J PREV MED HYG 2010; 51: 7-14

REVIEW

The intradermal vaccination: past experiences and current perspectives

L. STICCHI [*,**], M. ALBERTI[*], C. ALICINO[*], P. CROVARI[*]
[*] Department of Health Sciences, University of Genoa, Italy; [**] U.O. Hygiene, San Martino University Hospital, Genoa, Italy

Key words

Intradermal vaccination • Immune response • Influenza vaccine

Introduction

Over the centuries, a number of vaccines have been administered into the skin using a variety of instruments, from simple to sophisticated ones.
At the same time as these progresses in administration techniques, advances in the field of immunology have led to an increased understanding of the basic mechanisms of innate and adaptive immunity and the skin has been identified as an attractive site for vaccination, largely due to the presence of a dense network of immune-stimulatory antigen-presenting cells.
Recently the development of innovative devices makes intradermal (ID) administration of vaccines a less invasive, simple, rapid, reproducible and safe technique of inoculation, further promoting clinical trials on vaccines delivered by this route.
The current renewed interest in ID route of vaccination has been largely driven by the perception that it might offer several advantages in terms of both immunogenicity, such as the reduction of antigen concentration (dose-sparing), the ability to improve immune response in low-responders and the avoidance of the need for adjuvantes, and some practical issues as the easier and safer administration with the respect to conventional intramuscular route and the reduction in risk of needle-stick injuries for health care workers and blood vessels or nerves injuries for patients.

Immunological basis of intradermal vaccination

During the last century, the skin was the subject of numerous studies demonstrating the highly complex and dynamic interplay between the skin and the other components of the immune system [1] and for this reason has been proven to be suitable for vaccine delivery.
The rationale for ID route lies in the demonstrated ability of certain immune system cells (Langerhans Cell, Macrophages, Mast Cells, Dendritc Cell, Leukocytes, …) to stimulate the innate immunity thus amplifying the adaptive immune response. While the adaptive response is primordial in generating a response to vaccination and generally becomes more effective with each successive encounter with an antigen, the innate immune mechanisms also play a key role as they are first activated in response to pathogen invasion or contact with foreign antigens.
The dermis is also rich in micro-vascular systems that enable interaction between the cells of the immune system, activated by danger-signals, and the network of the regional lymph node. These exchanges occur, principally, in the micro vascular dermal units that are located in the papillary dermis near the dermal-epidermal junction.
The resident Dendritic Cells (DCs), located deeper in the dermis, are the principal immune cells target by ID vaccination.
After the vaccine administration through ID route, immature DCs residing in the papillary dermis, capture and process antigen, re-express part of the peptides in the groove of MHC class I/II on the surface and, subsequently mature and migrate to regional lymph node. This mechanism is activated and facilitated by pro-inflammatory cytokines, particularly IL-1 β and TNF-α, released in response to any danger non self signal such as a vaccine antigen [2]. These cytokines play a key role in DCs migration to the paracortical area of regional lymph nodes where DCs act as Antigen Presenting Cells. During the migration process through the draining lymphatics, dendritic cells undergo functional maturation, losing the ability to process antigen and acquiring immune stimulatory activities, aimed at recognition by naive T cell receptors and specific precursor B cells [3].
In paracortical area of regional lymph nodes, the complex peptide-MHC class I and peptide-MHC class II are specifically recognized by CD8+ T cells and CD4+ lymphocytes, respectively. Specific CD8+ T-cell precursors expand clonally in the draining lymph node and diffuse to the blood stream through the efferent lymphatic vessels and the thoracic duct. During this process they acquire skin-specific homing antigens (CLA and CCR4) and become effector and memory T cells [3].
Furthermore, recent studies have shown that ID administration of antigen improves the recruitment of DC precursors from blood stream into the dermis and their subsequent migration to the lymph node. This is an important phenomenon for the priming and differentiation of T cells, particularly CD8 + T cells into effector cells [4, 5].

With regard to CD4 + T cells, they promote the differentiation of B cells into plasma cells able to produce and release antibodies into the system circulation [6].
The antigen ID administered, can reach up free through the lymphatics to regional lymph nodes where it is able to activate directly, through interaction with the B cell receptor (BCR), specific B cell precursors. This is followed by internalization and processing of BCR-antigen complex and then by the expression of antigenic epitopes on the surface of B cells through the complex peptide-MHC class II. Therefore, B cells present the antigen to CD4 + T cells, which trigger the differentiation in antibody producing plasma cells [3].
Briefly, intradermal immunization develops two synergistic paths: by promoting the migration of antigen through the lymph ducts and subsequent stimulation of resident lymph node DCs and by triggering the activation and migration of dermis DCs that amplify the immune response and resulting in a potent activation of lymph nodes T cells (Tab. I.).

The experiences with intradermal vaccination

Targeting the skin to protect against infectious diseases has been practiced for many centuries. The inoculation of small amounts of powdered scabs from persons affected with smallpox, into the nose or skin of healthy subjects in order to protect them from disease, was extremely popular in many parts of the world, before a similar practice, called "variolation", was introduced in Europe in 1721 [7].
Few years later, in 1796, Edward Jenner performed the first vaccination against smallpox by the cutaneous administration of the milkmaid's virus. In the first time, the importance of Jenner's studies was not recognized by the scientific community and it took 57 years for vaccination against smallpox to be made compulsory in United Kingdom as public health strategy [7].
Next steps, towards the development of the modern ID vaccines, were taken at the beginning of the XX century, when the French doctor Charles Mantoux published his results of intradermal injection of tuberculin, used as diagnostic test for tuberculosis disease [8]. The adoption of this technique, initially used only for diagnostic purposes, was the rationale for the ID administration of different preventive vaccines and it is still used today for vaccines such as rabies (pre and post-exposition) and Bacillus of Calmette Guérin (BCG) [9, 10].
The first information of using a syringe and needle injection system for the ID vaccination was reported by Tuft in 1930 [11]. His studies on the ID administration of the typhoid vaccine at reduced dose of antigen demonstrated an equivalent immune responses and a better safety profile compared to subcutaneous (SC) injection.
In the following years numerous studies have been performed using other ID vaccines administered in order to evaluate their immunological efficacy, in terms of both (i) determining antibody responses at least equivalent to those elicited by available intramuscular (IM) vaccines, when administered at reduced dose of antigen,

Tab. I. Functions of the cells involved in the immune response following intradermal vaccination.

Immune response	Immune system cells	Functions
Innate	DCs resident in the dermis	Capture and process the antigen released in the dermis
		Migration to the paracortex of regional lymph node where they act as APC to T and B *naive* cells
	DCs recruited from blood stream	Recruitment in the dermis
		Migration to the paracortex of regional lymph node where they act as APC
		Key role for the priming and differentiation of CD8+ T cells into effector cells
	DCs resident in regional lymph node	Capture and process the antigen which reach up free to regional lymph node
		Amplification of the immune response
Adaptative	Lymphocytes T CD4+	Promote of differentiation of B cells in plamacells able to produce and release antibodies into the bloodstream
	Lymphocytes T CD8+	Expand clonally in the lymph node
		Diffuse to the blood stream through the efferent lymphatic vessels
		Acquire skin-specific homing antigens becoming effector and memory T cells
	B Cells	Present the antigen to CD4+ T cells
		Differentiation in antibody producing plasmacells

DCs: Dendritic Cells; APC: Antigen Presenting cells

and (ii) improving immunogenicity profile when administered at full dose.
These researches involved many of the current traditional vaccines, such as those against Measles [12], Rabies [13, 14], Polio virus [15, 16], Hepatitis A [17] and more recently Hepatitis B [18, 19]. In more recent years, the immune potential of intradermal vaccination has been extensively tested in humans for influenza vaccine: the results were promising and led to the marketing of ID influenza vaccine [20-25].
A summary of the most interesting researches has been reported below.

Measles vaccination

A small number of studies have been conducted to investigate the intradermal vaccine against Measles. The rationale of most of these trials was lower cost and easier administration of this vaccine. The results are conflicting: while some studies investigating reduced antigen dose ID vaccine *versus* standard dose SC vaccine, have shown the capability of both formulations to induce an equivalent immune response; others studies did not demonstrate the same [26-28]. Therefore, this route of immunization cannot be considered alternative to current methods of vaccine administration.

Rabies vaccination

The study of reduced dose ID Rabies vaccines were initiated to decrease the high cost of vaccines, originally produced in cell cultures.
Several trials have been conducted evaluating the ability of low-dose intradermal vaccine (usually 10% or 20% of the full dose used in IM vaccine) to induce a neutralizing antibody titre greater than the threshold of 0.5 IU/ml, known as a correlate of protection, without the use of a comparative arm of IM vaccine dose, whole or reduced. Most of these studies demonstrated the ability of low-dose intradermal vaccine to determine an equivalent immune response than vaccines administered at full dose intramuscularly; however, few researches compared the ID and the IM vaccines when administered at the same dose of antigen.
Particularly, in a study by Fishbein et al., vaccines were administrated either IM with 100% 10% and 3% of the standard IM dose of antigen or ID with 10%, 3% and 1% of the standard IM dose [29]. Although the full dose IM was able to induce higher antibody titers, 10% ID dose was significantly superior to 10% IM dose.
Two studies conducted by Bernard and colleagues have shown slightly inconsistent results. In both cases, a full dose of vaccine delivered IM was superior to reduced doses delivered ID or SC [30, 31]. In the first study, a reduced dose delivered ID was superior to the same reduced dose administered SC, whereas in the second study this difference was not statistically significant. In all cases, protective levels of antibody were reached.
Since 1991, World Health Organization (WHO) approved intradermal route of administration for both post-exposure and pre-exposure prophylaxis, provided that the ID vaccines meet the same requirements for production, control and potency required for IM vaccines [32].

Polio virus vaccination

In the 50s of last century, the intradermal administration was the standard via of inactivated Polio virus vaccine (IPV) in some countries. The possibility of IPV dose-sparing is currently of great interest to make the vaccine more accessible and increase its use post-Polio eradication, with the concomitant aim to gradually replace the use of oral Polio vaccine.
Few studies using ID Polio virus vaccine have been published in the literature, and some are ongoing. In two cases, satisfactory serum conversion rates were observed after ID administration of a low dose of antigen (20% of full dose); in these studies the control group of immunized with the IM vaccine was not included [33, 34]. Nirmal et al. reported that two or three doses ID vaccine containing 0.1 ml of antigen were equivalent to two IM doses of 0.5 ml [35].
Therefore, these data underline that 20% doses administered by ID route are likely to be not-inferior to the standard full-dose delivered IM.
Recently, in two Global Polio Eradication Initiative studies an innovative device was tested to administer an intradermal vaccine containing 20% of the full IM dose. Two different immunization programs have been investigated in Oman and Cuba: lower rate of serum conversion to each type of Polio virus vaccine, was observed after administration of ID vaccine according to the 6, 10 and 14 weeks of age schedule. When the vaccine was given at 2, 4 and 6 months, the rate of seroconversion to all three types of Polio virus was higher than 95% even with low-dose ID vaccine (20% of the full dose). The present data from these studies are still incomplete and the difference between the results are not clear [36].

Hepatitis A vaccination

Few studies published in the literature have evaluated the immunogenicity of Hepatitis A ID vaccine but the results are inconsistent. Two clinical trials were conducted using an inactivated whole-virus vaccine adjuvanted with aluminum salts [37, 38], while a third study used a virosomal formulation [39]. None of these studies compare equivalent doses of vaccine administered by different routes. In two cases, a reduced dose of antigen administered ID elicited an immune response similar to IM full dose vaccine [38, 39]. In contrast, Brindle et al. reported a lower immune response after 1-3 doses of 0.1 ml of intradermal Hepatitis A vaccine, compared with a single 1.0 ml dose of IM vaccine [37].
In 2009, Frosner et al. compared the immunogenicity and safety of an aluminum-free virosomal Hepatitis A vaccine administered by ID or IM routes. The vaccine resulted highly immunogenic and well tolerated when administered either via ID or IM. Local reactions were more common in subjects vaccinated ID but this route may confer significant cost savings over the conventional IM delivery [17].

Hepatitis B vaccination

Several clinical trials (over 100) investigated the intradermal Hepatitis B vaccine aiming to evaluate the immune response both in case of using formulation containing reduced dose of antigen and in subjects traditionally low responder to IM vaccination.

Most of these studies comparing ID vaccines with reduced dose of antigen (10% or 20%), with IM or SC full-dose vaccines. In a recently meta-analysis, Chen and Gluud analyzed eight studies that compared the immunogenicity of reduced dose ID vaccines *versus* IM/SC full-dose vaccine administered to health care workers [40]. Overall, 10 or 20 μg/dose administered by IM route where significantly more effective compared with 1 or 2 μg /dose delivered by ID route in terms of participants showing protective anti-Hepatitis B surface antigen (HBsAg) levels. The authors note, however, that the intradermal route of administration deserves further evaluation in the light of the potential cost savings. Moreover, the ID route caused significantly more local adverse events, while the IM one was responsible for significantly more systemic adverse events.

More recently, Sangaré et al. published a meta-analysis of 33 clinical studies on Hepatitis B vaccine delivered ID [41]. The results of this study demonstrated that the response to ID vaccination undergoes significant variations depending on age and gender of vaccinated subjects. In 11 of the 13 randomized trials, ID vaccination against Hepatitis B has been associated with a lower percentage of subjects achieving seroprotection level than those who received the IM vaccine. However, studies conducted after the ID vaccine in school-age children showed responses close to or equivalent to those obtained with the IM vaccination, suggesting that the ID route may be more immunogenic in younger populations. In addition 5 of the 6 studies assessing the serum level of protection by gender showed that females are more likely to reach the serum level of protection than males.

In six studies published in the literature, ID and IM Hepatitis B vaccines containing the same dose of antigen were evaluated [42-47]. All of these studies, except that conducted by Wahl and Hermodsson [47], showed that the intradermal route of administration is equivalent, but not superior to the intramuscular route of administration in terms of immune response. Wahl and Hermodsson reported that the response elicited by 2 μg of antigen ID delivered was equivalent to that induced by IM full dose (20 μg), and higher than that induced by 2 μg SC administration [47]. Finally, Rahman et al., administering by ID and IM route a standard dose of 20 μg, have achieved that, although some measures cell-mediated immunity were higher after ID vaccination, the two routes of delivery were equivalent in terms of concentration of serum antibodies [43].

Other clinical trials performed in patients with chronic diseases such as renal failure, with or without haemodialysis, suggest that the repeated injections of reduced dose (5 μg) of ID Hepatitis B vaccine were able to induce a better immune response than the IM vaccine administered according to the standard schedule [48].

In two studies involving HIV-positive patients, the ID administration of 3 or 4 doses of reduced dose vaccine (2 μg or 2.5 μg respectively) induced an antibody response equal to or higher than the full dose IM vaccination, in 39% and in 50% of subjects respectively [49, 50].

Influenza vaccination

The first studies upon an ID influenza vaccine were carried out in 1948, when Weller et al, just three years after the marketing of the first influenza vaccine in the United States, concluded that a reduced dose of antigen was able to induce a satisfactory antibody response in terms of seroconversion in most of the subjects tested [51]. During the pandemic of 1957 (commonly known as "Asiatic") other researchers tested the possibility of dose sparing the ID vaccine [52, 53]. In 1976 and 1977 the unexpected spreading of a new influenza virus of swine origin, brought a new interest into the ID administration. Some researchers found that the ID vaccination of mono- and bivalent vaccines, containing just one fifth of the standard dose of antigen, was able to achieve the same immune response obtained with a full dose of IM and SC vaccines [54, 55].

Recently, the worldwide growing demand of influenza vaccines, together with the well known difficulties of produce them in a short time, led to a renewed interest in ID vaccines with reduced antigen content [21, 24, 56-64]. Several studies demonstrated that ID vaccines containing different antigenic concentrations (3 - 6 - 7,5 and 9 μg of hemagglutinin (HA) per virus strain) were able to elicit immune response satisfying the European Medicines Agency (EMA) criteria, adopted for licensing the traditional products, with results very similar to those obtained with full dose IM vaccines [21, 24, 61-64]. In this regard, the studies by Leroux-Roels and by Beran, comparing a 9 μg HA trivalent split ID vaccine with a traditional IM one in a population of over 2000 healthy adults, have been of particularly importance [21, 61]. The first clinical trial showed that intradermal trivalent inactivated influenza vaccine induced non inferior humoral immune responses against all three strains and superior responses against both A strains (H1N1, H3N2) compared with the conventional IM vaccines [21]. The second study investigated the immunogenicity and safety during 3 successive years of different dosages (3, 6 and 9 μg) of a trivalent, inactivated, split-virion vaccine against seasonal influenza given intradermally with an intramuscular control vaccine (15 μg). The 3 and 6 μg intradermal formulations were less immunogenic than intramuscular 15 μg, while 9 μg ID vaccine was comparably immunogenic to 15 μg IM one for all strains and both vaccines have met the criteria of immunogenicity required by EMA [61].

The ID administration has been studied not only for dose-sparing purposes, but also in the view to improve the immunogenicity profile of the seasonal influenza vaccines: the suboptimal immunogenicity and protective efficacy of IM traditional seasonal influenza vaccines in some categories, particularly the elderly due to the ageing of immune system, but also in subjects with specific

conditions resulting in a state of immunosuppression, is well documented in the literature [65-66].
A number of strategies have been proposed to fill this gap with conventional non adjuvanted vaccines: (i) the addition of adjuvants, (ii) the administration of high doses or (iii) the use of more conserved antigens, such as conserved HA epitopes or M2 viral protein, and (iv) the use of alternative routes of antigen delivery, such as mucosal (intranasal and oral) or cutaneous (i.e., intradermal and transcutaneous) [67].
In this context, two interesting studies on ID seasonal influenza vaccine were recently published. Holland et al. have firstly demonstrated, in a phase II clinical trial conducted on over 1000 subjects aged > 60 years, that influenza ID vaccine containing 15 µg of HA per virus strain, was able to significantly improve immune response than conventional IM vaccines, thus opening new and important perspectives for the control of influenza within one of the categories with highest risk in the population [22].
This was the first study in the literature that clearly demonstrated the benefit of ID vaccine *versus* IM vaccine for immunization against influenza of the elderly. More recently, a phase III European multi-center clinical trial, was conducted in three consecutive seasons, over 3707 subjects aged ≥ 60 years. The enrolled subjects were randomized to receive two vaccines ID or IM, both containing 15 µg of HA per strain and in-depth analysis of the safety, tolerability and immunogenicity of the two vaccines were conducted. During the first year, both vaccines have met the criteria of immunogenicity required by EMA; in addition the ID vaccine resulted in significantly higher seroprotection rates for all three strains compared to the IM traditional one. A good safety, registered in terms of occurrence of serious adverse events, together with a better immunogenicity of the ID vaccine have been reported also during the two following seasons of the study. The tolerability profile of both vaccines was good, with incidence of systemic side effects similar in both treatment groups: greater local reactogenicity was observed in patients immunized with the prepared ID, although the extent of local effects described was mostly mild and of short duration (less than 1% of subjects reported a duration of reaction > 3 days) [20].
Another interesting study was carried out during the influenza season 2007-2008 on a population of 795 adults aged ≥ 65 years. In these subjects, randomized to receive an ID or a IM vaccine adjuvanted with MF59, both containing 15 µg of HA per virus strain, the ID group showed not inferior, in terms of immunogenicity, than the IM MF59-adjuvanted vaccine. Injection-site reactions were generally mild and transient; erythema, but not pain, were more frequent in the ID vaccine group as could be expected with the intradermal route of administration. The systemic safety profile was comparable between ID and IM groups [68].
The encouraging results with ID influenza vaccine led, in February 2009, to a positive response for the marketing of this new vaccine by the European Commission, also considering the previous positive response regarding the characteristics of the product expressed by EMA, in December 2008: the trade name of the ID influenza vaccine is Intanza (Sanofi Pasteur MSD, Lyon, France), with indication for use in adults < 59 years of age (formulation containing 9 µg of HA per viral strain) and in subjects ≥ 60 years of age (formulation containing 15 µg of HA per viral strain) [69].

Intradermal route: from Mantoux technique to Microinjection system

The intradermal injection technique, introduced in 1908 by Mantoux, remains today the standard ID administration and consists of entering into the skin tissue of a small short needle with the blunt up, connected to a 1ml syringe [8]. The needle is inserted almost parallel to the skin surface in order to penetrate the thin layer of skin and release the vaccine preparation. This technique, however, is not commonly used because it is difficult to do properly and requires special training of personnel with experience.
The ID vaccination through the bifurcated needle was widely used in the past and it gave a fundamental contribution towards the eradication of smallpox. This device, specifically developed to allow the use of vaccine in the dermis, has been of substantial assistance to health workers to correctly inoculate vaccine for active immunization against smallpox. After the bifurcated needle tip in the bottle containing the solution of the vaccine and the two brands have taken a small amount of antigen, which was, however, barely detectable, the immunization was carried out repeatedly pricking the skin [70].
To overcome the problems related to the difficult reproducibility of the two techniques described above, and to give greater reproducibility, reliability, accuracy and easiness to the practice of vaccination new devices for administration ID have been developed. These novel medical devices have kept a further benefit in terms of safety of the vaccination, in particular to reduce the accidental puncture of health workers and to prevent reuse of a syringe.
The new devices can be classified into: (i) Jet Injectors, (ii) Microneedles and (iii) Micro-injection system.

(i) Most Jet Injectors are formed by a syringe without needle; a propulsion system consisting of reusable and disposable cartridges of vaccine pre-filled is replaced with each dose. Although this device presents numerous advantages including the possibility of immediate use of the preparation without the need to reconstitute the vaccine, the reduction of space for storage and disposal, as the supply is mainly in the cartridges [71-72], some cases of parenteral transmission of infection diseases have been reported. Jet Injectors have been used for Polio Virus ID dose-reduction vaccine and in several clinical trials comparing ID and IM delivery of influenza, HPV, yellow fever, malaria vaccines.
(ii) The Microneedles can be classified by type, patch or syringe, both by the length of the needle. The

choice of different devices depends on the characteristics of the vaccine preparation: liquid vaccines are administered primarily through systems of Microneedles and syringes, while solids can be directly contained within the micro-needle or applied via a patch, after micro-puncture skin. Thanks to its small size, administration via micro-needle penetrates the skin to a depth of few millimeters, making it almost imperceptible to the patient and, unlike deep IM injection, eliminating the potential risk of injury to nerves or blood vessels. Furthermore, the system patches, being less bulky than conventional syringe and needle, means easier maintenance of cold chain [73].

Micro-needle devices have recently been tested for the ID administration of reduced doses of inactivated influenza vaccine in healthy adult volunteers [24]. In this study, the device consisted of an array of four silicon crystal Microneedles, each 0.45 mm in length, fixed to an adaptor that could be mounted on a standard syringe.

(iii) Microinjection system has a prefilled syringe ready for use, the volume of which can vary from 100 to 200 µl, with an integrated mini needle approximately 1.5 mm in length. The tip of the syringe has a system specifically designed to limit the penetration depth and injection to ensure proper placement of needle insertion perpendicular. The syringe also has an automatic needle shielding system that is activated after the completion of injection and thus help to reduce the risk of accidental puncture for the healthcare workers, and prevent the reuse of the device [74].

The above-mentioned devices are shown in Figure 1. A microinjection system, called Soluvia and producted by Becton Dickinson (BD), has been used for the approved ID influenza vaccine Intanza (Sanofi Pasteur MSD, Lyon, France). The BD Soluvia microinjection system is easy to use and reliable method of ID delivery, which integrates a 1.5 mm length micro-needle with a 0.1 ml injected volume. This microinjection system provides key safety benefits for patients and the healthcare system, such as a high level of sterility assurance and reduced risks associated with vaccine preparation and administration (Fig. 2).

Fig. 1. Medical devices for the intradermal administration.

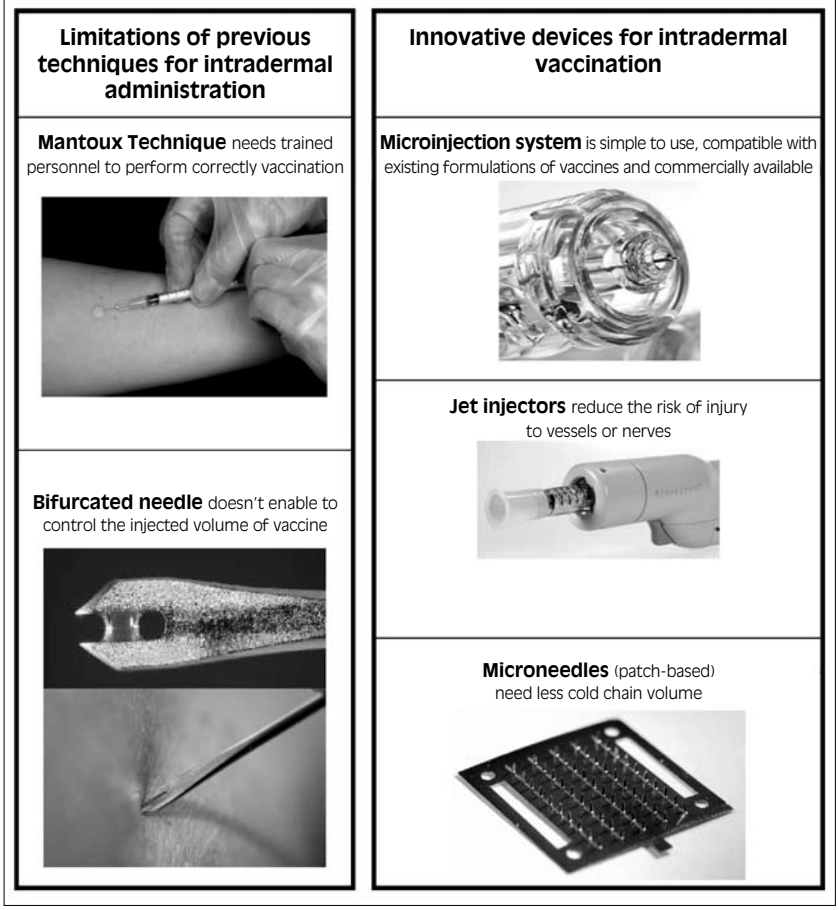
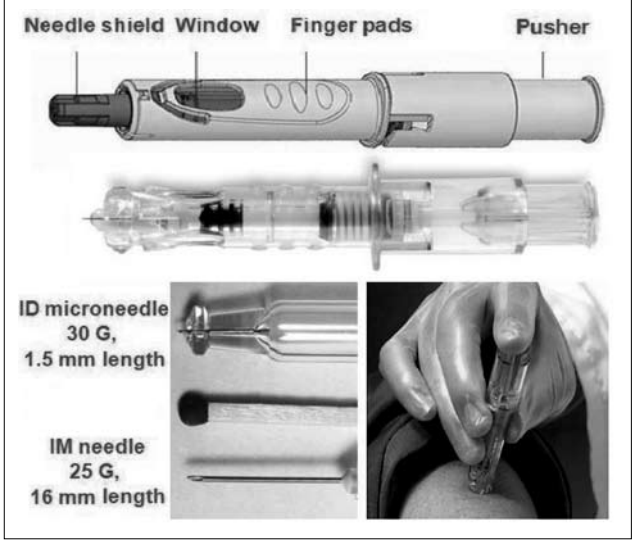


Fig. 2. Soluvia microinjection system.



Conclusion

Intradermal administration of vaccines represents a promising alternative to IM route both by immunological and practical standpoints. Extensive clinical experiences with ID vaccination has demonstrated the ef-

ficacy and the safety of this route and has underlined that the immunological advantages depend of several factors such as the type and the dose of antigen, the features of vaccinated subjects in terms of age and immune status.
Recently, ID influenza vaccine has been demonstrated to confer a better immune response than the conventional IM influenza vaccines in subjects traditionally considered as hypo-responder to influenza vaccination, such as the elderly.
In addition to immunological consideration, the recent development of novel devices which guarantee less invasive, reproducible and easier injection than IM, may lead to a broad use of ID vaccines in the clinical practice and also stimulate further researches on novel formulations delivered by this attractive route.

References

[1] Spellberg B. *The cutaneous citadel: a holistic view of skin and immunity.* Life Sci 2000;67:477-502.

[2] Lambert PH, Laurent PE. *Intradermal vaccine delivery: will new delivery systems transform vaccine administration?* Vaccine 2008;26:3197-208.

[3] Nicolas JF, Guy B. *Intradermal, epidermal and transcutaneous vaccination: from immunology to clinical practice.* Expert Rev Vaccines 2008;7:1201-14.

[4] Le Borgne M, Etchart N, Goubier A, et al. *Dendritic cells rapidly recruited into epithelial tissues via CCR6/CCL20 are responsible for CD8+ T cell cross priming in vivo.* Immunity 2006;24:191-201.

[5] Allan RS, Waithman J, Bedoui S, et al. *Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming.* Immunity 2006;25:153-62.

[6] Itano AA, Jenkins MK. *Antigen presentation to naive CD4 T cells in the lymph node.* Nat Immunol 2003;4:733-9.

[7] Plotkin SA. *Vaccines: the fourth century.* Clin Vaccine Immunol 2009;16:1709-19.

[8] Mantoux C. *L'intradermoréaction a la tuberculine et son interprétation clinique.* Presse Medicale 1910;18:10-3.

[9] Bricks LF. *Percutaneous or intradermal BCG vaccine?* J Pediatria 2004;80:93-8.

[10] Briggs DJ, Banzhoff A, Nicolay U, et al. *Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine.* Bull WHO 2000;78:693-8.

[11] Tuft L. *Active immunization against thyphoid fever, with particular reference to an intradermal method.* J Lab Clin Med 1931;16:552-6.

[12] Cutts FT, Clemants CJ, Bennett JV. *Alternative route of measles immunization: a review.* Biologicals 1997;25:323-38.

[13] Nicholson KG, Prestage H, Cole PJ, et al. *Multsite intradermal antirabies vaccination. Immune response in man and protection of rabbits against death from street virus by postexposure administration of human diploid-cell-strain rabies vaccine.* Lancet 1981;2:915-8.

[14] Warrell MJ, Nicholson KG, Warrell DA, et al. *Economical multiple-site intradermal immunization with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis.* Lancet 1985;1:1059-62.

[15] Connolly JH, Dirk GW, Corkin DL. *Antibody response following intradermal or oral administration of formalinised poliomyelitis.* Lancet 1958;2:333-6.

[16] Nirmal S, Cherian T, Samuel BU, et al. *Immune response of infants to fractional doses of intradermally administrated inactivated poliovirus vaccine.* Vaccine 1998;16:928-31.

[17] Frösner G, Steffen R, Herzog C. *Virosomal hepatitis a vaccine: comparing intradermal and subcutaneous with intramuscular administration.* J Travel Med 2009;16:413-9.

[18] Miller KD, Gibbs RD, Mulligan NM, et al. *Intradermal hepatitis B virus vaccine: immunogenicity and side effects in adults.* Lancet 1983;2:1454-6.

[19] Clarke JA, Hollinger FB, Lewis E, et al. *Intradermal inoculation with Heptavax-B immune response and histologic evaluation of injection sites.* JAMA 1989;26:2567-71.

[20] Arnou R, Icardi G, De Decker M, et al. *Intradermal influenza vaccine for older adults: a randomized controlled multicenter phase III study.* Vaccine 2009;27:7304-12.

[21] Leroux-Roels I, Vets E, Freese R, et al. *Seasonal influenza vaccine delivered by intradermal microinjection: a randomised controlled safety and immunogenicity trial in adults.* Vaccine 2008;26:6614-9.

[22] Holland D, Booy R, De Looze F, et al. *Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial.* J Infect Dis 2008;198:650-8.

[23] Kenney RT, Frech SA, Muenz LR, et al. *Dose sparing with intradermal injection of influenza vaccine.* N Engl J Med 2004;351:2295-301.

[24] Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, et al. *Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults.* Vaccine 2009;27:454-9.

[25] European Medicines Agency (EMA). *Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.* Available at http://www.emea.europa.eu/humandocs/PDFs/EPAR/intanza/H-957-en6.pdf - Accessed 10 February 2010.

[26] Whittle HC, Rowland MG, Mann GF, et al. *Immunisation of 4-6 month old Gambian infants with Edmonston-Zagreb measles vaccine.* Lancet 1984;2:834-7.

[27] Kok PW, Kenya PR, Ensering H. *Measles immunization with further attenuated heat-stable measles vaccine using five different methods of administration.* Trans R Soc Trop Med Hyg 1983;77:171-6.

[28] Burland WL. *Measles vaccination by the intradermal route.* Postgrad Med J 1969;45:323-6.

[29] Fishbein DB, Pacer RE, Holmes DF, et al. *Rabies pre-exposure prophylaxis with human diploid cell rabies vaccine: a dose-response study.* J Infect Dis 1987;156:50-5.

[30] Bernard KW, Roberts MA, Sumner J, et al. *Human diploid cell rabies vaccine. Effectiveness of immunization with small intradermal or subcutaneous doses.* JAMA 1982;247:1138-42.

[31] Bernard KW, Mallonee J, Wright JC, et al. *Preexposure immunization with intradermal human diploid cell rabies vaccine. Risks and benefits of primary and booster vaccination.* JAMA 1987;257:1059-63.

[32] World Health Organization (WHO). *Rabies vaccines. WHO position paper.* Wkly Epidemiol Rec 2007;82:425-35.

[33] Samuel BU, Cherian T, Sridharan G, et al. *Immune response to intradermally injected inactivated poliovirus vaccine.* Lancet 1991;338:343-4.

[34] Samuel BU, Cherian T, Rajasingh J, et al. *Immune response of infants to inactivated poliovirus vaccine injected intradermally.* Vaccine 1992;10:135.

[35] Nirmal S, Cherian T, Samuel BU, et al. *Immune response of infants to fractional doses of intradermally administered inactivated poliovirus vaccine.* Vaccine 1998;16:928-31.

[36] Eighth WHO/Unicef consultation with opv/ipv manufacturers and nras. 30 October 2009, Geneva, who/hq. Available at: www.who.int/entity/immunization_standards/vaccine_quality/ipvopv_summary3dec09.pdf. Accessed February 7, 2010.

[37] Brindle RJ, Morris CA, Berger R, et al. *Inadequate response to intradermal hepatitis A vaccine.* Vaccine 1994;12:483-4.

[38] Carlsson U, Brudin L, Eliasson I, et al. *Hepatitis A vaccination by intracutaneous low dose administration: a less expensive alternative.* Scand J Infect Dis 1996;28:435-8.

[39] Pancharoen C, Mekmullica J, Thisyakorn U, et al. *Reduced-dose intradermal vaccination against hepatitis A with an aluminum-free vaccine is immunogenic and can lower costs*. Clin Infect Dis 2005;41:1537-40.

[40] Chen W, Gluud C. *Vaccines for preventing hepatitis B in health-care workers.* Cochrane Database Syst Rev 2005;(4): CD000100.

[41] Sangaré L, Manhart L, Zehrung D, e al. *Intradermal hepatitis B vaccination: a systematic review and meta-analysis.* Vaccine 2009;27:1777-86.

[42] Heijtink RA, Knol RM, Schalm SW. *Low-dose (2 micrograms) hepatitis B vaccination in medical students: comparable immunogenicity for intramuscular and intradermal routes.* J Med Virol 1989;27:151-4.

[43] Rahman F, Dahmen A, Herzog-Hauff S, et al. *Cellular and humoral immune responses induced by intradermal or intramuscular vaccination with the major hepatitis B surface antigen.* Hepatology 2000;31:521-7.

[44] Milne A, Allwood GK, Pearce NE, et al. *Low dose hepatitis B vaccination in children.* N Z Med J 1986;99:47-9.

[45] Ayoola EA. *Hepatitis B vaccine in developing countries: problems and prospects.* IARC Sci Publ 1984;(63):297-305.

[46] Coberly JS, Townsend T, Repke J, et al. *Suboptimal response following intradermal hepatitis B vaccine in infants.* Vaccine 1994;12:984-7.

[47] Wahl M, Hermodsson S. *Intradermal, subcutaneous or intramuscular administration of hepatitis B vaccine: side effects and antibody response.* Scand J Infect Dis 1987;19:617-21.

[48] Micozkadioglu H, Zumrutdal A, Torun D, et al. *Low dose intradermal vaccination is superior to high dose intramuscular vaccination for hepatitis B in unresponsive hemodialysis patients.* Ren Fail 2007;29:285-8.

[49] Shafran SD, Mashinter LD, Lindemulder A, et al. *Poor efficacy of intradermal administration of recombinant hepatitis B virus immunization in HIV-infected individuals who fail to respond to intramuscular administration of hepatitis B virus vaccine.* HIV Med 2007;8:295-9.

[50] Ristola MA, Vuola JM, Valle M, et al. *Antibody responses to intradermal recombinant hepatitis B immunization among HIV-positive subjects.* Vaccine 2004;23:205-9.

[51] Weller TH, Cheever FS, Enders JF. *Immunologic reactions following the intradermal inoculation of influenza A and B vaccine.* Proc Soc Exp Biol Med 1948;67:96-101.

[52] Boger WP, Liu OC. *Subcutaneous and intradermal vaccination with Asian influenza vaccine.* JAMA 1957;165:1687-9.

[53] McCarroll JR, Kilbourne ED. *Immunization with Asian-strain influenza vaccine.* New Engl J Med 1958;259:618-21.

[54] Brown H, Kasel JA, Freeman DM, et al. *The immunizing effect of influenza A/NewJersey/76 (Hsw1N1) virus vaccine administered intradermally and intramuscularly to adults.* J Infect Dis 1977;136:(Suppl):S466-S471.

■ Received on February 10, 2010. Accepted on February 22, 2010.

■ Correspondence: Marisa Alberti, Department of Health Sciences, University of Genoa, Italy, via Pastore 1, 16132 Genoa, Italy - E-mail: marisa.alberti@unige.it

[55] Halperin W, Weiss WI, Altman R, et al. *A comparison of the intradermal and subcutaneous routes of influenza vaccination with A/New Jersey/76 (swine flu) and A/Victoria/75: report of a study and review of the literature.* Am J Public Health 1979;69:1247-5.

[56] European Medicines Agency (EMA). *Committee for proprietary medicinal products (CPMP). Note for guidance on harmonisation of requirements for influenza vaccines.* Available at: http://www.ema.europa.eu/pdfs/human/bwp/021496en.pdf Accessed on 7 February 2010

[57] Jo YM, Song JY, Hwang IS, et al. *Dose sparing strategy with intradermal influenza vaccination in patients with solid cancer.* J Med Virol 2009;81:722-7.

[58] Chiu SS, Peiris JS, Chan KH, et al. *Immunogenicity and safety of intradermal influenza immunization at a reduced dose in healthy children.* Pediatrics 2007;119:1076-82.

[59] Chiu SS, Chan KH, Tu W, et al. *Immunogenicity and safety of intradermal versus intramuscular route of influenza immunization in infants less than 6 months of age: a randomized controlled trial.* Vaccine 2009;27:4834-9.

[60] Belshe RB, Newman FK, Wilkins K, et al. *Comparative immunogenicity of trivalent influenza vaccine administered by intradermal or intramuscular route in healthy adults.* Vaccine 2007;25:6755-63.

[61] Beran J, Ambrozaitis A, Laiskonis A, et al. *Intradermal influenza vaccination of healthy adults using a new microinjection system: a 3-year randomised controlled safety and immunogenicity trial.* BMC Med 2009;7:13.

[62] Kenney RT, Frech SA, Muenz LR, et al. *Dose sparing with intradermal injection of influenza vaccine.* N Engl J Med 2004;351:2295-301.

[63] Belshe RB, Newman FK, Cannon J, et al. *Serum antibody responses after intradermal vaccination against influenza.* N Engl J Med 2004;351:2286-94.

[64] Auewarakul P, Kositanont U, Sornsathapornkul P, et al. *Antibody responses after dose-sparing intradermal influenza vaccination.* Vaccine 2007;25:659-63.

[65] Hannoun C, Megas F, Piercy J. *Immunogenicity and protective efficacy of influenza vaccination.* Virus Res 2004;103:133-8.

[66] Vu T, Farish S, Jenkins M, et al. *A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community.* Vaccine 2002;20:1831-6.

[67] Durando P, Icardi G, Ansaldi F. *MF59-adjuvanted vaccine: a safe and useful tool to enhance and broaden protection against seasonal influenza viruses in subjects at risk.* Expert Opin Biol Ther 2010;10:639-51.

[68] Van Damme P. *Evaluation of non-inferiority of intradermal versus adjuvanted influenza vaccine with two serological techniques.* Poster PB7 513 presented at IAGG World Congress – Paris, 5-9 July 2009.

[69] European Medicines Agency (EMA). *EPARs for authorized medicinal products for human use.* Available at: http://www.ema.europa.eu/humandocs/Humans/EPAR/intanza/intanza.htm Accessed on 10 February 2010

[70] Kravitz H. *A simplified technique for vaccination against small pox.* Pediatrics 1961;27:219-26.

[71] Giudice EL, Campbell JD. *Needle-free vaccine delivery.* Adv Drug Deliv Rev 2006;58:68-89.

[72] PATH. *Shots without needles. A safer, cheaper way to get vaccines where they are needed most.* Available at: http://www.path.org/projects/jet_injector.php Accessed February 7, 2010.

[73] WHO (World Health Organization). *Intradermal Delivery of Vaccines. A review of the literature and the potential for development for use in low- and middle-income countries.* August 27, 2009.

[74] Laurent PE, Bonnet S, Alchas P, Regolini, et al. *Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system.* Vaccine 2007;25:8833-42.