it has the possibility to bypass gastrointestinal degradation and hepatic first-pass elimination, and it shows better patient compliance (Chien 1987).

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21.2 Need for Safer Delivery of Vaccines

Despite the positive effects of vaccination on health, some of the adverse effects associated with injections of vaccines have been realized. Parenteral route causes a significant pain, trauma of needle injection, distress, common adverse reactions, tissue reactions, and an alarming rise of infections, which result in lack of patient compliance. Moreover, administration of vaccines by injections requires syringes, needles, and trained personnel (Pashine 2005). Other delivery routes like nasal (Slutter et al. 2008), percutaneous (Mikszta and Laurent 2008), oral (Simerska et al. 2009), and pulmonary (Giudice and Campbell 2006) route have also been explored for the vaccine administration. Among the different routes, the percutaneous route (vaccination through intact or pretreated skin) is predominantly interesting, as successful and effective immune response can be induced via the skin. In addition, prevention of the direct contact of potent (even slightly toxic) adjuvants with the blood circulation makes the skin a safer route for immune stimulation (Ponvert and Scheinmann 2003). However, the barrier nature of the stratum corneum (uppermost layer of the skin) imposes a major hindrance for the transport of antigens across the skin.

21.3 Transcutaneous Immunization

Transcutaneous immunization (TCI) is a new method for the introduction of antigens into the skin by topical application of vaccine formulations onto the skin, which provides access of antigens to the skin-associated immune system without the use of needles. TCI is an alternative novel method for the conventional vaccination routes, which has been shown to elicit systemic and mucosal antibody responses resulting in the induction of protective immunity against infectious pathogens (Mikszta and Laurent 2008). This is one of the most noteworthy benefits of TCI over the traditional vaccine administration.

It relies on the application of antigen with adjuvant onto the outer layer of the skin and subsequent delivery to underlying densely distributed and potent antigen-presenting cells (APCs), Langerhans cells (LCs), to generate robust

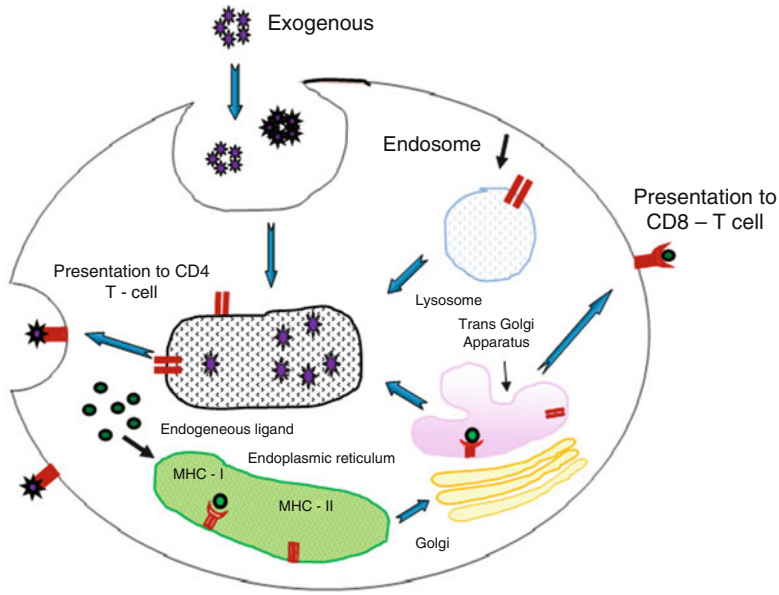

Fig. 21.1 Schematic presentation of the intracellular routes of antigen

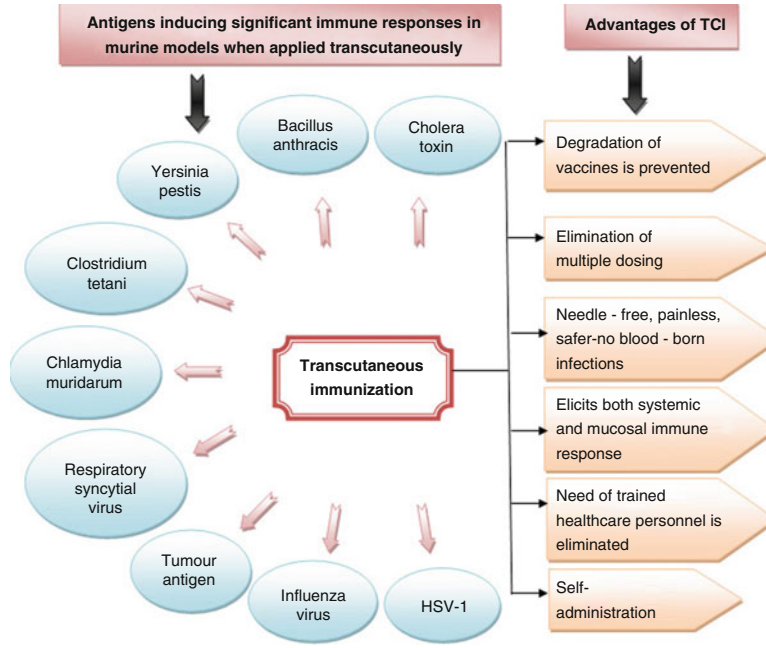

Fig. 21.2 Schematic representation of advantages of TCI and some of the antigens that can induce immune response when applied transcutaneously

immune responses (Fig. 21.1) (Glenn et al. 1999). However, although the vaccination through the skin is an eminent option because of the presence of a high amount of antigen-presenting cells, the stratum corneum hinders vaccine diffusion into the skin. The first investigation of using the skin as a site of vaccine administration was reported in 1997 (Tang et al. 1997), and since then, extensive reports have been published which supported that TCI is an effective vaccine delivery method in animals and humans. Immunocompetent LCs are found in close proximity to the stratum corneum and in abundance along the transdermal penetration pathways, whereas dendritic cells are present in high densities in the dermis (Gupta et al. 2004, 2005).

Presence of these cells makes the skin highly attractive as a target for vaccines by chemical, mechanical, or nanotechnological means and devices thereby promoting access of vaccine antigens to these APCs. Disruption of the stratum corneum using either physical disruption (e.g., tape stripping), penetration enhancers, or cytokine conditioning of the immunization site may provoke immune responses by TCI (Palamara et al. 2004). The advantages of TCI and antigens used for TCI are shown in Fig. 21.2.

21.4 Immunology of the Skin

The skin is an attractive target site for gene therapy protocols, drug, and vaccine delivery. The skin harbors a wide variety of immune cells and elicits a strong immunological response, when it comes in contact with any immunogen. Topical immunization may be an attractive option for both prophylactic and therapeutic vaccines. The skin is an active immune surveillance site rich in potent antigen-presenting dendritic cells (DCs), such as LCs in the epidermis and the immature dendritic cells in the dermis. Other cells present are mast cells in the dermis, resident antigen-presenting cells, and transient inflammatory lymphocytes. These cells altogether function in association with lymph nodes and are responsible for generation of both cellular and humoral immune responses (Gupta et al. 2004).

The skin is well set with a complex network of immune cells, described as "skin-associated lymphoid tissue (SALT)" and "skin immune system," and it constitutes a primary immunological barrier to the external environment. The presence of cytokines which have the capacity to regulate the immune responses confirms the existence of SALT in the skin. The skin immune system is

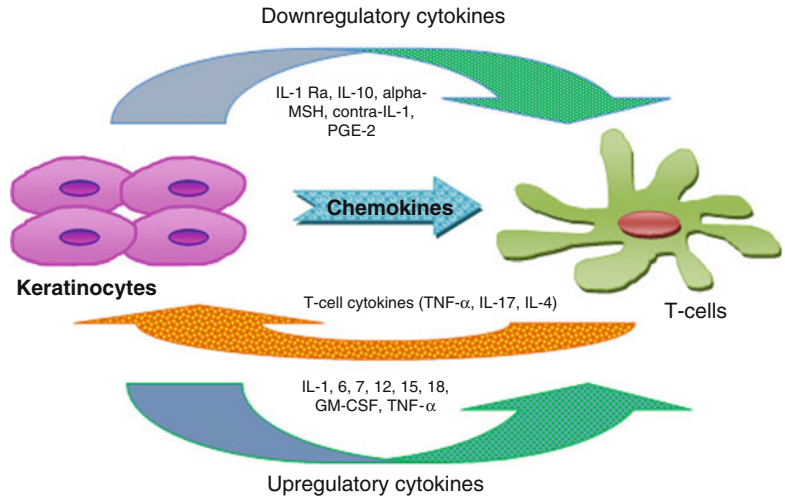

Fig. 21.3 Interrelation mediated by humoral factors between keratinocytes and T cell

capable of eliciting both innate and adaptive immunities (Nestle et al. 2009). The main gate keepers of the skin immune sentinels are DCs, as professional APCs which are capable of eliciting both innate and adaptive immunities. SALT is comprised of LCs, recirculating T lymphocytes, keratinocytes, and a set of draining peripheral lymph nodes. Lymphatics drive the antigens to the lymph nodes where they come in contact with the epidermis and with the APC because migratory T cells are attracted toward the peripheral lymph nodes (Chu et al. 2011) (Fig. 21.3).

21.5 Delivery Considerations

The transport and migration of antigen across the skin barrier, and consequently its uptake and maturation by DCs, are the two main challenges encountered by TCI. Since considerable variation occurs between different species in the structural characterization and lipid composition of the skin, it is imperative to have knowledge of the composition and characteristics of the skin of the species that is to be vaccinated. A minute understanding of the stratum corneum composition is required to facilitate the development of targeted and topically applied vaccine formulation. The highly compact structure of the stratum corneum with alternating hydrophilic and lipophilic area provides effective first-line defense against the invasion of chemicals, pathogens, and therapeutics topically applied to the skin. Alternating hydrophilic and lipophilic areas present a barrier in particular against large and hydrophilic molecules. The purified antigens are highly unstable when applied in their native state, hence innovative strategies are developed (i.e., suitable carrier devices or formulations), which enable antigen stabilization and facilitate their permeation (Combadière and Mahé 2008).

21.5.1 Skin Structure

The skin is composed of three major layers: the epidermis (about 50–150 μm thick), dermis (about 250 μm thick), and hypodermis or subcutis (Young et al. 2006). The stratum corneum being the outermost layer of the epidermis is composed of non-nucleated highly keratinized cells surrounded by densely packed lipid molecules which are responsible for the barrier function of the skin. The use of adjuvants (agents that stimulate the immune system) like bacterial exotoxins, disrupting the stratum corneum by tape stripping, swabbing with alcohol or other solvents, hydration, ultrasound, microneedles, and other physical or chemical permeation enhancers are the general techniques used to overcome the barrier nature of the stratum corneum. These

techniques not only weaken the skin barrier but also activate resident cells to augment expression of cytokine and to enhance antigen presentation. The variations exist in the thickness and composition of the skin at different sites in the human body which affect the permeability characteristics of applied antigens. There are many studies that confirm the difference in the role and responses of skin-resident DCs (Wang et al. 2007), serum antibody responses, and antigen-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) at different anatomical sites.

21.5.2 Transportation into and Across the Skin

Three possible routes via which the antigen can permeate through the skin by a passive diffusion process are transcellular, intercellular, and appendageal routes (Prow et al. 2011). Physicochemical properties such as molecular weight or volume, solubility, and the lipophilicity govern the diffusion rates of the antigen/adjuvants across the intercellular lipidic channels/routes, which have been estimated to be 19 nm. Numbers of approaches have been explored for the efficient delivery of bioactive molecules to the skin, which overcome the barrier properties of the stratum corneum, such as physical, chemical, and vesicular approach (Merwe et al. 2006).

21.5.3 Vesicular Systems for Transcutaneous Immunization

Novel drug delivery systems are being investigated which successfully overcome the problems regarding patient compliance and safety and opened up both opportunities/options for alternative therapeutic strategies to evoke immunological responses without breaching the skin barrier (Teichmann et al. 2007). Moreover, use of these carriers is also beneficial because they require no specially trained personnel and may avoid risk associated with needle-borne prick. Novel vesicular systems could improve vaccination programs by acting as adjuvants to enhance the immunogenicity of antigens, which otherwise induce "weak" immune response when applied topically (Gupta and Vyas 2012). Topical immunization includes the utilization of carriers like liposomes, niosomes, ethosomes, and transferosomes, since they are proficient in transferring immunogens (DNA and antigens) across the intact skin, by enhancing skin permeability for bioactives. These vesicular carriers utilize different pathways in the skin, i.e., either the intercellular lipidic route or the hair follicles to cross the skin barrier and reach the desired cells.

The advantage of using vesicles for vaccine delivery is also their ability to retain the antigen for a longer time and can act as local depot for sustained release of immunogens (Singh et al. 2002).

21.5.3.1 Liposomes

Liposomes are one of the most commonly and extensively studied vesicles representing a promising carrier system for topical delivery of drugs, biologically active molecules, and antigens. They are spherical vesicles made up of phospholipids (varying lipid composition) amenable for cutaneous delivery. Various mechanisms by which the antigen-loaded liposomes permeate through the skin are schematically presented in Fig. 21.4. These mechanisms may be attributed to the similarity in lipid composition of liposomes to the epidermis that enables them to penetrate deeper into the skin through the epidermal barrier. Dermal accumulation and depot formation of drugs/antigens by liposomes are responsible for the localized effect of antigens.

Liposomes encapsulating epitope from *Plasmodium falciparum* were evaluated in a clinical study (Fries et al. 1992), and they were found to be safe and effective as vaccine against malaria. Initially, simple liposomes were used for the introduction of genes (plasmids) and oligonucleotides into cells; however, later modified cationic lipids were used to improve the compaction of the DNA and to neutralize the negative charge.

Advanced types of liposomes like pH-sensitive liposomes may promote the fusion of the membrane of liposome with the cell

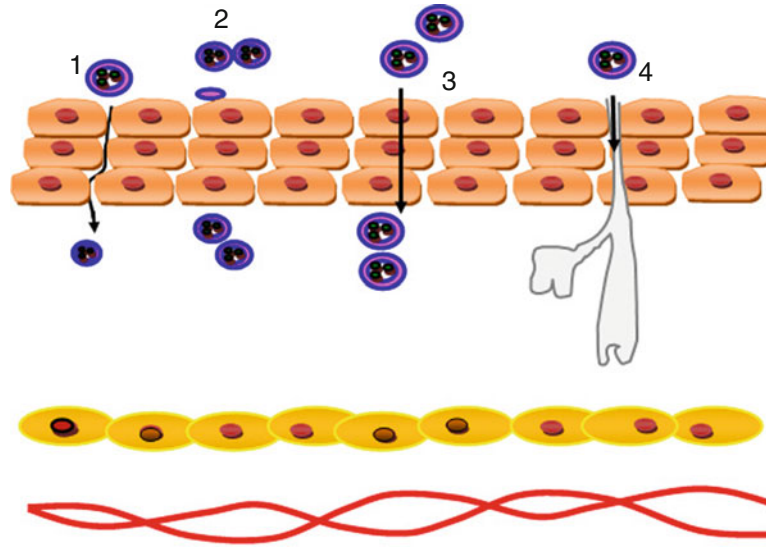

Fig. 21.4 Various mechanisms of penetration of antigen-loaded liposomes across the skin: (*1*) intercellular transport, (*2*) integration with skin lipids, (*3*) transcellular transport, (*4*) pilosebaceous-mediated delivery

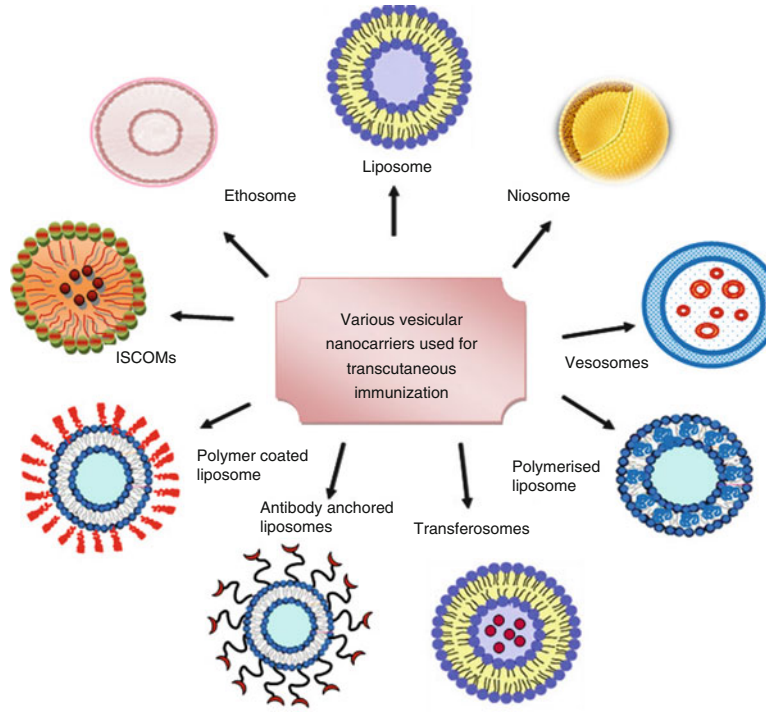

Fig. 21.5 Various vesicular nanocarriers that can be explored for transcutaneous immunization

membrane or within the lysosomal membrane at low pH and thus allow the DNA to escape into the cytoplasm of the cell (Couvreur et al. 1997). Oleic acid, palmitoyl-N-homocysteine, dipalmitoyl succinyl glycerol (DPSG), and cholesteryl hemisuccinate (CHEMS) are the agents that formulate pH-sensitive liposomes. As liposomes were successful in gene therapy trials, they can also be exploited for the delivery of plasmid-encoding genes from pathogens in order to stimulate immunity against that pathogen. Schematic representations of various vesicular carriers that can be used for TCI are shown in Fig. 21.5.

21.5.3.2 Immune-Stimulating Complexes (ISCOMS)

ISCOMs are spherical, micellar matrix constructs of about 40 nm diameter and have size comparable to that of viruses (Cui and Mumper 2002). ISCOMs incorporate amphiphilic antigens like membrane proteins, saponin mixture (Quil A), cholesterol, and phospholipids. These may promote endocytosis of antigens by DCs, monocytes, and macrophages, thus effectively promote T- and B-cell activation (Cui and Mumper 2001).

It was hypothesized that ring-shaped micelles with a diameter of about 10 nm are the building blocks of ISCOMs where the composition influences the aggregation behavior. In the early 1970s, Quil A, a potent adjuvant, has been used as such in veterinary vaccines. Hydrophilicity in the outer area is maintained by the high fraction of Quil A which is essential to prevent micelle–micelle hydrophobic interactions. Fluidity of the micelles is due to phospholipids that allow the formation of spherical structures: i.e., empty ISCOMS consisting of about 14 ringlike micelles. ISCOMs can only contain antigens if hydrophobic; electrostatic interactions or hydrogen bonding (between carbohydrates) is established and involved (Windon et al. 2002).

21.5.3.3 Niosomes

Niosomes are nonionic surfactant-based vesicles that have gained wide acceptance as the topical carrier for dermal or transdermal delivery of bioactives and immunogens (antigens or DNA). As compared to liposomes which cause corneocyte swelling and disruption of the intercellular lipid ultrastructures, niosomes made up of decycloethyleneoleylether result in fusion of corneocytes and formation of lipid stocks. Niosomes can also be used for targeting of immunogens to the pilosebaceous units in order to transfer the immunogens or other active substances to the deeper skin layers. Vesicles also protect antigen from degradation by enzyme attack and hence act as rate-limiting membrane barrier serving as a local depot for the sustained release of encapsulated antigen (Schreier and Boustra 1994). Niosomes of optimum size (2–6 μm) play an important role in the case of pilosebaceous targeting as they enter the pilosebaceous units against the sebum outflow. Drug transport across the skin depends on the vesicle composition and physicochemical properties as it was reported that liquid-state vesicles are more effective than gel-state vesicles in enhancing drug transport (Vyas et al. 2005).

In a study by our group, topical delivery of niosomes containing plasmid DNA encoding hepatitis B surface antigen (HBsAg) was investigated (Vyas et al. 2005). These niosome-based systems were applied topically to mice, and there serum anti-HBsAg titer and cytokine levels (IL-2 and IFN-c) were assessed for the immune-stimulating activity. Titer values obtained after 6 weeks were analogous and comparable to that elicited by intramuscular injection of pure HBsAg. In addition, a high thermodynamic activity gradient is created at the bioactive stratum corneum interface as a result of adsorption and fusion of niosomes onto the surface of the skin (Schreier and Boustra 1994). Surfactants used in the niosome formulation enhance penetration and reduce the barrier property of the stratum corneum (Valjakka-Koskela et al. 1998). Uptake of antigen-loaded nanocarriers by the LCs is represented in Fig. 21.6.

Niosomes containing HBsAg for topical immunization have been prepared by using reverse-phase evaporation technique with an entrapment efficiency of 58.11 ± 0.71 % (Maheshwari et al. 2011). In another study, to target LCs, niosomes were coated with a modified polysaccharide O-palmitoyl mannan (OPM) (Jain and Vyas 2005), and it was resulted that niosomal formulations showed a significantly higher serum immunoglobulin G (IgG) titer than alum-adsorbed BSA ($P < 0.05$) when applied topically. Moreover, it was also found that mannose-coated niosomes elicited appreciably higher serum IgG levels as compared with plain uncoated niosomes ($P < 0.05$).

21.5.3.4 Ethosomes

Ethosomes are interesting and innovative vesicular carriers that are soft and malleable, hence, enabling improved delivery of active agents. They represent ethanol-containing liposomes, which are able to provide an effective antigen

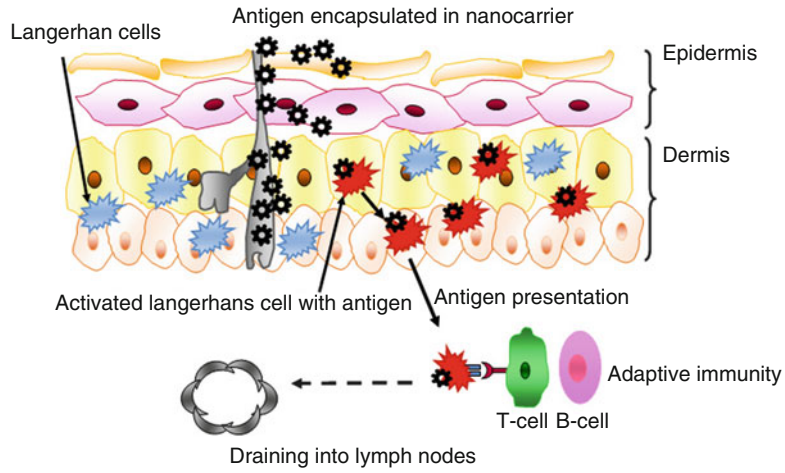

Fig. 21.6 Schematic of nanocarrier systems co-encapsulated with antigen taken up by Langerhans cells for transcutaneous immunization

delivery to deep skin strata more efficiently than conventional liposomes (Dubey et al. 2007; Dayan and Touitou 2000). In a study, a robust systemic and mucosal humoral immune response was elicited when HBsAg-loaded ethosomes were applied topically in experimental mice. In vitro permeation studies using human cadaver skin revealed that transcutaneous delivery of the antigen was much higher for antigen-loaded ethosomes in comparison to antigen-loaded liposomes and plain HBsAg solution. HBsAg-loaded ethosomes are reported to have the ability to carry the antigen(s) to target the immunological environment of the skin and are able to produce a protective immune response. Thus, it was shown that ethosomes possess a great potential in the development of a transcutaneous vaccines (Mishra et al. 2008).

21.5.3.5 Transfersomes

Transfersomes™ (IDEA AG, Germany) are specially designed unique lipid and surfactant-based vesicles that offer flexible characteristics and excellent approach for topical immunization. These ultradeformable carrier systems are highly efficacious in transferring the bioactive molecules across the stratum corneum by virtue of their high capability of changing shape and passing through the natural pores in the skin layer. Transfersomes™ of diameter 500 nm are able to pass through a skin pore of diameter less than 100 nm which clearly indicates that these carriers can permeate through the minute pores present in the skin having a diameter five times less than their own diameter.

After the transfer of antigen-loaded vesicles through the intact skin, antigen is delivered to the lymphatics from where they can be transferred to lymph nodes. It is found that Transfersomes™ with respect to other vesicular carriers give rise to elevated antibody titer. Moreover, when applied topically in a low dose, they show comparable titer values with their intradermally applied counterparts. The Transfersomes™ are under investigation for the development of human vaccines, and if designed suitably, they can have satisfactory immunoadjuvant action and ability to target macrophage. Transfersomes™ incorporating gap junction proteins of bacteria have been developed for the topical application and resulted in higher titer value of antibodies against the gap junction proteins than subcutaneous injection (Paul et al. 1998).

The structural flexibility of the Transfersomes™ is due to the presence of sodium deoxycholate. Cationic lipids being positively charged like DOTMA (N-[1-(2,3-dioleyloxy)-propyl]-N,N,N-trimethylammonium chloride) can be used along with sodium deoxycholate for preparing a novel vesicular construct that has the capability of penetrating intact skin and tends to form a composite complexation with anionic DNA. In a study by our group, the possibility of cationic Transfersomes™ to be used as topical

carriers for DNA vaccine was studied. The specific immunological response induced by plasmid DNA encoding for HBsAg antigen loaded in cationic Transfersomes™ was compared with that elicited by topical or intramuscular administration of liposomes and naked plasmid DNA. DOTMA was used as a cationic lipid, instead of egg PC, with sodium deoxycholate. Under an electron microscope, these cationic vesicles appeared as unilamellar vesicles as shown in Fig. 21.7 (Mahor et al. 2007). Results revealed that immune responses with DNA-loaded cationic Transfersomes™ were considerably higher as compared to naked DNA when mice were topically immunized. Moreover, the antibody titer obtained after 6 weeks was analogous and comparable to that elicited by intramuscular injection of pure HBsAg. DNase enzymes present in interstitial space hydrolyse the naked DNA (Perrie and Gregoriadis 2000); thus, the vesicles in addition to their intrinsic ability to be taken up by the APCs also shield DNA from hydrolytic attack by DNase.

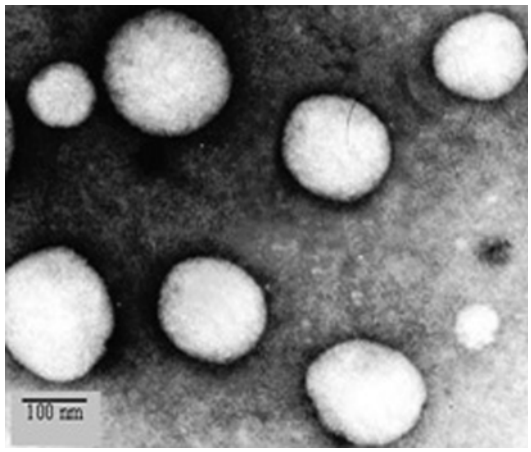

Fig. 21.7 Transmission electron microscopic image of DNA-loaded cationic transfersomes (Mahor et al. 2007)

The immunity induced by topical immunization appears to be long lasting, as indicated by persistence of serum antibodies. Sodium deoxycholate present in the Transfersomes™ is responsible for their deformability, whereas niosomes and liposomes usually contain cholesterol that imparts rigidity to the vesicle. Thus, niosomes and liposomes are not much capable of passing through the pores smaller than their own diameter.

In a study by our group, elastic vesicles Transfersomes™, niosomes, and liposomes were compared for their potential in noninvasive tetanus toxoid (TTx) delivery (Gupta et al. 2005). It was found that Transfersomes™ can entrap higher amounts of proteins as compared to liposomes and niosomes (Yoshioka et al. 1994). Schematic presentation of mechanisms of penetration of antigen-loaded transferosomes across the skin epithelium is shown in Fig. 21.8.

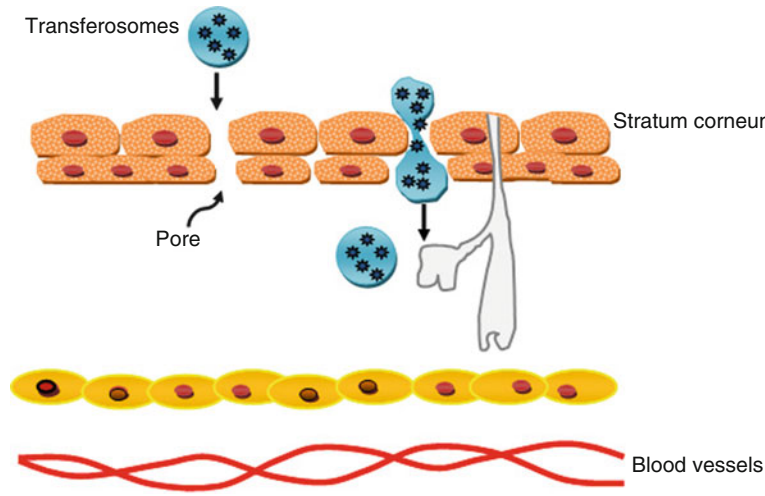

Fig. 21.8 Mechanisms of penetration of antigen-loaded transferosomes across skin epithelium

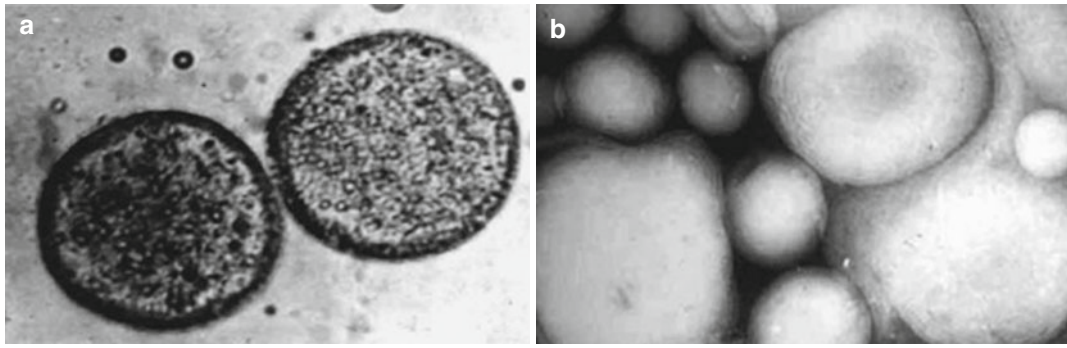

Fig. 21.9 Transmission electron microscopic image of vesosomes (**a**). Phase contrast image of fusogenic vesosomes (**b**) (Adapted from Mishra et al. 2006a, b)

In the case of liposomes and niosomes, the entrapment efficiency was almost equivalent; however, in case of niosomes, slightly less drug entrapment was estimated. This may be due to the presence of surfactants in niosomes that are responsible for the pore formation in the outer layer leading to lower drug entrapment. However, presence of nonionic surfactants in niosomes is responsible for the enhanced permeation effect which is reflected by the better immune response elicited by niosomes than by liposomes. Deformability, a unique property of Transfersomes™, is combined with sensitivity of immunization. Numerous pores in the horny region of the skin may act as permeability shunts and locally lower the skin barrier potential. Transepidermal water gradient strongly drives the deformable Transfersomes™ through these pores. Because of the low deformability of liposomes and niosomes, they are not able to enter the intact skin spontaneously as Transfersomes™. Thus, it can be concluded from the reported studies that Transfersomes™ can be regarded as superior delivery systems for TCI owing to the higher entrapment efficiency and maintenance of better immune response.

21.5.3.6 Vesosomes

Vesosomes, i.e., fusogenic liposomes, are a class of novel carriers which have the unique property of fusing with the target cell and can potentially aid the intracellular delivery of encapsulated antigen/proteins. Lipids, such as dioleoylphosphatidylethanolamine (DOPE), that are able to form non-bilayer phases, contribute in the formation of vesosomes which can promote destabilization of the bilayer of vesicles, inducing fusion events (Kono et al. 2000). Mishra et al. 2006b have studied tetanus toxoid (TTx) containing vesosomes, which can deliver it effectively in order to produce an effective immunization via topical administration. Structural studies on the shape of the prepared vesosomal system using transmission electron microscopy and phase contrast microscopy have indicated that the systems were almost spherical with smooth surface and unilamellar in nature (Fig. 21.9). The developed novel fusogenic vesosomes, composed of inner cationic liposomes contained in an outer liposomal bilayer, have the potential to microinject entrapped antigen directly into the cytoplasm of target cells through fusion with the plasma membrane.

Fusion of vesosomes with the APCs results in cytosolic delivery of the antigen. It is evident by the earlier studies that highly charged particles with size greater than 10 μm are unable to reach deep into hair follicle due to chemical environment present in hair follicle. Moreover, microparticles less than 3 μm are distributed

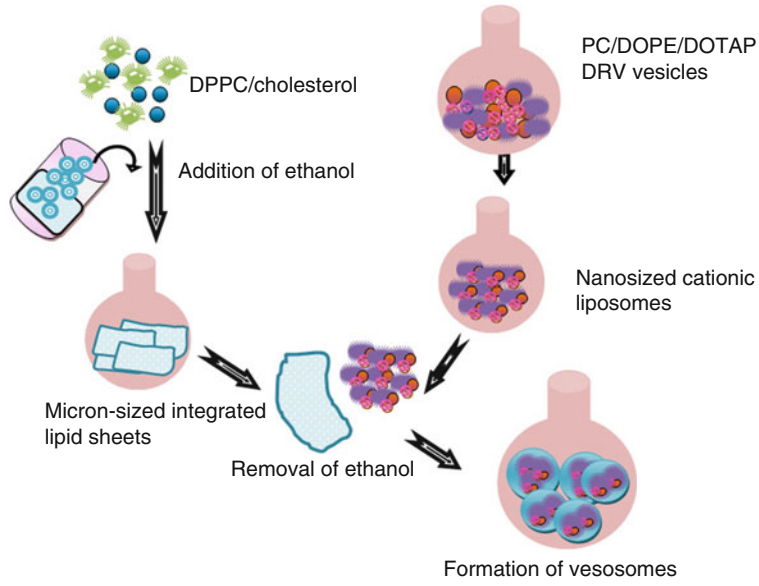

Fig. 21.10 Preparation of vesosomes

randomly into hair follicles and stratum corneum, and those ranging from 3 to 7 μm could selectively penetrate follicular ducts (Rolland et al. 1993). Taking these facts into consideration, vesosomes have been designed and optimized in respect of size and charge. Level of IgG in the skin was increased considerably with vesosomal systems compared to conventional liposomal formulations administered topically. This may be possible due to the release of encapsulated cationic vesicles within hair follicles and subsequently fusion of these vesicles with immune-responsive cells (e.g., LCs, epidermal T cells) for better and more effective antigen presentation. Furthermore, encapsulated antigen may be released through cationic fusogenic liposomes in the vicinity of these cells (Mishra et al. 2006a).

In a study by Baraka et al. 1996, the fusogenic properties of non-phospholipid liposomes containing dioxyethylene acyl ethers and single-tailed non-phospholipid amphiphiles as principal membrane constituents were prepared. These liposomes can fuse with phosphatidylcholine liposomes at neutral pH. Scheme presentation of preparation of vesosomes is shown in Fig. 21.10. Further, studies indicated that these non-phospholipid liposomes could fuse effectively with the plasma membranes of erythrocytes and fibroblasts.

Table 21.1 shows some of the nanocarriers used for the transcutaneous delivery of antigens.

Table 21.1 Schematic overview of nanocarrier-mediated gene/antigen delivery after topical application onto the skin

Delivery system	Vesicle composition	Antigen/DNA/plasmid encoding	Tested on	Effect	Reference
Cationic Transfersomes™	DOTMA and sodium deoxycholate	Hepatitis B surface antigen (HBsAg)	HepG2 cell line has >90 % cells were viable	Higher anti-HBsAg antibody titer and cytokines level compared to pure HBsAg	Mahor et al. (2007)
Cationic liposomes	Soybean phosphatidylcholine (SPC), 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP) and 1,2-dioleoyl-sn-glycero-3 phosphoethanolamine (DOPE)	Ovalbumin (OVA) and a toll-like receptor (TLR) ligand	HEK293 cells	Interferon production by restimulated splenocytes, OVA/CpG liposomes shifted the IgG1/IgG2a balance more to the direction of IgG2a	Bal et al. (2011)
Ethosomes	SPC, Span® 80	HBsAg	NIH3T3 cells (embryonic fibroblast cells)	Stimulate the T lymphocytes and generation of TH1-type immune response higher than liposomes and soluble HBsAg	Mishra et al. (2010)
Vesosomes	Phosphatidylcholine, DOPE, DOTAP, dipalmitoyl phosphatidylcholine (DPPC)	Tetanus toxoid (TTx)	Murine macrophage cell line J774 A.1	Serum IgG titer was higher than alum-adsorbed TTx given intramuscularly and topically administered plain tetanus toxoid solution, plain liposomes, and cationic fusogenic liposomes. The vesosomal systems could elicit combined Th1 and Th2 immune responses following topical administration	Mishra et al. (2006b)
Elastic liposomes	SPC was mixed with Span® 80	HBsAg	–	Induces robust systemic and mucosal antibody response, efficient delivery of antigens to LCs cells and lymphatics	Mishra et al. (2006a)

Elastic liposomes	SPC was mixed with Span® 80	HBsAg	Murine bone marrow cells	Induced approximately a two- to threefold increase in IL-2 levels, four- to fivefold increase in IFN-γ levels and twofold increase in TNF-α levels	Mishra et al. (2007)
Niosomes	Span® 80 and cholesterol	HBsAg	–	Serum IgG titers and higher IgG1/IgG2a ratio suggest induction of both Th1 and Th2 responses	Maheshwari et al. (2011)
Novel-modified liposomes (ethosomes)	SPC	HBsAg	Murine bone marrow cells	Predominantly TH1 type of immune response	Mishra et al. (2008)
Niosomes	Sorbitan monostearate/sorbitan trioleate (Span® 60/Span® 85), cholesterol, and stearylamine	Bovine serum albumin (BSA)	–	Significantly higher serum IgG titer upon topical application as compared with topically applied alum-adsorbed BSA, eliciting both humoral and cellular responses	Jain et al. (2005)
Elastic vesicles	Sucrose-laurate ester, octaoxyethylene-laurate ester	Diphtheria toxoid (DT)	–	Surfactant-based vesicles served as adjuvants	Ding et al. (2008)
Deformable liposomes		HBsAg plasmid DNA		Elicited a comparable serum antibody titer and endogenous cytokine levels compared to other vaccines	Wang (2007)
Transfersomes™	DOTAP:NaC	GFP reporter	Mouse skin	GFP expression in the liver and lungs	Kim et al. (2004), Lee et al. (2005)
Hybrid nonionic cationic liposomes	GDL:Chol:POE-10:DOTAP	Human interleukin-1 receptor antagonist (IL-1ra)	Hamster skin	Transgene expression in perifollicular cells	Niemiec et al. (1997)

(continued)

Table 21.1 (continued)

Delivery system	Vesicle composition	Antigen/DNA/plasmid encoding	Tested on	Effect	Reference
Biphasic vesicles	SPC:Chol:DC-Chol SPC:Chol:DMPC	Glycoprotein D (gD)	Mouse skin	Elevated anti-IgD IgG gD-specific cellular response (IL-4) in spleen cells	Babiuk et al. (2002)
Niosomes	Tween®61:Chol: DDAB	Luciferase	Reporter in vitro rat skin	High cumulative amounts in skin and high transdermal fluxes	Manosroi et al. (2009)
Niosomes	GDL:Chol:POE-10	bGr	Rat skin	Intense staining of follicular and epidermal cells	Raghavachari and Fahl (2002)
Niosomes	GDL:Chol:POE-10	IL-1ra	Hamster ears	IL-1ra expression in hair follicles	Ciotti and Weiner (2002)

HepG2 hepatocellular carcinoma, *IL-1ra* human interleukin-1 receptor antagonist, *SPC* soybean phosphatidylcholine, *bGr* b-galactosidase reporter, *DOTMA* N-[1-(2,3-dioleyloxy)-propyl]-N,N,N-trimethylammonium chloride, *Span® 80* sorbitan monooleate, *Tween®61* PEG-4 sorbitan monostearate, *HEK293* human embryonic kidney 293 cells, *GDL* glyceryl dilaurate, *CHOL* cholesterol, *POE-10* polyoxyethylene-10, *DOTAP* N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium, *GFP* green fluorescent protein, *DDAB* didecyldimethylammonium bromide

Conclusion

Topical immunization appears to be an attractive vaccine delivery strategy that enables the use of a variety of antigens and adjuvants. Noninvasive vaccination has proved to be an efficient option for the successful expression of antigen followed by enhanced immune response, making the subject immunized against the disease. Antigens in conventional delivery systems containing classical penetration enhancers are unable to penetrate through the intact skin. Immunologically rich cutaneous surface containing the immune-responsive cells are responsible to initiate an adaptive immune response. Immunization through cutaneous surfaces takes advantage of the assortment of immune-responsive cells in the skin to initiate an adaptive immune response.

Deeper understanding of the cutaneous cells and the antibody and cell-mediated responses promoted the research for more options for TCI. Both human and murine studies support the use of TCI for the induction of protective systemic and mucosal immune responses. In the recent years, significant researches have explored the immune mechanisms and modes of action of adjuvants and thus made vaccine delivery a well-defined science with a potentially immense medical and economic impact. The knowledge of immune mechanism involved and optimization of administration route, delivery system, immune modulator, and formulation stability led to the development of safer and better vaccines. Clinical research on adjuvants for noninvasive delivery and ex vivo use of human material still has to be unraveled and requires further efforts for further exploration. Moreover, humanized animal models should also be exploited for the understanding and better development of transcutaneous vaccine. Site of vaccine administration, type of pretreatment if any, dosing, and the selection of appropriate adjuvant, concentration, and type of penetration enhancer to be used are many variables to be considered. Noninvasive immunization has shown its applicability and success in multiple mucosal compartments (respiratory, digestive, and female genitourinary tract) making it a promising vaccine delivery strategy for safe and effective immunization against a variety of pathogens.

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