Vaccine Research
ISSN: 2423-4923
eISSN: 2383-2819

The Skin Immune System and Intradermal Delivery of Vaccines: A Review

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ARTICLE INFO

Review Article

VacRes, 2020
Vol. 7, No.2, 15- 21
Received: June 25, 2020
Accepted: September 20, 2020
Pasteur Institute of Iran

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KEYWORDS: Vaccines, Intradermal, Immune system, Adverse effects.

ABSTRACT

The skin is considered as the largest organ in the body, functioning as a barrier providing protection from the outside environment, but also performing essential immune functions through a complex network of epidermal and dermal cells that interact with each other. The basic principle of the vaccines is to induce protection against pathogens by simulating its interaction with the immune system, allowing to generate a memory immune response. To achieve this protection, the interaction and binding of both innate and adaptive immune responses is required. Intradermal (ID) delivery of vaccines achieves direct injection of the antigen into the dermis, where the largest numbers of immune cells are found (macrophages, dendritic cells, Langerhans cells, B and T lymphocytes, and mast cells, among others). It is a novel route that elicits antibody responses equivalent to other routes of administration but at lower doses, a phenomenon known as "dose saving". This route also allows for better thermo-stability of the antigen, fewer booster immunizations and, as a consequence, increased adherence to the vaccination regimens with less burden on the medical personnel. There are currently several vaccines for the ID administration on the market, and several more under development; with good safety profiles and efficacy rates. In this article, we review the most important aspects of the immune system within the skin, the pathways by which vaccines are applied to the skin intradermally to produce an adequate immune response, and also their advantages and disadvantages.
The skin has important immune machinery, thanks to which both innate and adaptive immune responses merge. This interaction allows for the basis of vaccination: development of memory responses to various antigens, providing protection for the future re-exposures.

Citation:
Carmona-Cruz S A, García-Romero M T. The Skin Immune System and Intradermal Delivery of Vaccines: A Review. vacres. 2020; 7 (2) :15-21
URL: http://vacres.pasteur.ac.ir/article-1-197-en.html

The Skin Layers and Immune Cells/Systems

The immune system within the skin is found in the epidermis and dermis. The most notable immune cells that reside in the epidermis are Langerhans cells (LC) and melanocytes. Several subpopulations of dendritic cells (DC), macrophages, and T cells are established in the dermis. The efficacy of the immune response is based on the interaction and communication of these cells with other neighboring cell types, such as keratinocytes and fibroblasts, as well as the compliance of the vessels and lymph nodes that drain the skin [1, 2].

Epidermis

The keratinocytes are the structural element of the skin. They are involved in the immune responses, both innate and adaptive. Keratinocytes along with the epithelial cells and neutrophils are important producers of the antimicrobial peptides (AMPs), which are molecules that act as a first line of defense. The AMP expression can be regulated by pro-inflammatory cytokines, like IL-22 and IL-17. In opposition, the AMPs are down-regulated by acetylcholine through the cholinergic anti-inflammatory pathway [3]. There are numerous families of the AMPs that perform specific functions, such as cathelicidin 37 (LL-37), which plays an important role in angiogenesis and wound healing, and is produced by keratinocytes. These cells also express Toll-like receptors (TLRs) both inside and on their surface, which promote Th1 responses and secretion of interferons (IFN) when activated. Keratinocytes additionally produce pro-inflammatory cytokines such as IL-18 and IL-1β through the inflammosome's signaling pathway [4]. The IL-1 increases the intercellular adhesion molecules (ICAM)-1 and the migration of leukocytes into the skin. Furthermore, keratinocytes can secrete tumor necrosis factor (TNF), IL-6 and IL-10 through stimulation by both IL-1 and IL-18 [2].

This ability of keratinocytes to produce receptors and chemokines enables them to interact and cooperate with other cell types like LC, neutrophils, or T cells during the immune response [2]. The LCs are the first line of defense in the outermost layers of the skin. They perform a tolerogenic, rather than inflammatory, function. They have the ability to produce IL-10 and induce regulatory (CD4+) and tolerance (CD8+) T cells [2]. The LCs are characterized by the expression of

[DOI: 10.52547/vacres.7.2.15]

langerin, CD45, major histocompatibility complex (MHC) class II molecules, E-cadherin, epithelial adhesion molecules and CD1a. The CD1a can present non-peptidic microbial antigens to the T cells. The LCs are responsible for activating the innate adaptive response and generating memory responses for carcinogenic antigens, avoiding the risk of relapse [3]. After being stimulated, they can lengthen the size of their dendrites, to increase the capture of the antigens at the epidermal junctions [3]. They can increase their migration rate from the vessels in the dermis to the lymph nodes that drain the skin during inflammation. The LCs are vital for capturing protein antigens and converting to a local Th2 environment [2].

Dermis

The dermal components include several subsets of dendritic cells (DC) and T cells that are located close to the hair follicles; as well as other critical cells such as macrophages, mast cells, basophils, and eosinophils [2].

Unlike the LCs, dermal dendritic cells (dDCs) are located deeper and express adhesion molecules for the epithelial cells, IL-10 and low-density lipoprotein-related protein 1 (CD91). They are capable of stimulating B cells in IgM-secreting plasma cells [4].

Depending on the environment, they can create different phenotypes of the cells. There are two main types of dDCs: langerin+ CD103+ and langerin− CD103− [5].

The dDCs can remain in an immature state when expressing TLR2, TLR4, CD206 and CD209; or acquire a mature state when expressing CD83 co-stimulatory molecules but almost no TLRs [5]. Their primary role is immuno-surveillance against pathogens through their involvement in the inflammatory responses via a network of cytokines and chemokines and the interaction with monocytes and macrophages of the skin [5].

Mast cells are found mainly in the superficial dermis. They contain histamine and are associated with the allergic reactions. However, they also play parts in wound healing, angiogenesis, inflammation and immune tolerance [1].

Type C mast cells (tryptase positive, chymase positive) are found on the skin. Tryptase affects fibronectin and breaks down the extracellular matrix proteins, conceding neutrophils, mononuclear cells, and lymphocytes to invade the epidermis. Tryptase likewise has a pro-angiogenic activity. Chymase, on the other hand, is a pro-inflammatory molecule that works through IL-1β and IL-18 [1].

Both enzymes regulate the immune response negatively by their capacity to break down various pro-inflammatory factors like cytokines and chemokines [1].

They have been shown to express TLR and function as complex antigen presenting cells (APCs). Also, mast cells express MHC class I and class II and present antigens when expressing co-stimulatory molecules such as CD86 and DC80. They consequently migrate to the lymph nodes where they enroll more cells [1].

It is also known that mast cells can calibrate the immune response through the capacity to incite tolerance. They secrete IL-10 and transforming growth factor (TGF)- β, and also increase the quantity of the regulatory T cells (CD4+, CD25+, Foxp3+) through TGF-β mediated mechanisms [1].

The skin is a reservoir for around 20 trillion T cells, about double of that of total blood volume. These cells can react to any antigen, migrate to all tissues, and produce countless cytokines and functions to kill pathogens and tumors efficiently [6]. It is known that >95% of the cells are memory T cells (CD45RO), and <5% are native, of which the majority express cutaneous lymphocyte antigen (CLA), approximately half of them express CCR8 and some express CC37 and CCR10. The resident T cells can initiate complete immune responses and their migration to lymphatic tissues is not necessary [6]. The T αβ and T CD8+ cells are memory cells and live among keratinocytes near the LCs. About the same amount of CD4+ and CD8+ T cells exist in the dermis, restricted to the dermal capillaries and the dermo-epidermal junction. A large proportion of them are memory cells that express antigens associated with the cutaneous lymphocytes [5].

Memory-resident T cells are long-term: they form after infections are resolved, and reside in the skin to provide protection. They accumulate both at the location of primary inflammation and on the distant unaffected skin. In this way they allow for more extensive protection of the host against the secondary challenge [2][6].

Th17 cells are a particular lineage of T cells that produce IL-17A, IL-17F, TNF-α, IL-21, and IL-22 (Th17 cytokines) and rely upon IL-23 for their development, survival, and expansion [5, 6]. Th17 lymphocytes, along with Th1 and Th2 lymphocytes, are effector cells in inflammatory skin pathology. Both IL-17 and IL-22 produced by Th17 cells can induce keratinocyte differentiation [5]. Th17 cells protect the skin from pathogens such as *Candida albicans*, *Klebsiella pneumoniae*, and *Staphylococus aureus*. The extent of the skin immune response is efficiently calibrated by the regulatory T cells (T regs), which constitute 5-10% of all skin-resident T cells. These cells transit between the skin and the lymph nodes and regulate T-cell responses, APCs such as DC and macrophages, and the accumulation of neutrophils during the initial phases of inflammation. T regs are essential for the development of self-tolerance, as well as its maintenance. They express the transcription factor FOXP3 and proliferate in inflammatory conditions. However, they are ineffective in curbing the inflammatory response of T cells receiving high avidity signals of T cell receptor (TCR), such as those found in memory responses to dangerous pathogens. Furthermore, IL-6 may interfere with inflammation suppression by T regs. Finally, T regs also allow latent infection of some parasitic infections [6].

T γδ cells reside in the epidermis in a pre-activated state and promote immunoregulation and inhibition of the tumor response in the skin compartments. These cells are fundamental in wound repair due to their capacity to secrete growth factors [6].

On the other hand, the antimicrobial immune response is attributed to T natural killer (NK) cells, which can activate keratinocytes, produce large amounts of TNF-α and increase the transit of DCs from the skin to the lymph nodes [6].

Tissue and Core Resident T Cells

When the skin is encountered by an antigen for the first time, the skin resident DCs englobe the antigen and migrate to the lymph nodes that drain the skin; here, DCs present the antigen to native T cells. Upon antigen recognition, native T cells differentiate and polarize into memory effector (TEM) and central memory T cells (TCM)[7].

TEM cells travel in the bloodstream and disseminate to all parts of the skin, although the greatest burden is at the site of pathogen exposure. These cells stimulate pathogen clearance and then remain as local residents on the skin [7].

Proliferating T cells are also discharged from lymph nodes that drain the skin and disseminate to antigen-free lymph nodes that drain other tissues, where they proliferate and evolve to

[DOI: 10.52547/vacres.7.2.15]

new subpopulations of effector T cells that migrate and settle in peripheral tissues like the intestine, lungs and others. Thus, immunization through the skin actually produces generalized immunity through the creation of different populations of tissue-resident TEM cells [7].

In Memory Immune Responses, T Cell Responses Can be Divided into 3 Phases:

1. Local re-exposure to a pathogen prompts its presentation by tissue resident DCs. This event potentiates the expansion and effector function of antigen-specific skin resident T cells, producing prompt pathogen neutralization.

2. Local inflammation leads to up-regulation of vascular adhesion receptors in the endothelium of the skin vessels, causing unspecific enrollment of T cells from the bloodstream: only a fraction of these cells will be antigen-specific.

3. The migration of antigen-filled DCs to the lymph nodes that drain the skin will generate stimulation of TCM cells, thus ensuing the production of large amounts of TEM that target the skin. TEMs will migrate through the bloodstream, reach the inflamed areas and aid in the elimination of the pathogen [7].

Methods of Vaccine Delivery

The basic principle of the vaccines is to induce protection against pathogens by simulating their normal interaction with the immune system, allowing to generate a memory immune response [8, 9]. The purpose of vaccine administration is to produce a faster, more intense and more specific response after re-exposure to the pathogen to which it was formulated. To achieve this protection, an essential requirement is the interaction and binding of both innate and adaptive immune responses, which is mediated by the APCs. Thus, the quality of immunity achieved by vaccination will depend on the ability of CD4+ T cells to induce a memory response by activating B lymphocytes, and on the quality of B cells to generate protective immunoglobulins [8] (Fig.1).

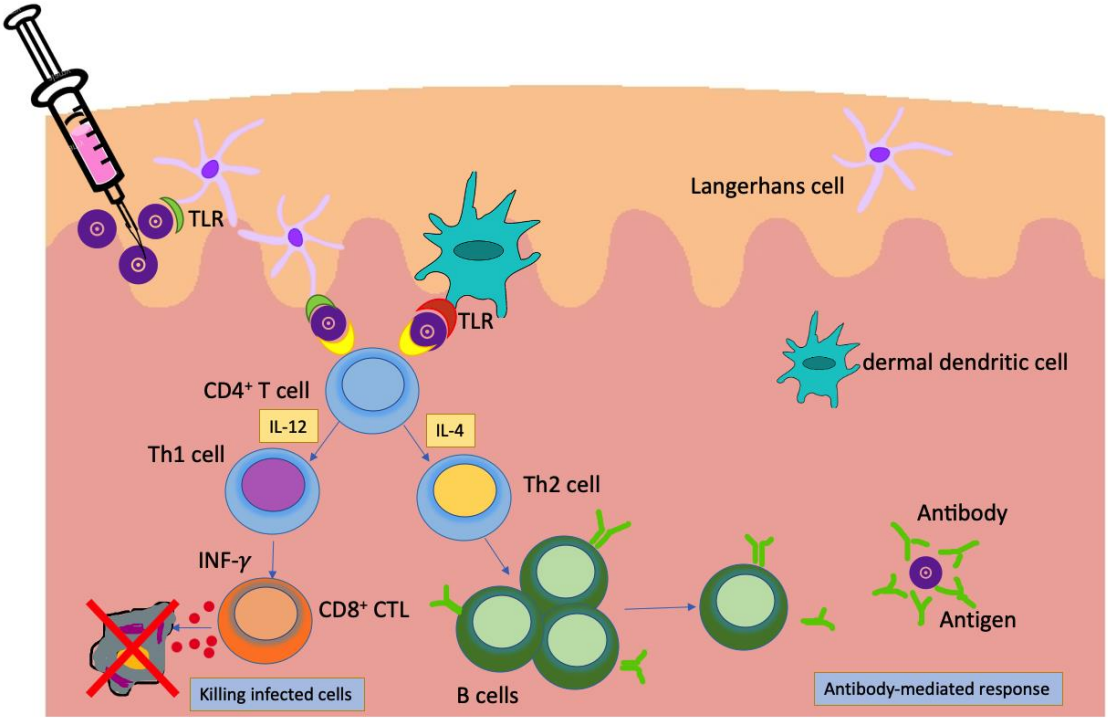

Fig.1. Mechanisms of immune response generation through vaccination

Inoculation of the antigen activates Langerhans and dermal dendritic cells which function as APCs, activating CD4+ T cells. The release of IFN-γ generates a Th1 response that exhorts the death of infected cells. The release of IL-4 generates a Th2 response which causes a memory immune response, through the generation of antibodies.

Advantages and Disadvantages of Intradermal Approach

Despite good response to the conventional administration of vaccines [intramuscular (IM) or subcutaneous (SC)], new technologies and application sites such as intradermal (ID) are being investigated to improve the host's immune response, taking advantage of the immune network present in the skin [4, 10, 11].

Conventional administration of the vaccines is associated with the systemic immunity but with the lack of response at the mucosal level, which is considered a disadvantage, since many pathogens infect through this route [11]. Furthermore, routes of administration that involve injections are usually painful and uncomfortable for the people with needle phobia [4].

Taking this into account, vaccines have been developed for the ID administration to skin, demonstrating rapid and wide bio-distribution of the antigen, the ability to induce protection in mucosa, measured by secretory IgA (sIgA), and cellular and humoral responses [11]. It is also suggested that ID administration of vaccines may be more effective in generating memory-resident T cells [12, 13].

The ID delivery of vaccines achieves the direct injection of the antigen into the dermis (where the largest numbers of immune cells are found) [14]. The techniques of ID application through micro-needles that break the cutaneous barrier imposed by the stratum corneum facilitate delivery of the antigen. Once

[DOI: 10.52547/vacres.7.2.15]

the antigen is inoculated, Langerhans cells trigger the inflammatory cascade in the skin, involving other cells such as mast cells. In this way, they directly restrict the early replication of the pathogen at the infection site [7, 11, 14]. Furthermore, they offer protection against future infections, by promoting the recruitment of T cells [7].

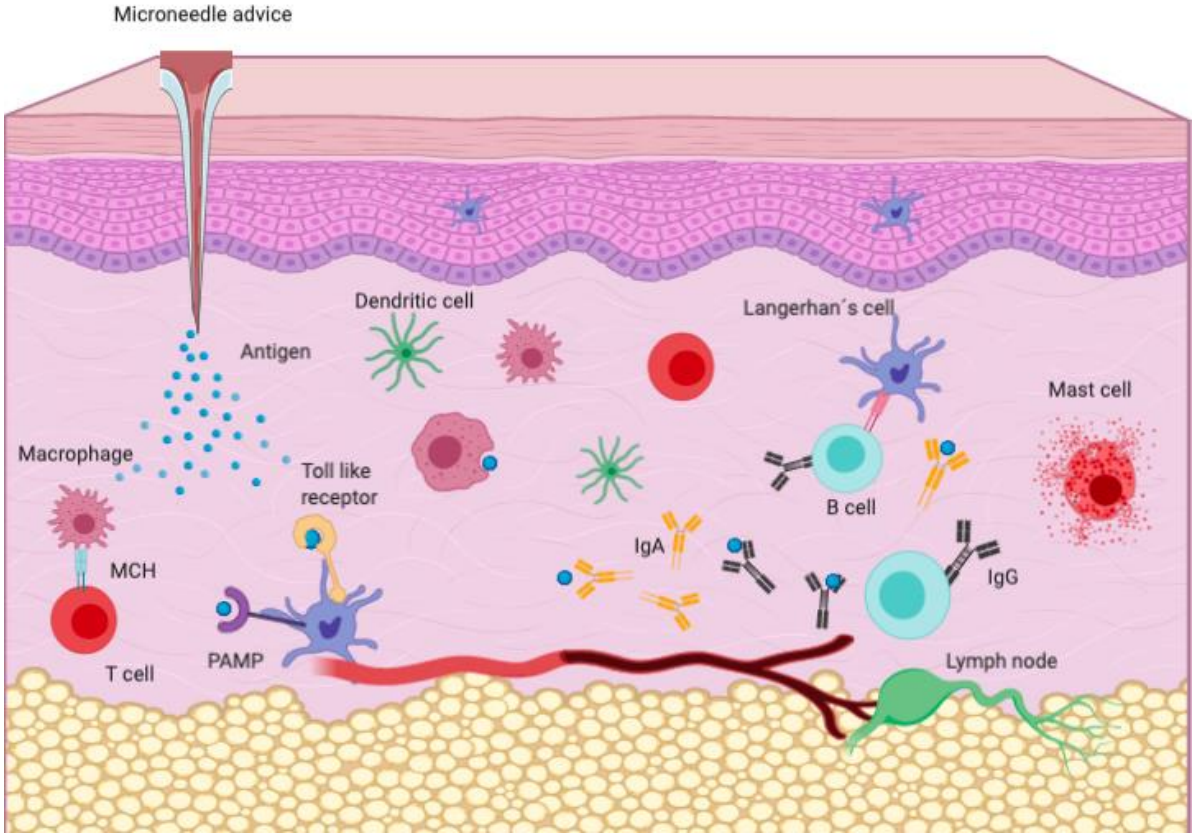

Fig.2. Intradermal vaccine delivery and immune response

The delivery of the antigen is made directly into the dermis, which thrives with immune cells (macrophages, dendritic cells, Langerhans cells, B and T lymphocytes, mast cells) that trigger a response. Langerhans and dendritic cells recognize the antigen through pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs) are responsible for carrying the antigen to the lymph nodes to initiate the expansion of B and T cells. Thus, an adaptive immune response is mainly generated through production of IgA and IgG antibodies.

On the other hand, mast cells increase the initial innate immune response by releasing inflammatory mediators through their degranulation.

The ID administration of vaccine is accomplished using needles, micro-needles (MNs) or particle pressure injectors, and recently developed self-administered patches with coated micro-projections, dissolving micro-needles (DMNs), or even dry solid needles that allow for the storage at room temperature [3, 4, 11]. The DMNs consist of rapidly dissolving materials such as polymers or sugars, so that the antigen is mixed in the matrix. The most frequently used are those of sodium hyaluronate, carboxymethyl cellulose, polyvinyl alcohol, poly vinylpyrrolidone, maltose, and trehalose [3, 4].

The MNs are approximately 25 μm in length, pierce the stratum corneum, create transient micropores for the antigen injection, and are short enough not to reach the pain receptors [4, 11]. Through these MNs, low molecular weight molecules such as lidocaine and naltrexone and even biotherapeutic products, such as insulin and human growth hormone can be administered [3]. In the field of vaccines, the used antigens include peptides, proteins, and DNA vectors that encode attenuated viruses and antigenic proteins [4].

The ID delivery of vaccines has the ability to elicit antibody responses equivalent to other routes of administration but at lower doses, a phenomenon known as "dose saving" [4, 11, 14, 15].

In the case of infectious outbreaks, where rapid vaccination is required, these innovative vaccine administration systems have advantages since they confer thermostability of the antigen, fewer booster immunizations and, as a consequence, increased adherence to the vaccination regimen with less burden on medical personnel [4, 12].

Another advantage of this route of administration is the possibility of improving the immunogenicity of various vaccines in immunocompromised hosts or in pregnancy [11].

However, other factors such as anatomical sites, the correct calculation of the antigenic dose, vaccine storage requirements,

[DOI: 10.52547/vacres.7.2.15]

or the length of the needles must be considered for the correct administration. There are studies that have measured the thickness of the skin by high-frequency ultrasound and found that this is influenced by anatomical site and age, but not the other factors such as sex, body mass index (BMI) or phototype, even in the children under 5 years [14, 15]. The importance of this issue is that there is stable skin thickness even in the pediatric population, which allows for standardization of the needles used for the ID administration of vaccines.

Preclinical Studies

The ID administration of vaccines through micro-needling methods have generated both comparable and superior cellular and antibody responses that are longer lasting compared to the conventional methods in preclinical studies [3, 4, 12]. Zhu and colleagues used a biodegradable micro-needle patch with 3-component influenza vaccine in mice. They observed a higher IgG1 and IgG2 antibody response, a larger population of IL-4-producing cells, IFN- γ and a larger amount of antigen-specific plasma cells compared to the IM administration; as well as cross-reaction for the other heterologous species of the virus [5].

Another study conducted by Louis et al., compared ID vs IM administration of vaccines in mice and showed the advantages of ID vaccine administration. The sequences of genetic material from six Leishmania parasites (*L. braziliensis*, *L. donovani*, *L. infantum*, *L. major*, *L. mexicana*, and *L. panamensis)* were inoculated. The sequences encoded the enzyme Leishmania carboxykinase phosphoenolpyruvate (PEPCK). This enzyme is known to be critical to the gluconeogenesis of the parasite and CD4+ T lymphocytes and peripheral blood mononuclear cells react to it during the peak of infection. The objective was to assess the immunity and protection after an infectious challenge in mice vaccinated by both routes. The results indicated that mice immunized intradermally were better protected against *L. major* after the challenge, and that they had similar levels of protection (same lesion size and parasite loads) as the immune mice that had been previously infected with parasites. This suggests that the ID delivery method is efficient in generating memory-resident T cells and protecting against Leishmania parasites [13].

The ID route of administration is novel and has raised high expectations in other areas of medicine, in addition to the prevention of infectious diseases. Its use in vaccine administration for the breast cancer was investigated. Chablani et al., used a highly tumorigenic strain of breast cancer cells to create a complete cell lysate and formulate a vaccine using spray-drying technique and ID inoculation through a micro-needle system (AdminPatch®). They compared a group of mice inoculated by this technique to another group of mice that were not immunized. Later, all mice were challenged to massive inoculation of the same strain. A higher adaptive immune response was observed in the vaccinated mice, evidenced by a higher number of IgG antibodies, as well as the generation of tumors up to 5 times smaller than unvaccinated mice [3].

Clinical Studies

Currently, the anatomical areas approved by the World Health Organization (WHO) for ID administration of vaccines are the deltoid and the suprascapular areas [14].

There are 3 vaccines on the market for the ID administration for influenza prophylaxis (Intanza®, Fluzone®, IDFlu®) [3, 16]. Intanza® was approved in Europe in 2009 and has been used since then in other countries such as US, Australia, Canada, and Korea. Its use is endorsed in adults over 18 years, considering the average of 2 mm skin thickness for this population. The documented benefits are dose saving and increased immunogenicity [14, 17]. In 2014, the FDA approved Fluzone®, which uses a 1.5 mm needle attached to a syringe pre-filled with influenza antigens [3, 12].

There is also an ID rabies vaccine, which is applied as post-exposure prophylaxis. It provides the same safety profile and immunogenicity of the IM administration, with 20-40% less volume and cost [14, 17].

Other vaccines that are applied intradermally are the Bacillus Calmette-Guérin (BCG) vaccine [17], as well as hepatitis B virus (HBV) vaccine that uses half dose compared to the IM administration in children aged 3 - 12 years old [14, 18].

Table 1. Examples of the ID vaccines approved/are under development

Vaccine status	Indication	Clinical trial (phase)	Ref.
Approved	Influenza A and B	Intanza®, Fluzone®, IDFlu®	[16]
Clinical trials	Hepatitis B, Gastroenteritis, Dengue fever, Ebola	Phase 1	[18, 19, 20, 21]
	AIDS*	Phase 2a	[22]
	Poliomyelitis	Phase 3	[23]

*Acquired immunodeficiency syndrome

We have listed some advantages that favor the ID administration of vaccines. However, the safety profile of this route of administration is not yet clearly known. Several studies indicate increased skin reactions at the site of application compared to the IM or SC routes of administration. These include erythema, edema, induration and pruritus [24-31].

Although increased local adverse effects have been reported with these devices (mild pain, redness, swelling), no systemic side effects have been mentioned [11, 14, 17].

It is well known that ID route limits the transfer of vaccine components into the blood circulation and possible toxicity due to hepatic first pass effect [11].

Regarding the safety profile of ID administration route, a French study evaluated the trivalent seasonal ID influenza vaccine in two groups, one with subjects 18 to 59 years old and another group with subjects >60 years old. In the first group, 78% reported ≥1 injection site reactions (pain, erythema, pruritus), and 60% reported ≥1 systemic reactions (headache and myalgia). Very few (3.8%) presented tremors, general malaise, nausea and headache. In the second group, 54% documented a reaction at the site of application and 32% reported a systemic reaction. In both groups the reactions were mild and transitory [32].

In a study by Hung et al., the ID vs IM administration of a trivalent influenza vaccine was compared. The redness and edema at the vaccination site and vaccine leakage were significantly more common in the group that received the ID vaccinations. The presence of edema was correlated with the subsequent long-term immunogenicity, thus, it could be considered an effective marker of vaccination by this route. In this study, general symptoms such as general malaise, myalgia and arthralgia were more frequent for the group that received

[DOI: 10.52547/vacres.7.2.15]

vaccines intradermally, however, this was not statistically significant. There were no serious adverse effects for either group [33]. Similarly, in the study by Seo et al., the presence of muscle pain was more frequent after the ID administration of influenza vaccine compared to the IM route [29]. On the other hand, Coleman et al., documented a lower frequency of systemic symptoms (16.4% vs. 9.5%; P=0.002) and a lower probability of severe myalgias, arthralgias or general discomfort with ID when compared to IM influenza vaccine [28].

Although erythema and edema are widely reported side effects for the ID vaccination, in a study by Henderson et al., which compared IM with ID administration for the HBV vaccine, the main adverse effect after ID vaccination was hypopigmentation of 1 to 5 mm without induration at the vaccine site for at least 2 years after vaccination [34].

Table 2. Advantages and disadvantages of the ID administration of vaccines

Advantages	**Disadvantages**
Rapid and wide bio-distribution of the antigen	Lack of standardization (anatomical sites, skin depth) in its administration
Good cellular and humoral responses	Increased skin reactions at the site of application (erythema, edema, induration and pruritus)
Dose saving	
No systemic adverse effects	

In none of the studies the adverse effects associated with the ID administration were considered serious to not recommend its usage. In most cases the pain associated with the application of the vaccine was less as compared to the IM vaccination [27,28,31].

Some techniques have been developed for the ID application that reduce the local adverse effects, such as that studied by Chen et al., who tested micro-fractional epidermal powder delivery using ablative fractional laser or microneedles to create microchannel arrays in the epidermis followed by the topical application of powder antigen-coated array patches to deliver vaccines into the skin. There was a decrease in the local adverse effects, probably associated with the slower release of antigens, complete recovery of the skin in a few weeks, and a preserved immune response [17].

A scale was recently proposed to measure the adverse effects: the Vaccine Site Appearance Grading Scale (VSAGS), based on the most commonly reported adverse reactions in the literature after ID vaccination. This scale incorporates characteristics such as erythema, induration, edema, bruising, papules or plaques, vesicles or blisters, hypo or hyperpigmentation, with a score of 0 to 5 for each item. Although not yet validated, it provides more specific characteristics which could serve to better classify the severity of the skin reactions [30].

CONCLUSIONS

The immunological properties of the skin have been exploited for the ID administration of various antigens. Several studies have shown that this route of administration can generate more efficient immune responses, and more intense and lasting memory responses that, in the long term, may decrease the costs and dosage requirements allowing for greater coverage for the populations at risk.

As the ID route of administration is relatively new, there are still several clinical trials comparing its safety and efficacy with the conventional routes of application. However, current evidence supports that ID administration of vaccination is safe, with few or no serious systemic effects reported to date; and in different areas of medicine may be a safe, easy and effective way for preventing various diseases, both infectious and non-infectious.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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[DOI: 10.52547/vacres.7.2.15]