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Vaccine 21 (2003) 776–780

Vaccine

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# Immunity under the skin: potential application for topical delivery of vaccines

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**Abstract**

With the technological advances in biomedical sciences and the better understanding of how the immune system works, new immunisation strategies and vaccine delivery options, such sprays, patches, and edible formulations have been developed. This has opened up the possibility of administering vaccines without the use of needles and syringes. Already topical immunisation is a reality and it has the potential to make vaccine delivery more equitable, safer, and efficient. Furthermore, it would increase the rate of vaccine compliance and greatly facilitate the successful implementation of worldwide mass vaccination campaigns against infectious diseases. This review gives a brief account of the latest developments of application of candidate vaccine antigens onto bare skin and describes some of our recent observations using peptide and glycoconjugate vaccines as immunogens.


*Keywords* Skin immunisation; CTL responses; *Haemophilus influenzae* type b

## 1. Advantages of skin delivery of vaccines

Application of antigens onto bare skin is a simple immunisation procedure that promises to revolutionise the way vaccines would be administered in the future. There are several advantages that make this approach of immunisation attractive: (1) it increases compliance due to the elimination of multiple dosing schedules and usage of needles and syringes. The practical importance of compliance is apparent. If people do not comply, the prophylactic goal of vaccination would not be achieved and the cost for emergency care required would become enormous; (2) the fact that no needles and syringes are needed would make the immunisation practice painless. This is particularly relevant for children that normally associate the site of a needle with pain; (3) vaccine delivery is safe, since reuse of needles and syringes that is a common practice in developing countries has the risk of transmission of bloodborn infections [1]; (4) vaccine administration would become practical and simple, since they can be self-administered with a patch without requiring any medical personnel and finally; and (5) topical immunisation elicits both systemic and mucosal immunity. The latter is of great importance, since the majority of pathogens enter the host via the mucosal surfaces.

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## 2. The immune barrier of the skin

The skin is the principal interface with the external environment, keeping water and nutrients in, and unwanted toxic substances and pathogens out. It also acts as an immune barrier, protecting the host from invading pathogens. For this purpose, the skin is equipped with immunocompetent cells, such as keratinocytes, Langerhans cells (LCs), subsets of T lymphocytes and strategically located lymph nodes that constitute the skin-associated lymphoid tissue (SALT) [2]. Keratinocytes, apart of being responsible for establishing the physical barrier of the skin and guaranteeing the structural integrity of the epidermis, produce a wide range of cytokines upon activation by various stimuli [3]. These cytokines shape the local microenvironment to help maintain the appropriate balance of skin immune responses, and stimulate the maturation and migration of LCs.

The LCs are powerful antigen-presenting cells that cover nearly 20% of the surface area through their horizontal orientation and long protrusions. They are located at the basal layer of the epidermis as immature cells playing a sentinel role in the epidermis. LCs capture and process antigens and during their migration via the efferent lymphatics to the paracortical T cell areas of the draining lymph nodes, they mature and present antigenic peptides to naïve T cells [4]. At this stage, they express co-stimulatory molecules of the B7 family, they upregulate the surface expression of MHC

0264-410X/02/$ – see front matter 
PII: S0264-410X(02)00597-2

class I and class II molecules bound to peptides, and secrete high levels of proinflammatory cytokines, such as IL-12 and IL-1.

## 3. Immunogenicity of antigens applied onto bare skin

The skin represents a readily accessible surface area for absorption (2 $m^2$ in adult humans). This offers a distinct advantage of exploiting its immune system for delivering vaccines. Antigens applied onto bare skin penetrate across the continuous stratum corneum mainly via the intracellular or intercellular routes [5]. However, appendages including hair follicles, sebaceous or sweat glands can also serve as portal of antigen entry.

For a long time it was thought that the skin barrier was impermeable to large molecules, but studies by Glenn et al. [6] have demonstrated that topical application of cholera toxin (CT) (secreted by *Vibrio cholerae*) onto hydrated skin can induce strong systemic and mucosal immune responses and confer protection against lethal mucosal toxin challenge [7]. A similar effect was demonstrated with another ADP-ribosylating exotoxin, the heat-labile enterotoxin (LT) of *Escherichia coli* [8] or its mutants LTK63 and LTR72 [9]. Also preliminary results from a phase I trial conducted in human volunteers [10] have shown that topical application of LT with a patch: (i) induces only minimal local or systemic adverse reactions; (ii) elicits anti-LT IgG responses that were boosted after the second and third topical application of LT; and (iii) induce long lasting immunity and detectable anti-LT IgG or IgA antibodies in urine and stools.

Table 1
Type of antigens that can be applied onto bare skin

| |
|---|
| Viruses: HSV, adenovirus, inactivated rabies virus, recombinant Mengo virus |
| Parasites: *Dirofilaria immitis* |
| Plasmid DNA: HBsAg, influenza Ags |
| Bacterial toxins (CT, LT), toxoids (TTx, DTx) |
| Proteins: BSA, β-galactosidase |
| Peptides: Th, B, CTL epitopes |

Parallel to their immunogenic potential, CT and LT act as adjuvants, enhancing immune responses to topically co-applied antigens, including toxoids, proteins, peptides and viruses [7–14]. It is the combination of their binding activity and built-in adjuvanticity that make CT and LT powerful immunogens and adjuvants. Several other adjuvants, including CpG motifs, lipopolysaccharide (LPS), muramyl dipeptide (MDP), alum, IL-2, and IL-12 have been shown to enhance the antibody titres to topically co-applied antigens [13]. However, the responses were short lived and weaker to those induced in the presence of CT or LT [13]. Following these initial observations, the potential of the skin as a non-invasive route for vaccine delivery has been demonstrated with several types of antigens (Table 1).

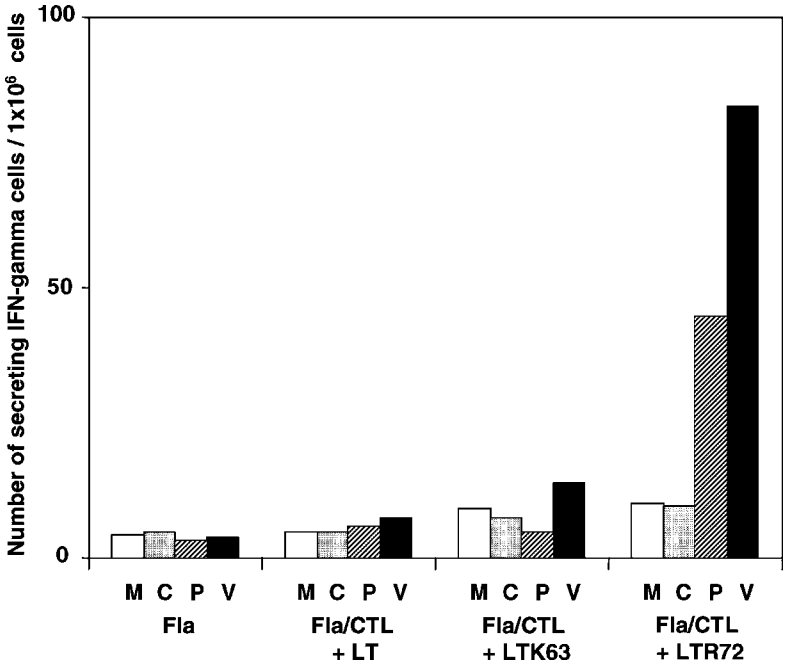


Fig. 1. Peptide- and virus-specific IFN-γ secreting T cells. Groups of BALB/c mice (two mice per group) were immunised onto bare skin with 25 μg empty flagella (Fla) or 25 μg flagella expressing the influenza nucleoprotein CTL epitope (Fla/CTL) with 25 μg of either LT or its mutants LTK63 or LTR72. A total volume of 50 μl was applied onto bare skin. Mice were boosted 2 weeks later by the same route and dose of antigen. Four weeks after the priming, splenocytes were collected and IFN-γ secreting T cells were measured by a standard ELISPOT assay. Results represent the mean of triplicate cultures. Cells were restimulated in vitro with 10 μg peptide or $3 \times 10^3$ pfu of heat-inactivated influenza virus (strain A/NT 60/68; H3N2)/culture. M: medium, P: test peptide, C: control peptide, V: virus.

## 4. Induction of CTL responses following immunisation onto bare skin

$CD8^+$ cytotoxic T lymphocytes (CTL) play a critical role in eliminating virus-infected cells. CTL responses can be elicited after systemic or mucosal immunisation with peptide epitopes administered with various delivery systems. Flagella is a bacterial component that has been extensively tested as a carrier protein to cloned epitope sequences that are expressed at the surface of flagellin, the flagellar major subunit [15]. This has prompted us to study the efficacy of a flagella fusion protein expressing a conserved cytotoxic T cell epitope from influenza virus nucleoprotein representing residues 147–158 (TYQRTRALVRTG) [15] to elicit peptide- and virus-specific CTL responses after application onto bare skin. To potentiate immune responses, LT or its LTK63 and LTR72 mutants were used as adjuvants. Two weeks following the booster application, splenocytes were tested for peptide- and virus-specific IFN-γ secreting T cells. As shown in Fig. 1, the flagella fusion in the presence of mutant LTR72 elicited higher numbers of peptide- and virus-specific IFN-γ secreting T cells. However, when the flagella fusion was applied topically in the presence of LT or LTK63, the number of peptide- and virus-specific IFN-γ secreting T cells was very low and not higher to the number produced by the flagella fusion given alone. These findings are in a good agreement with a recent report demonstrating the preferential stimulation of IFN-γ by LTR72 mutant after immunisation onto bare skin [9].

## 5. Topical application of a *Haemophilus influenzae* type b conjugate vaccine with CT elicits protective immunity

*Haemophilus influenzae* type b (Hib) conjugate vaccines have successfully reduced the burden of invasive Hib disease in developed countries and there is clearly a case for implementation of these vaccines worldwide. Since topical delivery of vaccines simplify vaccine administration and has the potential to promote compliance and cost-effectiveness, we were interested in evaluating the immunogenicity and efficacy of a Hib conjugate vaccine following skin immunisation. Two applications of 50 μg of poly-ribosyl-ribitol phosphate (PRP) oligosaccharide conjugated to the non-toxic, cross-reacting mutant protein of diphtheria toxin ($CRM_{197}$) (PRP-$CRM_{197}$) onto bare skin of rats with or without CT (50 μg/rat) as an adjuvant elicited substantial levels of both IgG anti-PRP and anti-$CRM_{197}$ antibodies as measured by ELISA [16] (Fig. 2). However,

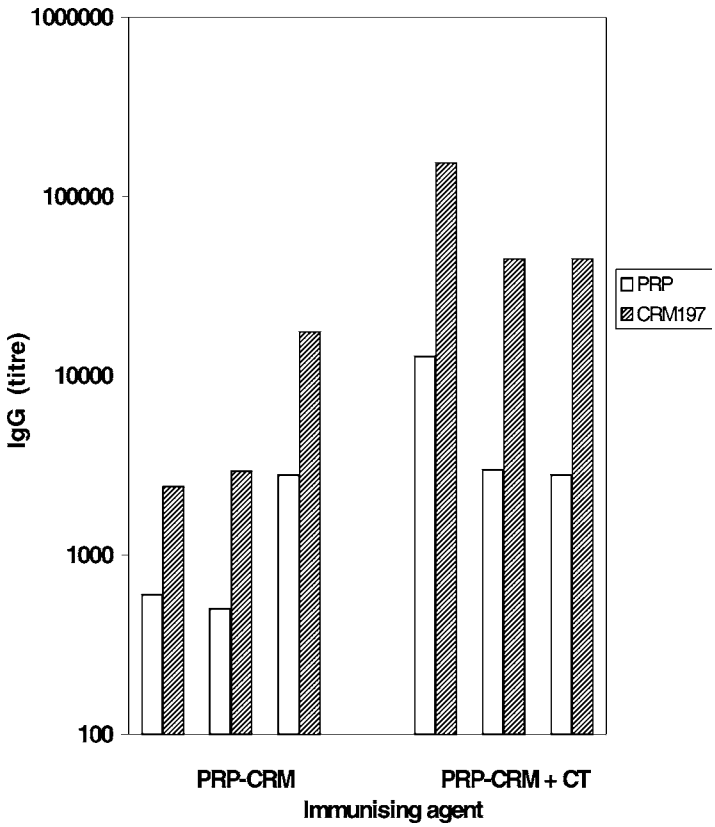


Fig. 2. Immunogenicity of *Haemophilus influenzae* type b conjugate vaccine following skin immunisation. Rats (Sprague Dawely, three per group) were immunised with 50 μg of PRP-CRM with or without CT (50 μg/rat) on days 0 and 21 and bled 3 weeks after the boost. Each bar represents antibody responses from individual rats.

Table 2
Passive protection of infant rats from *Haemophilus influenzae* type b bacteraemia by anti-PRP antibodies induced after immunisation onto bare skin

| Immunogen | Number of rats bacteraemic at 24 h/number of rats challenged |
|---|---|
| PRP-CRM | 1/4 |
| PRP-CRM + CT | 0/3 |
| Normal rat serum | 4/4 |

Immune sera from rats immunised with two doses of PRP-CRM or PRP-CRM + CT were injected i.p. (50 μl/rat) into 5-day-old infant rats (Sprague Dawely) 24 h before challenge (i.p.) with $10^4$ *Haemophilus influenzae* type b. Rats were bled 24 h after challenge and blood was cultured on chocolate agar for the presence of bacteraemia.

responses against both the polysaccharide (PRP) and the carrier protein ($CRM_{197}$) were higher in the group of rats receiving CT as an adjuvant. Moreover, adoptive transfer of immune sera from both groups protected infant rats from Hib-induced bacteraemia (Table 2).

## 6. Perspectives for skin delivery of vaccines

The realisation that the skin is easily accessible has an effective immune system, and its physical barrier is not so impermeable as previously thought makes it an attractive route for non-invasive delivery of vaccines. Studies in several animal species and clinical trials in humans have established the proof of principle. However, for effective vaccine delivery several variables related to the nature of the antigen and characteristics of the skin barrier must be first overcome. For example, the diffusion of an antigen through the stratum corneum is dependent on its physicochemical properties and its molecular interactions with skin constituents. This could explain the differences in immunogenicity of several antigens after their application onto bare skin. For example, in a recent study where the safety and immunogenicity of a prototype enterotoxigenic *E. coli* vaccine was assessed in adult volunteers after topical application, only 68 and 53% were found to have serum anti-colonising factor CS6 IgG and IgA antibodies, respectively, while all responded to LT [17].

Furthermore, the skin of humans and animals poses a unique barrier due to differences in anatomy and physiology between species [18]. Therefore, these variables make the task of extrapolating the findings of studies performed in different animal species to the target species difficult.

Current research efforts are focused: (i) in the selection of suitable delivery systems that will allow efficient penetration across the stratum corneum and selective uptake of antigens by LCs; (ii) in understanding the mechanisms involved in the induction of systemic and mucosal immune responses after topical immunisation; and (iii) to identify approaches to enhance and modulate immune responses.

## Acknowledgements

The authors would like to thank Prof. Ruth Arnon (Weizmann Institute of Science, Rehovot Israel) and Dr. Rino Rappuoli (IRIS Research Center, Chiron S.P.A, Siena, Italy) for kindly providing the recombinant flagella construct and the LT mutants, respectively.

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