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SECTION THREE: Vaccines in development and new vaccine strategies

Alternative vaccine delivery methods

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The earliest known route of vaccination was respiratory, by intranasal insufflation of powdered scab material containing variola virus from smallpox patients, reportedly practiced in China as early as the 10th century AD (see Chapters 1 and 32).¹ The cutaneous route for such variolation involved breaking the skin with a sharp instrument, and it was used in India perhaps as early as in China, but it was not documented until the 16th century.² Variolation was supplanted by safer cutaneous application of material from cowpox lesions, the method of “vaccination” known in the 18th century and first published by Edward Jenner.

After 15th-century experiments with hypodermic injection,³ the introduction of the needle and syringe (N-S) in the mid-19th century by Pravaz,^{4,5} Rynd,⁶ and Wood⁷ began a new era in medicine. Pasteur used a Pravaz syringe to inoculate sheep in the famed controlled challenge experiment demonstrating protection against anthrax, after which he honored Dr. Jenner by broadening his predecessor's term—vaccination—to mean the administration of immunizing agents for various diseases, not just smallpox.⁸

With acceptance of the germ theory and resulting sterilization of medical equipment by the early 20th century,⁹ and with mass production of needles and glass (later plastic) syringes by mid century, hypodermic injection became the norm for convenient, accurate, and certain administration of most vaccines and many drugs. Regrettably, aseptic practice was ignored in many developing countries,^{10,11} and by nonmedical-intravenous-drug users everywhere,¹² leading to widespread iatrogenic and self-inflicted disease transmission during that era once decried as the Injection Century.¹³

Other drawbacks of N-S include needlestick injuries to health care workers,^{14,15} needle-phobia and discomfort for patients facing increasingly crowded immunization schedules,^{16,17} and the costs and complexity of safe disposal of sharps in the medical waste stream.¹⁸ In the early 21st century, new targets for disease control and eradication, the expansion of philanthropic efforts to make expensive new vaccines affordable for the world's children, and promising results for novel techniques have stimulated research on vaccine delivery that avoids N-S and may be dosage sparing. Preparedness efforts for threatened pandemics and bioterrorism have also rekindled^{19,20} past interest²¹ in novel needle-free methods for mass vaccination campaigns.

Existing and potential alternatives to conventional intramuscular (IM) and subcutaneous (SC) vaccination by N-S, as well as by oral ingestion, are classified here into three major categories: *cutaneous vaccination*, *jet injection*, and *respiratory vaccination*.²² The cutaneous route may be subdivided into *classical intradermal* (ID) via conventional needle, passive diffusion with or without chemical enhancers or adjuvants, and disruption or penetration

of the stratum corneum by mechanical contact, heat, electricity, or light. Jet injection involves pressurizing liquid into high-velocity streams to reach targeted IM, SC, or ID tissues. Respiratory vaccination delivers airborne particles via the nose or mouth for deposition onto the mucosal surfaces of the upper or lower airways.

Cutaneous vaccination

As mentioned, the skin was one of the first tissues into which variola (smallpox) virus and, later, cross-protecting cowpox virus were introduced to prevent smallpox. This route remains the standard for smallpox vaccine (now containing vaccinia virus) (see Chapter 32), as well as for administering bacille Calmette-Guérin (BCG) to prevent tuberculosis (see Chapter 35).

The cutaneous route has both demonstrated and hypothetical advantages over other delivery methods, as described here and as reviewed by others.^{23–44} Reduced dosages of various vaccines into the skin, compared with full dosages into muscle or fat, have shown this tissue's dosage-sparing ability, which is useful when vaccines are scarce, or unaffordable in full dosages. The skin is also the least invasive route, and thus, in theory, cutaneous delivery of new antigens is less likely to result in unanticipated serious adverse reactions—for example, intussusception after the first American oral rotavirus vaccine,⁴⁵ Bell's paralysis of the seventh cranial nerve after the first European intranasal influenza vaccine,^{46,47} and the occasional abscesses and nerve injury from needle injections into muscle and fat.

Of course, BCG and smallpox vaccines delivered into the skin are not always benign, and rarely they may result in uncontrolled replication and spread of the live antigen, causing serious complications, particularly in immunocompromised patients.^{48–50} Nevertheless, skin reactions in general are easier to detect early and access with palliatives or active therapeutic or anti-inflammatory agents than are reactions in deeper tissues.

Finally, successful delivery of antigen by cutaneous vaccination is relatively sure, although not as certain as the “gold standard” of needle injection. And, as with needles, lack of cooperation by some infants and children can be overcome with firm restraint. In contrast, oral doses can be spit out or vomited, and intranasal doses sneezed out or blocked by mucoid or purulent rhinitis. Some pulmonary delivery methods require patient-initiated inhalation (see Figure 61-8E), or they may take from 30 seconds to over 2 minutes to administer by mask or prong (see Figure 61-7A,B,E,F). Such drawbacks may raise doubts about successful delivery of the antigen.

An unstandardized and inconsistent nomenclature to describe vaccination targeting the skin is found in the literature

(eg, *cutaneous*, *dermal*, *epicutaneous*, *epidermal*, *intracutaneous*, *intraepidermal*, *intraepithelial*, *patch*, *percutaneous*, *skin*, *topical*, *transcutaneous*, and *transdermal*). Often, prefixes of Latin (*intra*, *per*, *trans*) or Greek (*epi*) origin are paired hetero-lingually with root terms for skin of the other etymology, *derma* (G.) and *cutis* (L.). Some recent coinage results from commercial intent to claim trade names from among this synonymy. In this chapter, *cutaneous vaccination* is the preferred term to encompass all methods for delivery of antigen anywhere into or onto the skin. *Classical intradermal* injection, or just *intradermal* (ID), is generally reserved for a type of cutaneous vaccination in which a bolus of liquid is deposited into the dermis to raise a visible bleb, as in the traditional Mantoux injection (discussed below).

Anatomy and immunology of the skin

The outermost section of the skin is the epidermis, a stratified squamous epithelium that is usually about 0.1 mm thick but can be from 0.8 to 1.4 mm on the palms and soles (Figure 61-1A). The *stratum malpighii* layer comprises the primary component of the epidermis, and its dividing and growing keratinocytes serve both a structural function—limiting the passage of water

and other molecules—and an immunologic role. Keratinocytes germinate just above the basement membrane, which demarcates the boundary between epidermis and deeper dermis. These cells then grow, flatten, mature, and senesce in increasingly superficial strata until they reach the surface and are sloughed. The main product of this cell is keratinohyalin, a dense lipid that helps form a waterproof barrier. The lateral edges of adjacent keratinocytes are tightly linked by desmosomes, which maintain the strength of the epidermis and also contribute to its resistance to the passage of foreign matter or molecules.^{51,52}

The topmost horny layer of the epidermis is the *stratum corneum*, comprised of staggered courses of dead keratinocytes—also known as corneocytes—in a lipid bilayer matrix. This stack of 10 to 20 cells, 10 to 20 μm thick, is the principal obstacle to the introduction of vaccine antigen for cutaneous vaccination.

Below the epidermis and basement membrane lies the dermis, about 1.5 to 3 mm thick, in which fibroblasts, fine collagen, elastic fibers, and most skin organelles, including small blood vessels, lymphatic vessels, nerves, hair follicles, and sweat and sebaceous glands, are found. The subcutaneous tissue below the skin, sometimes referred to as the hypodermis, consists primarily of fat; it varies widely in thickness between different body surfaces and, of course, individuals.

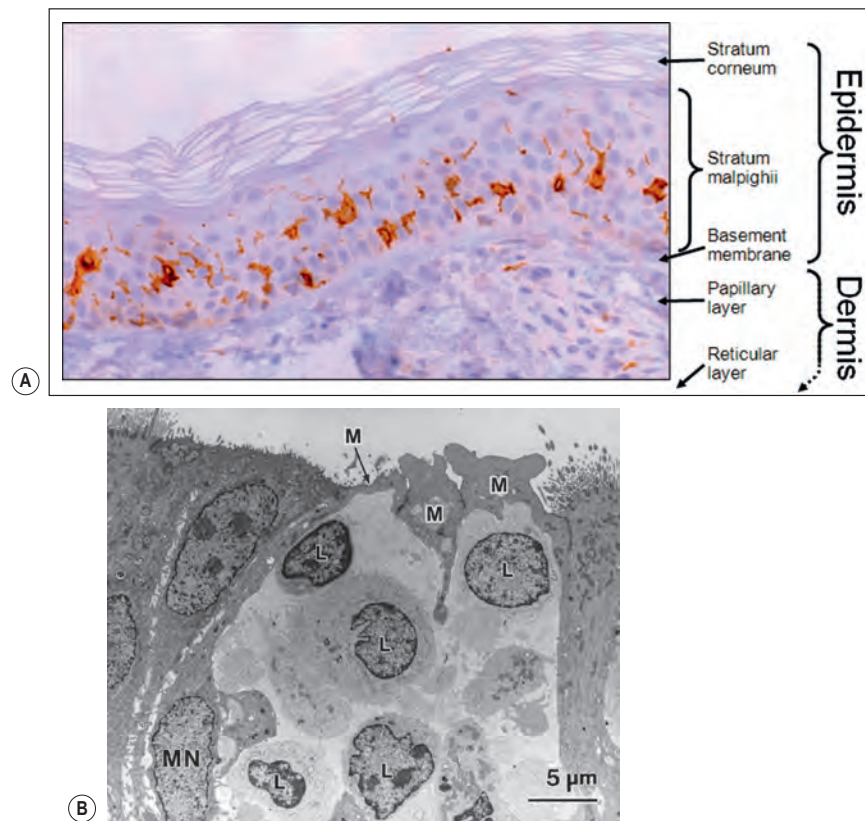


Figure 61-1 Key Antigen-presenting Cells of the Immune System for Cutaneous and Respiratory Vaccination. (A) Activated Langerhans cells (dark stain) in the epidermal Malpighian layer 48 hours after immunization by application of cutaneous patch containing heat-labile enterotoxin (LT) of *E. coli*. Full depth of dermis not shown. (From Glenn GM, Taylor DN, Li X, et al. Transcutaneous immunization: a human vaccine delivery strategy using a patch. *Nat Med* 6:1403-1406, 2000 [Fig. 3b, p. 1405]; from Glenn GM, Kenney RT, Hammond SA, et al. Transcutaneous immunization and immunostimulant strategies. *Immunol Allergy Clin North Am* 23:787-813, 2003 [Fig. 1, p. 788]; and from Glenn G, Kenney R. Mass vaccination: solutions in the skin. *Curr Top Microbiol Immunol* 304:247-268, 2006 [Fig. 1, p. 249].) (B) Transmission electron micrograph of nasal-associated lymphoid tissue (NALT) from excised human adenoids, showing lack of apical cilia at the endothelial lumen (top) of an M cell (M), the M cell nucleus (MN), and the lymphocytes (L) enfolded in the cell's invaginated pocket, which remains contiguous with the extracellular space. M cells sample particulates from the lumen, presenting them to lymphocytes, macrophages, and dendritic cells, which congregate in the pockets. (From Fujimura Y. Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. *Virchows Arch* 436:560-566, 2000 [Fig. 3, p. 563]; and from Kraal G. Nasal associated lymphoid tissue. In: Mestecky J, Lamm ME, Strober W, et al, eds. *Mucosal Immunology*. 3rd ed. Amsterdam: Elsevier, 415-434, 2005 [Fig. 23.3, p. 417].)

Skin thicknesses have been mapped in children to identify the histologic suitability of sites for cutaneous vaccination.⁵³ Equally important is selecting skin sites that are easily accessed so as to minimize disrobing and loss of privacy. In smallpox eradication, the volar surface of the forearm was commonly used because it was quickly accessible, the vaccinator could hold the vaccinee's wrist for stabilization (and to prevent escape), and the scar was easily visible to verify prior vaccination.¹

The speed of diffusion of therapeutic substances transcellularly through the dead and living keratinocytes, and via the intercellular channels between them, correlates with smaller molecules (< 500 Da), lower melting points, increased lipophilicity (and correspondingly lower water solubility), higher (saturated) concentrations, and a relative lack of pendant groups that form hydrogen bonds that slow diffusion.^{31,54}

The specific mechanisms that produce the resulting immune response when vaccine antigen is introduced into the skin are not entirely clear. With stimulation, keratinocytes can produce pro-inflammatory cytokines (eg, interleukin 1 [IL-1]) and can themselves function as antigen-presenting cells by displaying major histocompatibility complex (MHC) class II antigens (human leukocyte antigen [HLA]-DR), as well as intercellular adhesion molecules (ICAM-1).⁵⁵ Epidermal Langerhans cells are believed to play a key role in cutaneous immunization, although dendritic cells and other well-known immune system players, such as CD8⁺ and CD4⁺ T lymphocytes, mast cells, and macrophages, also circulate or reside in the epidermis or dermis.^{36,37,39,56-60}

The immature Langerhans cells reside like sentinels among the keratinocytes in the epidermis, comprising about a quarter of the skin surface area,⁶¹ where they efficiently capture foreign antigens by phagocytosis or endocytosis. Like dendritic cells in other tissues (see Chapter 5), on activation (see Figure 61-1A) these professional antigen-presenting cells (APC) process the antigen as they migrate to draining lymph nodes. There, now mature, they express high levels of class II MHC molecules, and present the antigen brought from the skin to T-helper (Th) lymphocytes, a critical step for the subsequent immune responses orchestrated by the latter cells.

Delivery by sharp instruments or needles

Traditional vaccination for smallpox

During the more than 200 years of cutaneous vaccination against smallpox (see Chapter 32), a variety of sharp instruments have been used to cut, scratch, poke, and otherwise penetrate into the epidermis (and unnecessarily deeper into the dermis), for inoculation of cowpox or vaccinia virus (Figure 61-2A,B,C,D).¹ In the 18th and 19th centuries, the scarification method involved scratching one or more lines into the skin with a needle, scalpel (lancet), or knife and rubbing vaccine into the resulting lesion. A rotary lancet first described in the 1870s consisted of a shaft attached to the center of a small disk, the opposite (patient's side) of which contained a central tine surrounded by smaller satellite tines. The twirling of the disk in a drop of vaccine on the skin produced much abrasion of the skin and often severe reactions from both vaccine and common bacterial contaminants. In the less traumatic multiple-pressure method introduced in the early 1900s, liquid vaccine was placed onto the skin and a straight surgical needle, held tangentially to the skin with its tip in the drop, was repeatedly and firmly pressed sideways into the limb 10 times for primary vaccination, and 30 times for revaccination.⁶² Multi-tine devices have also been used.^{63,64}

Bifurcated needle

In the 1960s, Benjamin Rubin invented the bifurcated needle (see Figure 61-2D),⁶⁵ for which Wyeth waived the royalties so that the World Health Organization (WHO) could produce it for smallpox eradication.^{1,66} The device holds approximately

2.5 μ L by capillary action between its tines, which is applied perpendicularly into the skin. This uses one fifth of the typical dose volume needed by earlier multiple-pressure methods, but it requires a higher virus concentration. Its simplicity, portability, and economy greatly facilitated the latter half of smallpox eradication, particularly in Asia and East Africa.

Tuberculosis vaccination

The bacille Calmette-Guérin vaccine for the prevention of disease from *Mycobacterium tuberculosis* was originally administered orally in the 1920s (see Chapter 35). Safety concerns prompted a shift to cutaneous administration by ID needle injection (1927),⁶⁷ and later multiple puncture (1939),⁶⁸⁻⁷¹ scarification (1947), and multi-tine devices (Figure 61-2, images A, C, G, F),^{64,72,73} as described earlier for smallpox vaccine. BCG has also been delivered cutaneously by jet injectors⁷⁴ and bifurcated needles.⁷⁵

Mantoux method

The needle technique for *classical intradermal* injection, as used for BCG, was developed in the early 20th century by Felix Mendel⁷⁶ and separately by Charles Mantoux⁷⁷ for the administration of tuberculin (now replaced by purified protein derivative) used for diagnosis of tuberculosis infection. Now referred to as the *Mantoux* method, this procedure has become the common route for ID injection of various antigens (see Figure 61-2G). A short-bevel, fine-gauge needle, usually 27 gauge (0.016 inch, 0.4060 mm diameter), is inserted, bevel up, at a 5 to 15-degree angle into slightly stretched skin, often the volar surface of the forearm.⁷⁸ The tip is advanced about 3 mm until the entire bevel is covered. Upon injection of fluid, proper location of the bevel in the dermis creates a bleb, or a wheal, as the basement membrane and epidermis above are stretched by the fluid. Leakage onto the skin indicates insufficient penetration to cover the bevel. Failure to produce a bleb indicates an improperly deep location of the fluid in the subcutaneous tissue. Drawbacks to the Mantoux method for mass vaccination campaigns are the training, skill, and extra time needed to accomplish it correctly.

Reinventing the wheal

The potential dosage-sparing effect of ID vaccination, reducing the amount of antigen needed by up to 80% (by reducing the volume from 0.5 to 0.1 mL), has prompted renewed attention to this route because of concerns about emerging threats such as pandemic influenza, severe acute respiratory syndrome (SARS), and bioterrorism, which may leave populations vulnerable because of insufficient vaccine supply.^{23,24} Both old and new techniques can more easily achieve the *classical intradermal* injection of the Mantoux method, depositing the injectate into the skin to produce a raised bleb or wheal of temporary induration. Since the 1960s, multiuse-nozzle jet injectors (see "Jet injection", later) have allowed ID delivery of smallpox, BCG, and other vaccines by using these specialized nozzles (see Figure 61-2E).^{66,79-81} Some adaptations of modern disposable-syringe jet injector technology also achieve *classical intradermal* injection, namely the Tropis⁸² and the Bioject ID-Pen⁸³ (see Figure 61-2J,K, and Table 61-1).

Mini-needle

To circumvent the amount of skill and time needed for successful Mantoux injection, Becton, Dickinson (BD)⁸⁴ developed a prefilled glass syringe with a staked, 30-gauge (outer diameter [OD], ~ 0.305 mm) mini-needle, which projects only 1.5 mm beyond its depth-limiting hub for intuitive perpendicular insertion into the skin (see Figure 61-2H).^{85,86} Termed the Soluvia Micro-Delivery System, it was licensed exclusively by Sanofi Pasteur⁸⁷ for certain vaccine applications.



Figure 61-2 Devices for Smallpox Delivery and Classical Intradermal Vaccination. (A) Vaccinostyle (no longer used for smallpox vaccine), which scratches the skin before or after applying liquid vaccine. (B) Rotary lancet (no longer used for smallpox vaccine), twirled between thumb and fingers to abrade skin. (C) Surgical needle (no longer used for smallpox vaccine), pressed parallel to skin in multiple-pressure method. (D) Pronged end of bifurcated needle (full length, 5 to 7 cm), the current preferred device for smallpox vaccination, holds between its tines approximately 2.5 μ L fluid by capillary action. (E) Intradermal nozzle of Ped-O-Jet⁶⁰⁷ multiuse-nozzle jet injector (no longer used, see Figure 61-5C) (Keystone Industries), showing 0.127-mm-diameter orifice bored into inset sapphire. Recessed cone in the nozzle directs jet stream across a short air gap at ~45-degree angle into skin. (F) "Kuchiki needle" multi-tined applicator for administration of Japanese BCG vaccine by a method termed *percutaneous* delivery.⁷² (G) Traditional Mantoux method for creating intradermal wheal using 26-gauge hypodermic needle and conventional 1-mL syringe. (H) Prefilled version of Soluvia mini-needle intradermal syringe (BD Micro-Delivery System; Becton, Dickinson and Co.⁸⁴) used for intradermal delivery of Sanofi Pasteur⁸⁷ brands of inactivated influenza vaccine (Intanza, IDflu,⁹⁴ Fluzone Intradermal⁹⁷). (From Kis EE, Winter G, Myschik J. Devices for intradermal vaccination. Vaccine 30:523-538, 2012 [Fig. 3, p. 526].) (H inset) The 30-gauge staked mini-needle projects 1.5 mm beyond its hub to limit the depth of injection upon perpendicular insertion into the skin. Marketing of vaccine-device combination product approved in European Union in 2009 and in United States in 2011. (I) Investigational adaptor for conventional tuberculin syringe-needle for quick and consistent Mantoux intradermal injection (SID Technologies,¹⁰⁷ West,¹¹¹ PATH¹⁰⁸), to be used for rabies vaccination in the developing world,¹⁰⁸ among other indications.¹¹² The fixed gap of < 1.0 mm underneath the "Ski-tip" guide manipulates the skin to optimize needle placement regardless of bevel orientation. (J) Investigational new version of Tropis needle-free intradermal jet injector (PharmaJet⁹²). (J inset) Unfolded cocking "wings" are used to compress its metal spring between injections. Original model cleared by FDA for US marketing in 2011. (K) Investigational Bioject ID Pen needle-free jet injector for intradermal delivery of 0.1 mL (0.05-mL-dose model not shown) (Bioject Medical Technologies⁸³). Powered by metal spring cocked with built-in lever. Disposable polypropylene spacer on disposable syringe creates the desired air gap to weaken the jet stream for intradermal delivery. (Figure 61-2A, B, C, E, G, courtesy of James Gathany, Greg Knobloch [CDC Photographic Services]; 61-2D, I, courtesy of Bruce G. Weniger; 61-2F, courtesy of Japan BCG Laboratory;⁷³ 61-2H, courtesy of Sanofi Pasteur;^{27,87} 61-2J, courtesy of PharmaJet;⁸² 61-2K, courtesy of Bioject Medical Technologies.⁸³)

The Soluvia's first major clinical trial (although unidentified in the publication) was for the ID trial arm using an investigational GlaxoSmithKline (GSK) influenza vaccine.⁸⁸ Later, Sanofi Pasteur undertook a series of clinical trials with its own trivalent, inactivated influenza vaccine,⁸⁹⁻⁹³ which led to marketing approval in Europe in 2009⁹⁴ for ID delivery of its Intanza and IDFlu products.⁹⁵ These contained either 9 μ g of viral hemagglutinin per strain per 0.1 mL for adults through age 59,^{92,93,96} or a full (non-dosage sparing) 15 μ g for those 60 and older.⁸⁹⁻⁹¹

In the US trials cited in the product insert,⁹⁷ Sanofi Pasteur's US-made Fluzone Intradermal product, containing 9 μ g per strain, was found to induce geometric mean titers (GMTs) of hemagglutination-inhibiting antibody that were non-inferior to those of control patients receiving conventional Fluzone by the IM route with 15 μ g per strain. In 2011, the US Food and Drug Administration (FDA) licensed the vaccine and its unique pre-filled delivery system, with an indication that it be used only for patients 18 to 64 years of age. Several other countries (eg,

Canada, Australia, New Zealand) have also licensed a Sanofi influenza vaccine in the Soluvia mini-needle delivery system.

A plastic, non-prefilled, empty, sterile version of the Soluvia mini-needle syringe is potentially available to others for end-user filling.⁹⁸ Detaching its hub exposes the full needle length to access conventional vials. Potential applications include post-exposure rabies prophylaxis in the developing world, for which a clinical trial demonstrated protective seroconversion comparable to a full dosage by the IM route⁹⁹ (see "Other conventional vaccines", later), as well as delivery of protein-based therapeutics,¹⁰⁰ among others.⁴⁰

A 34-gauge (OD, ~ 0.178 mm) version of the Soluvia, without the bulky plastic emballage required to shield the needle for health workers, is sized for preclinical animal experiments. These produced good immune responses to anthrax recombinant protective antigen (rPA),^{101–103} conventional hemagglutinin and plasmid DNA antigens for influenza,¹⁰⁴ and live recombinant yellow fever vector for Japanese encephalitis vaccines.¹⁰⁵ Rabbits immunized intradermally and challenged with about 100 LD₅₀ of *Bacillus anthracis* spores had survival rates (no adjuvant, 100%; aluminum salt adjuvant [alum], 100%; CpG, 83%) that were identical to those of IM-immunized controls.¹⁰¹ Rhesus macaques were protected from aerosol challenge with lethal dosages of anthrax, botulism, plague, and staphylococcal pathogens or toxins.¹⁰³

Adapter for Mantoux injection

A novel syringe adapter (see Figure 61-2I), designed for quicker insertion and improved consistency over the traditional Mantoux technique (see Figure 61-2G), guides the needle to its appropriate position in the skin to produce the desired bleb. In human trials conducted by PATH (once known as the Program for Appropriate Technology in Health),¹⁰⁶ the adapter's Luer interface was fitted to conventional 1.0-mL syringe, and injections of 0.1 mL produced desired blebs in 100% of 20 bevel-up and 20 bevel-down injections, yielding mean diameters of 9.3 mm, ± 0.9 mm SD (range, 7 to 12 mm), with ID deposition confirmed by ultrasound in all patients.^{107–109} The device was developed by SID Technologies,¹¹⁰ with financial and technical support from the Centers for Disease Control and Prevention (CDC), West Pharmaceutical Services,¹¹¹ and PATH,^{106,112} which has rights in the developing world for rabies vaccination and other applications. At licensure, West will manufacture and market the adapter in the United States and other developed countries.

Other intradermal vaccines

In addition to smallpox and BCG, and a combined BCG-and-smallpox vaccine,^{113,114} over a dozen other vaccine types have been administered intradermally.

Influenza

A substantial literature documents equivalent immunogenicity, occasional superiority, and, less commonly, lower responses to influenza vaccination by the ID route using needle-syringe compared with larger dosages by the SC and IM routes.¹⁷⁴ Studies took place in two eras. The first started in 1937 with a report by Thomas Francis (of Salk polio vaccine trial fame)¹¹⁵ and extended until 1979, when the last two of the 1976–77 season's influenza A/New Jersey/76 (swine flu) papers^{116,117} were published. Of these, 19 indicated equivalence or superiority,^{115,116,118–134} but not with the sample sizes and analytical rigor of modern clinical trials. Six studies found the ID route less immunogenic than the SC or IM route for some or all of the antigens studied,^{117,135–139} but some of these had attempted 10 to 1 dosage sparing.

When the ID route was compared with either the IM or the SC route using identical amounts of reduced antigen, the results conflicted with those of mid-century trials using the whole-cell products of that era. Bruyn and colleagues found GMTs in children receiving 0.2 mL intradermally of influenza vaccine to be

higher than in those receiving the same dosage subcutaneously,¹²¹ as did Davies and coworkers¹⁴⁰ and Tauraso and colleagues¹³¹ administering 0.1 mL by both routes. When administering by the ID route, one-tenth (0.1 mL) the SC dose (1.0 mL) in varying dilutions below the labeled dosage of 800 chick cell agglutinating (CCA) units/mL, Stille and coworkers also found greater ID responses, but only when the SC dosage was low, at 8 or 0.08 CCA (ID dosage: 0.8 and 0.008, respectively).¹²⁷ In contrast, SC responses exceeded ID ones when the standard SC dosage was used or reduced by only one log (80 CCA; ID, 80 and 8 CCA, respectively). This suggested a linear ID dosage-response curve, but a sigmoid SC one, which favored the ID route at the lower-dosage end. On the other hand, when identical reduced dosages for a new shifted "Asian" strain were given by the two routes (80, 40, or 20 CCA, compared with 200 per full 1.0 mL), both McCarroll and colleagues,¹⁴¹ studying hospital employees 18 to 65 years of age, and Klein and coworkers,¹⁴² studying infants 2 months to 5 years of age, found little difference in responses between the ID and SC routes. McCarroll speculated that the ID superiority in earlier studies was the result of an anamnestic effect not present that season. Klein simply doubted any ID superiority when equal volumes are used.

Regarding systemic reactions, among 101 infants from 2 months to 2 years of age receiving 0.1 mL of influenza vaccine in the study by Klein and Huang, febrile reactions were reported among 34.7% (17/49) in the intradermal group and only 19.2% (10/52) in the SC group getting the same reduced dosage.¹⁴² Similarly, local reactions of small areas of erythema and induration with slight tenderness and itching within 2 to 3 days were described for "all" intradermal participants (ages 2 month to 5 years, *N* = 96), whereas only 2 of 94 children vaccinated by the SC route had local pain and induration. Considering the entire reduced-dosage, ID influenza literature, this route might be considered when antigen shortages and distributive equity demand the use of the lower end of the dosage-response curve, where ID may outperform the SC or IM route. The increased reactions described in these whole-virus studies would perhaps be mitigated by use of today's less reactogenic split-virus products.

Twenty-five years after the final mid-20th-century ID influenza studies, two papers were published simultaneously in 2004,^{88,143} soon after several national shortages¹⁴⁴ had revived interest in dosage sparing.^{145,146} Among 240 hits on literature searches through May 2011 for intradermal influenza vaccination studies published since 1950, Young and Marra¹⁴⁷ culled 205 that reported on nonseasonal vaccines (such as avian H5N1 or pandemic H1N1), or were duplicates or otherwise inappropriate. From the remaining 35, they excluded 22, which were either animal studies, were nonrandomized, used obsolete whole-virus antigen, or assessed immunity outside the selected window of 21 to 28 days after vaccination. They comprehensively compared the remaining 13 reports of split-virus studies, all from 2004 onward among adults 18 years of age and older.^{88–93,96,143,148–152} As in 20th-century reports, Young and Marra¹⁴⁷ found dosages 40% to 80% smaller by the ID route in most studies to be comparably immunogenic with full 15-µg dosages given intramuscularly (seven of eight in the 18- to 60-year age range, four of six trials among those older than 60 years). ID superiority was found (without dosage sparing^{90,103}) in the remaining two studies in the older group. As usual, local reactions were consistently more frequent by the ID route.

Among published 21st-century ID influenza studies not included in the Young and Marra¹⁴⁷ review was a study of children in Hong Kong given 2005–06 seasonal trivalent vaccine (Fluarix, GSK).¹⁵³ It found 0.1 mL ID dosages to be comparably immunogenic to full 0.5-mL IM ones, with increased but tolerable induration and erythema after ID delivery. Another study in Texas administered investigational, monovalent avian A/H5N1 antigen to adults in dosages of 3 and 5 µg by the ID route, and 15 and 45 µg by the IM route.¹⁵⁴ All dosages less

than 45 µg by either route induced very poor responses, whereas 45 µg by the IM route induced a fourfold titer rise and titers of 40 or greater in 56% after two doses.

One multicenter study encompassed a range of four dosages of hemagglutinin per vaccine strain of the 2004–05 formulation of Fluzone, comparing 15 µg by IM needle, 9 and 6 µg intradermally by Soluvia mini-needle syringe, and 3 µg by Mantoux injection.¹⁵⁵ By GMT, the 6- and 9-µg ID doses were non-inferior to the IM control for all three strains, but the 3-µg ID dose was non-inferior only for the A/H3N2/Wyoming strain. Other studies of the Fluzone ID vaccine delivered by Soluvia mini-needle^{89–93} were described above (see “Reinventing the wheel”, earlier).

When low-dosage ID vaccine is compared only with full-dosage by the IM route, it cannot be ascertained whether a low dosage into the muscle (usually with fewer local reactions) would have performed as well. Belshe and colleagues addressed that question by adding a third arm to a trial of low-dosage influenza vaccine by traditional Mantoux injection by the ID route, versus control. They found that low dosages by either the ID or the IM route were almost as immunogenic as the full-dosage IM control.¹⁴⁹

Poliomyelitis

In Salk's first clinical trials with inactivated polio vaccine, it was administered by the ID route,^{156,157} which was routinely used for millions of Danes in the mid 1950s,^{158,159} and responses in studies were good up to the early 1990s.^{160–164} As polio eradication nears its goal, it will be necessary to remove from circulation the live Sabin strains of oral polio vaccine (OPV), with their propensity to revert to virulence and to circulate from vaccinees to others, and to replace them with injectable, inactivated polio vaccine (IPV). However, the latter vaccine in full 0.5-mL dosages costs about 20 times as much per dose as OPV, promoting a search for cost-saving strategies that also avoid the introduction of needles into the polio eradication program.¹⁶⁵

Recent clinical research in Oman sponsored by WHO and others found seroconversion rates equivalent to full dosages given intramuscularly when IPV was delivered intradermally by needle-free jet injectors in 0.1 mL dosage-sparing volumes into the skin to infants at 2, 4, and 6 months of age,¹⁶⁶ but GMTs were consistently lower. At the earlier ages of 6, 10, and 14 weeks, studied in Cuba, the ID responses were somewhat lower, perhaps from maternal antibody interference.¹⁶⁷ A study in the Philippines compared one-fifth dosages by Mantoux injection with full dosages by the IM route at 6, 10, and 14 weeks of age, finding inverse titers of 8 or greater to all three types in 99% to 100% of all participants, concluding that the reduced-dosage ID route was non-inferior to the IM route.¹⁶⁸

An Indian study of older children, 6 to 9 months of age, using a different investigational jet injector for ID delivery, deemed more than half of the injections “inadequate” because of a wheal diameter of less than 3 mm, or because more than a “small drop” of vaccine remained on the skin surface.¹⁶⁹ Overall, seroconversion rates and GMTs to all polio types were lower by reduced-dosage by the ID route than by full IM dosages, especially for such “inadequate” injections. Another study in the Netherlands of the same device is underway. More are planned.¹⁷⁰

Yellow fever

The ID route was used extensively for the live attenuated yellow fever French neurotropic vaccine, which was given by ID scarification in the 1940s and 1950s in Francophone Africa (see Chapter 38).¹⁷¹ The 17D strain showed both good¹⁷² and poor¹⁷³ immune responses when jet-injected by the ID route. A recent review discussed evidence for dosage-sparing equivalence in skin using one-fifth the usual dosage.¹⁷⁴

Other conventional vaccines

Inactivated vaccines with good immune responses after ID injection include typhoid¹⁷⁵ and rabies.^{99,176–183} The latter has been used widely for dosage-sparing purposes in the developing world.^{184,185}

Generally good results have been reported for ID hepatitis B,^{186–192} with exceptions when antigen mass was prepared by a 10 to 1 reduction instead of the more common 5 to 1 reduction for the ID route,¹⁹³ in infants,^{194–196} and with recombinant vaccine.^{197–199} A recent meta-analysis among five comparable, randomized clinical trials totaling 757 subjects (in 234 published studies) found a “slight” (14%) decrement in seroprotection rates for hepatitis B by the ID route compared with the IM route.²⁰⁰ In contrast, another meta-analysis found hepatitis B by ID route somewhat more immunogenic than by IM route among dialysis patients.²⁰¹

For meningococcal disease, one 1972 paper on group A vaccine²⁰² and unpublished data posted at ClinicalTrials.gov for a 2002 to 2004 study of the modern, non-protein-conjugated A/C/Y/W-135 combination (Menomune)²⁰³ found good results. These two are the only reported studies of any polysaccharide vaccines (including *Haemophilus influenza* type b, and conventional or conjugated pneumococcal) by the cutaneous route.

Mixed results for the ID route have been reported for cholera²⁰⁴ and hepatitis A vaccines.^{205,206} Other nonliving antigens studied rarely by this route include diphtheria-tetanus-pertussis,^{207,208} tetanus,^{209,210} tetanus-diphtheria,²¹¹ tetanus-typhoid,^{212,213} tick-borne encephalitis,^{214,215} and Rift Valley fever.²¹⁶ Similarly mixed results were found for live measles vaccines by the ID route.^{217–229}

Investigational vaccines

In the mid to late 2000s, the ID route was pursued for a wide variety of investigational vaccines, including dengue,²³⁰ human immunodeficiency virus (HIV),²³¹ malaria,²³² and tuberculosis.²³³ The ID route—as well as the IM—had led to the serendipitous discovery in an influenza model²³⁴ that viral genes encoded into bacterial DNA could express their protein antigens in vivo, a seminal event in the modern era of recombinant nucleic acid vaccinology.²³⁵ Gene proto-antigens to prevent influenza,²³⁶ HIV or acquired immunodeficiency syndrome (AIDS),^{237,238} smallpox,²³⁹ and many other diseases are being inserted into both naked DNA/RNA²⁴⁰ and various vectors such as modified vaccinia Ankara virus, for delivery by the ID route. ID jet injection has been used for immunomodulators such as interferon.²⁴¹

Novel methods to deliver antigen into the skin

Various commercial patch delivery systems developed since 1981 have demonstrated the ability of certain therapeutic agents (eg, scopolamine, nitroglycerin, clonidine, estradiol, fentanyl, nicotine, testosterone) to diffuse passively into bare, untreated skin without the use of the active technologies or enhancers described in the following paragraphs.⁵⁴ However, such passive diffusion usually works only for small molecules with certain physical characteristics. Thus, there are but a few animal models of immunization onto bare, untreated skin.^{242–244} Newer methods to facilitate antigen delivery to the epidermis involve painlessly stripping, abrading, scraping, piercing, vaporizing, shocking, vibrating, bombarding, and otherwise permeabilizing the barrier of the stratum corneum. Some methods combine several processes. These have been detailed in reviews by others.^{27,29,31,33,35–37,39–43,54,60,245–248}

Stripping and abrading

Tape and friction

A variety of simple tools have been used to remove the stratum corneum. Common cellophane adhesive tape may be applied to the skin and pulled away, carrying away dead keratinocytes with each repetition. Such tape-stripping has been shown to enhance cytotoxic-T-cell and cytokine immune responses on subsequent application of various antigens and adjuvants to

the skin in mice.^{249–255} Similarly, rubbing gauze, emery paper, electrocardiographic (ECG) pads, or pumice on the skin removes cells by their abrasive effects, and this has been found to enhance immune responses in humans.²⁵⁶ Application of cyanoacrylate glue followed by stripping the skin to apply antigen to the exposed hair follicles has been described,²⁵⁷ but its practicality has been questioned.³⁷

Skin preparation system and transcutaneous immunization

Among methods that strip the skin, perhaps the most advanced is one that combines this step with the use of a remarkably potent adjuvant, the heat-labile enterotoxin (LT) of *Escherichia coli* (see “Bacterial exotoxins”, later). This effort was originally championed by Gregory M. Glenn, first at the Walter Reed Army Institute of Research, then at Iomai Corporation, and later at Intercell.²⁵⁸

The vaccinator or the patient holds against the skin a device, the Skin Preparation System, developed by Ideo (Figure 61-3A).²⁵⁹ With the push of a button and the pull of a tab, a controlled pressure is applied to a sandpaper strip, which gently abrades and removes about 25% of the stratum corneum.^{260,261} Then, a patch containing LT as antigen alone, or containing LT as an adjuvant for another antigen, is applied to the skin; the process is called *transcutaneous immunization*.^{262–265} LT alone is intended to induce immunity against enterotoxigenic *E. coli* (ETEC), the cause of traveler's diarrhea, or against *Vibrio cholera*, with²⁶⁶ or without^{267,268} ETEC colonization factor.

An initial, randomized, blinded field trial among travelers to Guatemala and Mexico found 75% efficacy for the patch with LT alone in protecting from moderate to severe diarrhea.²⁶⁹ In 2010, Intercell reported mixed results from two follow-up field studies.^{270,271} In a pivotal phase 3 trial for travelers diarrhea

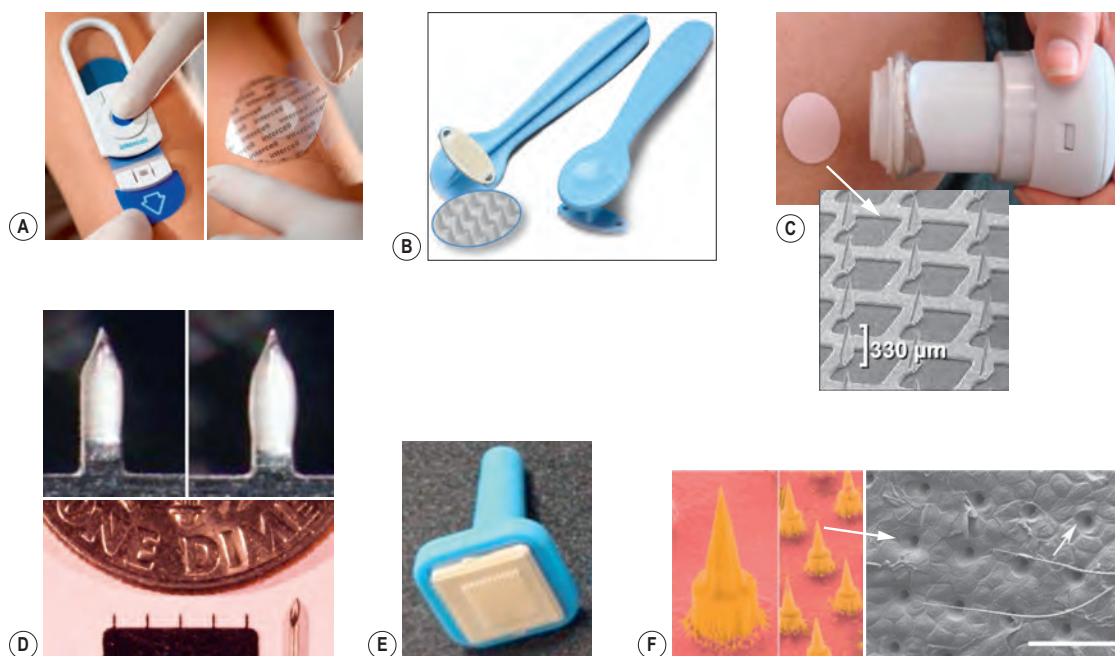


Figure 61-3 Investigational Devices for Disrupting Stratum Corneum by Friction or by Penetration with Uncoated or Coated Solid Microneedles, for Potential Cutaneous Vaccination. (A, left) Investigational Skin Preparation System (SPS) for *transcutaneous immunization* (Intercell AG,²⁵⁸ originally developed by Iomai Corporation). Blue push-button requires the correct amount of abrasion pressure on the stratum corneum by a sandpaper-like strip pulled with the blue tab. (A, right) After the skin is abraded, the vaccine or adjuvant-containing patch is applied within guide marks of the temporary dye (not shown) left by the SPS to indicate the pretreated area. (Photographs by Andi Bruckner [www.andbruckner.com] for Intercell AG, with permission; from Kim YC, Jarrahian C, Zehrung D, et al. Delivery systems for intradermal vaccination. *Curr Top Microbiol Immunol* 351:77–112, 2012 [Fig. 4a2-3, p. 97].) (B) 3M Microchannel Skin System is an uncoated microneedle device licensed in the United States in 2011 and elsewhere “to create microchannels in the skin” for dermatologic or other medical use.^{295,296} The device contains 351 solid microneedles (B inset).^{297,298} (C) Investigational Zosano Pharma ZP Patch (formerly Macroflux) applicator and patch. (C, inset) Scanning electron microphotograph of titanium tines, 330 µm in height, embedded in the patch, coated with drug or antigen, and applied into the skin. (From Sachdeva V, Banga AK. Microneedles and their applications. *Recent Pat Drug Deliv Formul* 5:95–132, 2011 [Fig. 2B, p. 105]. Inset from Matriano JA, Cormier M, Johnson J, et al. Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm Res* 19:63–70, 2002 [Fig. 1B, p. 64].) (D, top) Investigational BCG-coated microtines after 6 (D, left) and 9 (D, right) coating cycles (Georgia Institute of Technology³²³), by brightfield microphotography. (D, bottom) An array of five such microtines compared with a 26-gauge hypodermic needle and a US 10¢ coin, 18 mm in diameter. (From Hiraishi I, Nandakumar S, Choi S-O, et al. *Bacillus Calmette-Guérin* vaccination using a microneedle patch. *Vaccine* 29:2626–2636, 2011 [Figs. 1 and 3Aii,iii, pp. 2629, 2630].) (E) Investigational Press&Patch finger-thumb device for applying (< 30 sec) the solid Microstructured Transdermal System (sMTS) containing drug-coated or uncoated microneedles. 3M Corporation.^{297,319–321,294} (F, left, and F, middle): Investigational Nanopatch³⁴⁷ microneedle array of silicon, after application to mouse skin. Microprojections are 30 µm wide at base and from 65 to 110 µm in height, and sputter-coated with 100 nm of gold. The red coating of antigen/adjuvant elutes to reveal the original gold coating. (From Prow TP, Chen X, Prow NA, et al. Nanopatch: targeted skin vaccination against West Nile virus and Chikungunya virus in mice. *Small* 6:1776–1784, 2010 [Fig. 1-g/h, p. 1777].) (F, right): Cryogenic scanning electron micrograph of projection holes produced in mouse ear skin by Nanopatch. White arrow shows the indentation left by the shoulder on the microprojection. Scale bar, 100 µm. (From Crichton ML, Ansaldo A, Chen X, et al. The effect of strain rate on the precision of penetration of short densely-packed microprojection array patches coated with vaccine. *Biomaterials* 31:4562–4572, 2010 [Fig. 3D, p. 4565].) (Figure 61-3A, left and right, Andi Bruckner, Intercell AG;⁴³ 61-3B, E, 3M Corporation;²⁹⁴ 61-3C, Zosano Pharma;²⁹¹ 61-3C inset, Zosano Pharma;³⁰⁶ 61-3D, top left and right, Georgia Institute of Technology;³²³ 61-3D bottom;³³⁵ 61-3E, 3M Corporation;²⁹⁴ 61-3F, University of Queensland/Vaxxas.^{351,344})

($N = 2,036$), again in Guatemala and Mexico, the trial's primary target endpoint of greater than 60% efficacy against moderate to severe ETEC diarrhea was not met, finding only about 35% protection. Nor was there an effect on the frequency of all causes of diarrhea. However, there was a 60% reduction in the incidence of LT-positive diarrhea of all degrees of severity, along with a significant reduction in duration and severity of all diarrhea causes. The patch also induced measurable immune responses and was well tolerated.^{270,272}

In a smaller phase 2 trial in India ($N = 723$),²⁷¹ the LT patch also did not reach its targeted endpoint, perhaps because of a low attack rate (about 1%) for LT-positive ETEC. As a result of these two trials, Intercell discontinued work on the LT patch for traveler's diarrhea but still pursues its use with the Skin Preparation System device for other applications.

Applying the Intercell LT patch near the site of injection of parenteral influenza vaccine (an application referred to as a vaccine-enhancement patch) was found to improve hemagglutination inhibition (HI) titers in the serum and mucosa of both young and aged mice,^{273,274} and to increase the HI titer or show an improving trend for adult volunteers older than 60 years.²⁷⁵ In May 2011, a partnership between Intercell and GSK²⁷⁶ began enrolling 300 volunteers for a study to compare the patch with AS03 adjuvant in boosting responses to pandemic H5N1 influenza vaccine.²⁷⁷

In preclinical studies of other applications, use of LT or a structurally similar cholera toxin as cutaneous adjuvants resulted in improved immune responses or challenge protection in animal models for tetanus,²⁷⁸ anthrax,^{279,280} malaria,²⁸¹ *Helicobacter pylori*,²⁸² and Shiga toxin-producing strains of enterohemorrhagic *E. coli*.²⁸³

In regard to safety, early clinical trials found no serious reactions,²⁶⁷ but pruritus and maculopapular rash at the patch site were found in 13%,²⁷⁵ 74%,²⁶⁶ or 100%²⁶⁸ of patients exposed to LT-containing patches for 6 hours, and in one study 17% of rashes progressed to vesicle formation.²⁶⁸ Delayed-type hypersensitivity contact dermatitis was observed when using recombinant colonization factor.²⁶⁶ Later clinical trials found the LT patch to be "well tolerated and consistent with previous studies".^{270,272}

Microrasps

Other methods take advantage of low-cost fabrication techniques adapted from the microelectronics industry to convert silicon, metal, or other material into arrays of micrometer- to millimeter-size microrasps designed to abrade the stratum corneum (as distinct from creating holes in it; see "Poking and piercing", later).^{31,35,39–41,99,284} One example is the *microenhancer array* (MEA, also known as Onvax), an investigational technology that scrapes the skin before or after topical application of the antigen or therapeutic agent.^{84,285} The MEA consists of a square or round chip containing about 1-cm² area of silicon or plastic microprojections mounted on a finger-held applicator.^{22,101,247}

Preclinical studies of the MEA device using mice inoculated with hepatitis B surface antigen (HBsAg) or DNA plasmids encoding firefly luciferase found similar or greater immune responses or light emission, respectively, compared with control IM and experimental ID injections. Anthrax rPA with alum or CpG adjuvants applied with the MEA device to mouse skin produced equivalent or better immune responses than IM controls (although not as good as an ID microneedle), whereas immune responses and challenge survival were significantly less among MEA-immunized rabbits compared with IM controls.¹⁰¹ Among cynomolgus monkeys vaccinated by six "swipes" of the MEA, with SC and 34-gauge, microneedle-based ID controls, all animals seroconverted to an investigational recombinant Japanese encephalitis vaccine.¹⁰⁵ Those vaccinated by swiping the MEA through a drop of vaccine already on the skin showed neutralizing antibody responses in the same range as the SC controls, whereas applying vaccine after the abrasion appeared to be less effective.

A clinical trial of the MEA measured transepidermal water loss (TEWL) as a surrogate indicator for removal of the stratum corneum after each of five consecutive swipes across the same site on the volar forearm of volunteers. Projection heights of 100, 150, and 200 μm showed steadily increasing rates of TEWL, with the tallest projections producing the greatest water loss. Control swipes with fibrous and sandpaper ECG pads showed little or no TEWL.²⁸⁵ A human trial, however, in which rabies vaccine was applied before or after four "rubs" of the device over four separate deltoid skin sites did not detect any immune response after three dosings on days 0, 7, and 21.⁹⁹

Shaving and brushing

The razor and the brush can also remove layers of the stratum corneum. In a clinical trial of adenovirus vectors encoded to express influenza hemagglutinin antigen, the abdominal skin of 24 adults was shaved with a disposable, twin-blade razor, followed by "gentle brushing with a soft-bristle toothbrush for 30 strokes" and application of the antigen with an occlusive Tegaderm patch.²⁸⁶ Two doses 28 days apart at the highest dosage level produced fourfold rises in HI titer with 67% of the cutaneous vaccines (there was no control group receiving conventional parenteral delivery of either the recombinant vaccine vector or a licensed inactivated influenza vaccine). Occasional mild erythema at the abdominal site was reported in 61% and rash or itching in 39% of patients.

This same research team,²⁸⁷ studying mice, substituted an electric trimmer for shaving but otherwise used similar brushing to demonstrate that topical application of nonreplicating *E. coli* vectors overproducing antigens for *Clostridium tetani* and *B. anthracis* were immunogenic.^{288,289} Control animals demonstrated that depilation alone had little effect; what made the difference was the mild brushing, which produced minimal irritation (Draize score, 1).²⁹⁰ Others studying Japanese encephalitis vaccine in an animal model supplemented skin shaving with a commercial depilatory cream, followed by occlusion of the site with an impermeable covering.²⁹¹ The practicality of such steps in routine immunization of humans is uncertain.

Poking and piercing

As with cutaneous vaccination in general, a diverse terminology is applied to microscopic projections for perforating the superficial skin to deliver the drug.^{31,41,43,247,248,292,293} In addition to the most common term *microneedles*, terms such as *microblades*, *microknives*, *micropins*, *microtines*, *microtubes*, and *nanopatches* have been used. This chapter uses *microneedles* for the broad category of all such projections shorter than 1,000 μm , reserving *mini-needles* for those of 1 mm or longer, whether solid or hollow (see "Mini-needles" and "Microrasps", earlier). The following sections divide microneedles into functional subcategories.

Uncoated microneedles

Earlier, we described methods in which vaccine or drug is applied to the site after it is prepared. The 3M Corporation²⁹⁴ developed an uncoated microneedle device to prepare the skin by perforating it. Although not licensed (or even intended) for vaccine or drug, its *3M Microchannel Skin System* of microneedles appeared on the US market in 2011 as a "pretreatment method for professional medical or cosmetic dermatologists to create microchannels in the skin" (see Figure 61-3B).^{295,296} Each application creates 351 holes through the stratum corneum into the epidermis.^{297,298} Other investigational technologies for uncoated microneedles are the MicroCor,^{299,300} the Functional MicroArray patch,³⁰¹ and the Micro-Trans.³⁰²

Coated solid microneedles

A common strategy pursued by a number of commercial and academic teams to carry antigen across the stratum corneum is to coat it onto solid microscopic projections, which are held

for variable periods of time in the epidermal layer while antigen or other drug elutes and diffuses.^{27,30–32,35,39–43,246–248} To date, only limited published data have demonstrated suitability for human vaccination, in contrast to therapeutic drugs.

One example of drug-coated microneedles that appears closest to marketing approval is the investigational Zosano Pharma ZP Patch platform (formerly known as Macroflux) (see Figure 61-3C).³⁰³ Its titanium projections vary from 225 to 600 μm in height and are packed into an area of 1 to 2 cm^2 at densities from 140 to 650 times per square centimeter. They are inserted by a spring-mounted applicator and held in place by an adhesive patch. The most advanced applications for these microneedles are delivery of parathyroid hormone to treat osteoporosis,³⁰⁴ already studied clinically, and erythropoietin to treat anemia.

Regarding vaccine applications,³⁰⁵ a graph from a human study of Zosano Pharma's ZP-Flu influenza vaccine patch, applied for 5 or 10 minutes onto the skin, trended toward increased titers and seroprotection compared with an IM control injection^{306,307} (no further details were provided, nor could a public clinical trials registration be found).

A hairless guinea pig model was used to study ovalbumin on the patch's microneedles as a representative, large antigenic protein.^{305,308} It was administered in two doses 4 weeks apart. It induced post-booster titers comparable to those of control IM, SC, and ID Mantoux-style injections at higher dosages, and it surpassed IM and SC routes at lower dosages. Other pre-clinical studies of the system demonstrated delivery of oligonucleotides³⁰⁹ and the peptide hormone desmopressin.³¹⁰ The company reports animal work with tetanus, diphtheria, Lyme disease, and hepatitis B (DNA) vaccine antigens.

Another coated-microneedle platform is the *solid micro-structured transdermal system* (sMTS)^{26,311–315} from 3M.²⁹⁴ Its drug-coated pyramidal projections vary from 250 to 750 μm height, in arrays of 300 to 1,500 microneedles mounted on an adhesive patch at a density of 1,300 per square centimeter.^{315–318} Application to the skin is by a manual finger-thumb *PressoPatch* device^{294,297,319–321} (see Figure 61-3E) or by a spring-powered applicator, shown elsewhere.²² Coatings of the microneedles are said to hold up to 0.5 mg of active pharmaceutical ingredient.

In a rabbit model, coatings of tetanus toxoid and alum adjuvant in various ratios induced antibody levels an order of magnitude higher than the presumed protective threshold (> 0.2 IU), using just a fraction of the standard IM dosage.³²² Ovalbumin as a surrogate vaccine applied to hairless guinea pigs by sMTS using the *PressoPatch* applicator was reported to induce antibody, as measured by enzyme-linked immunosorbent assay, equivalent to that induced by IM-needle injection.³¹⁹ A second study using hairless guinea pigs compared three doses of 1.5 μg of HBsAg by sMTS ID and by IM injection; at 8 weeks, after two doses, seroconversion was 100% and GMT was 158 for the ID route, and 20% and GMT 0 for the IM route.³²⁰ After dose 3, seroconversion for IM rose to 80% and GMT to 34, while the ID route remained at 100% and GMT rose to 410. In swine, a model virus-like protein (HBsAg) demonstrated dosage sparing via sMTS compared with antigen delivered by IM control route.³²¹

Experimental placement of the sMTS microneedles device on human volunteers found it to be “well-tolerated” and “non-intimidating and not painful”.³¹⁵ A more recent public registration described a safety trial without antigen.²⁹⁶ Otherwise, no further clinical data were found in public registries or reports.

The Georgia Institute of Technology (GA Tech),³²³ a pioneering center for microneedle technology, has worked with Emory University to conduct numerous studies of coated microneedles^{247,324} in animal models for cutaneous vaccine delivery. In a series of murine studies using solid metal microneedles coated with inactivated influenza viruses, cutaneous vaccination induced robust immune responses—often better than equivalent dosages in controls injected by the SC route—as well as protection against lethal viral challenge.^{325–334}

When coated with BCG, the same microneedle platform (see Figure 61-3D) was highly immunogenic in guinea pigs, with robust cell-mediated responses in lungs and spleen comparable to those with Mantoux injection.³³⁵ Similarly, plasmid DNA antigen for hepatitis C, coated on 500- μm -long needles, primed specific cytotoxic T lymphocytes in vaccinated mice more readily than did typical “gene gun” delivery^{336,337} or conventional needle.³³⁸ Inactivated rotavirus vaccine—developed to avoid the inhibitory effect of breast milk on live, oral vaccines³³⁹—was coated onto this microneedle platform and found immunogenic in an animal model.³⁴⁰

For most of these formulations prepared at GA Tech, a key ingredient of the carboxycellulose matrix of the dried coating was trehalose, one of several sugars, including sucrose, that have been found useful in protecting protein antigens from damage by drying and freezing, and thereby improving vaccine thermostability.³⁴¹

Another center for microneedle research, in Australia,³⁴² developed a novel nitrogen gas jet-drying method for coating antigen onto silicon that overcomes the challenges of dip-coating closely spaced projections,^{324,343,344} but it still elutes within 2 to 3 minutes upon skin entry (see Figure 61-3F). It has achieved 1/30th to 1/100th dosage sparing compared with the IM route in a mouse model for influenza.^{345,346} Other antigens studied with good results in murine models with this platform—called the Nanopatch and recently transferred to industry³⁴⁷—include human papillomavirus,³⁴⁸ herpes simplex type 2,^{349,350} and the West Nile and chikungunya viruses.³⁵¹

Coulman and coworkers studied nanoparticles and DNA plasmids expressing β -galactosidase and fluorescent proteins applied to the epidermal surface of ex vivo human breast skin donated at mastectomy.³⁵² After applying the microneedles to the skin for 10 seconds, they were able to verify epidermal penetration and gene expression by a variety of histologic and photometric means. Later work by this Welsh group reported decreased pain in clinical studies with 180- μm and 280- μm microneedles compared with the 25-gauge conventional needle,³⁵³ as well as morphologic changes suggestive of immune activation in human Langerhans cells after intradermal injection of influenza virus-like particles into excised human skin.³⁵⁴ This group also found that both public and private immunization providers were positive, in focus-group discussions, toward microneedles as a change from conventional needle-syringe delivery.³⁵⁵ Research on and development of coated microneedles for vaccination are also underway by many other groups.^{292,293,302,356}

Dissolving microneedles

An elegant strategy to decrease risk from intentional reuse of, or inadvertent contact with, used microneedles is for the sharps to dissolve in the skin with hydration, thus releasing the antigen.^{32,43,357–361} The most common matrix for dissolvable microneedles hard enough to penetrate skin is carboxymethylcellulose, “generally recognized as safe” for parenteral delivery by the FDA, among other compounds.^{357,358} Chu and Prausnitz molded arrowhead-shaped antigen carriers of blended polyvinyl alcohol and polyvinylpyrrolidone, and mounted them on metal shafts (Figure 61-4A).³⁵⁸ The lower corners of the “arrows” act as barbs to keep the carrier in the skin when the patch is removed, which is done immediately. From the same group at GA Tech and Emory, Sullivan and coworkers encapsulated inactivated influenza vaccine virus into biocompatible polymer, which dissolved within minutes after its application to mouse skin (see Figure 61-4B).³⁵⁹ Robust antibody and cellular immune responses provided complete protection from lethal challenge.

Several sugars, such as trehalose, sucrose, and maltose, have been found to be key ingredients in stabilizing and maintaining the potency of antigen during the process of forming dissolvable microneedles,^{341,362,363} but thermostability studies have not yet been reported to assess whether such formulations would

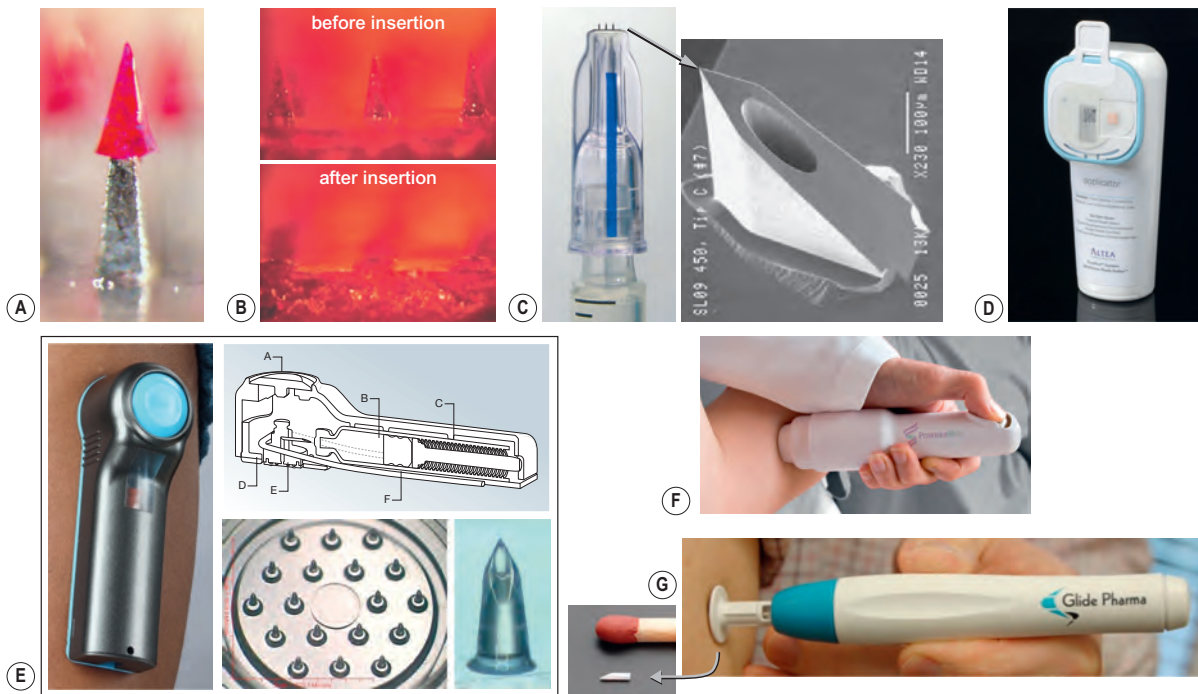


Figure 61-4 Dissolving and Hollow Microneedles, Electromagnetic Devices, and Kinetic Devices for Potential Cutaneous Vaccination.

(A) Investigational dissolving 600- μ m-tall "arrowhead" microneedle composed of polylactic co-glycolic acid (PLGA) encapsulating sulforhodamine B color indicator, atop 300- μ m-tall metal shaft base shaft. (From Chu LY, Prausnitz MR. Separable arrowhead microneedles. *J Control Release* 149:242-249, 2011 [Fig. 6A, p. 247].) (B) Investigational dissolving microneedle of biocompatible formulation material containing sulforhodamine B before (top) and after (bottom) skin insertion, demonstrating disintegration upon exposure to tissue moisture (Georgia Institute of Technology³²³). (C) MicronJet adapter with Luer fitting onto conventional syringe for ID delivery via hollow MicroPyramid microneedles (inset) (NanoPass Technologies Ltd.³⁷¹). Cleared for marketing in the European Union and the United States. The blue line on the hub indicates to the user that the bevel and lumen of the microneedle needle are on the opposite side. (C, inset) Microphotograph shows the pyramidal shape and lumen of an individual microneedle. (From Prausnitz MR, Mikszta JA, Cormier M, et al. Microneedle-based vaccines. *Curr Top Microbiol Immunol* 333:369-393, 2009 [Fig. 4 right, p. 375], and from Van Damme P, Oosterhuis-Kafeja F, Van de Wielen M, et al. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. *Vaccine* 27:454-459, 2009 [adapter, Fig. 1, p. 455; MicroPyramid, Fig. 2A, p. 456].) (D) Investigational PassPort thermoporation device and patch applied to patient arm. Heat induced by the device in metallic filaments embedded in the patch creates micropores in the stratum corneum for subsequent entry of drug within the patch (Altea Therapeutics⁴⁰⁰). (E) Investigational *Hollow Microstructured Transdermal System* (hMTS) injection system (3M Corporation).^{294,313,376,314} (E, left) The wearable device is fixed by adhesive to skin. On activation, prefilled liquid drug is forced over 5 to 40 minutes through lumina of (E, bottom center) 18 microneedles of (E, bottom right) 500 to 900 μ m height. Blue button ("A" in E, upper right schematic) releases spring "C", triggering piercing of glass dose chamber "B" and transfer of liquid into reservoir of patch, which is applied to skin by delivery spring "F", and held in place by adhesive "D". (From Hansen K, Burton S, Tomai M. A hollow microstructured transdermal system (hMTS) for needle-free delivery of biopharmaceuticals. *Drug Deliv Technol* 9:38-44, 2009 [Figs. 1, 2, pp. 38, 40]; and from Burton SA, Ng CY, Simmers R, et al. Rapid intradermal delivery of liquid formulations using a hollow microstructured array. *Pharm Res* 28:31-40, 2011 [Figs. 1, 2, p. 33].) (F) Investigational Particle-Mediated Epidermal Delivery (PMED) device (PowderMed⁴⁴¹) propels (usually gold) microparticles coated with (usually DNA) antigen into skin using a stream of supersonic helium gas. (G) Investigational *Solid Dose Injector* (SDI) from Glide Pharma⁴⁷² is powered by a metal spring, which is compressed and released as the disposable drug cassette (white component extending beyond blue hub) is pressed fully against the skin. It shoots a (G, inset) pointed, hardened, $\sim$ 1-mm-diameter drug formulation (shown compared with conventional matchstick tip) into subcutaneous tissues, where it dissolves.^{473,474} (Figure 61-4A, courtesy of Leonard Chu, Georgia Institute of Technology;^{323,358} 61-4B, courtesy of Georgia Institute of Technology³²³ [Jeong-Woo Lee]; 61-4C, courtesy of Bruce G. Weniger; 61-4C inset;^{41,150} 61-4D, Altea Therapeutics;⁴⁰⁰ 61-4E, 3M Corporation;^{294,313,314} 61-4F, PowderMed;⁴⁴¹ 61-4G, Glide Pharma.⁴⁷²)

resist heat degradation and allow transport and storage outside the cold chain.³⁶⁴ A hydrogel polymer is proposed by Corium International as a binder-cum-adhesive for active pharmaceutical ingredients, and it will dissolve with delivery into the skin.²⁹⁹ In Japan, CosMED markets a cosmetic MicroHyal microneedle array containing hyaluronate, which dissolves in 60 to 90 minutes,³⁶⁵ and vaccine applications are planned. Many others also pursue dissolvable microneedles.^{366,367}

Hollow Microneedles

Hollow microprojections of similar sub-millimeter lengths to those of the solid ones just described are designed to inject therapeutic liquids through their tiny lumens.^{29,40,43,247,368}

(In this chapter, only needles less than 1 mm long are classified as *microneedles*, as opposed to the still-small but longer *mini-needles*, such as those in the Soluvia system [see Figure 61-2H]—see "Reinventing the wheel", earlier.) Although harder to manufacture and more easily broken and clogged,^{247,284} flow rates of microneedles have been measured up to a remarkable 1 mL per minute per cannula.³⁶⁹ Common lengths of 200 to 500 μ m are short enough, in theory, to be painless, as they would not reach nerve endings in the dermis.^{31,353,368,370} However the quite perceptible stretching of skin with the injection of liquid may eliminate any such advantage.

The MicronJet 600 device³⁷¹ is unique in its availability as a licensed, sterile, disposable device for end users to inject liquids

for cutaneous delivery. It consists of three hollow 600- μm -tall microneedles of beveled pyramidal shape mounted on an adapter with Luer interface for fitting onto a conventional syringe for liquid vaccine or drug (see Figure 61-4C). In 2010, it was cleared by the FDA for injection of any drug approved for ID delivery. It also holds a CE mark for marketing in Europe.

Adult volunteers vaccinated intradermally by a similar MicronJet version (four microneedles of 450 μm height each) received reduced 3 or 6- μg -per-strain single doses, or full 15- μg doses by IM route, of licensed alpha-RIX (Fluarix, GSK) 2007–08 seasonal influenza vaccine. By day 21, all three study arms developed comparable increases in GMTs and satisfied European criteria³⁷² for relicensure of seasonal influenza vaccines in full.¹⁵⁰ Local reactions were more frequent than by the IM route, but they were mild and transient. Similar dosage-sparing trials for 2009–10 monovalent H1N1 influenza vaccine³⁷³ and 2010–11 trivalent vaccine³⁷⁴ confirmed comparable or superior immune responses for the ID route versus IM.³⁷⁵

Another hollow microneedle system is 3M's *hollow micro-structured transdermal system* (hMTS).^{26,294,311,313,314,376} Its patient-contact surface contains 18 microneedles of 500 to 900 μm in length, whose lumina of 10 to 40 μm in diameter deliver liquid volumes ranging from 0.3 to 1.5 mL (see Figure 61-4E). A spring-powered device contains liquid drug prefilled into a glass dose chamber. Upon triggering, the stopper of the chamber is pierced by a spike, through which the dose passes and is forced slowly over a period of 5 to 40 minutes through the microneedles into the skin of the upper arm or thigh. Adhesive on the patch keeps the system in place until delivery is complete. Delivery of equine tetanus antitoxin to swine as a model for delivery of monoclonal antibodies resulted in pharmacokinetic profiles of tetanus antitoxin similar to dosages via SC injection.³⁷⁶ Other groups have also pursued hollow microneedles.^{22,302,356}

Tattoo technology

Preclinical studies using commercial cosmetic tattoo machines delivered experimental DNA vaccine antigens on multineedle arrays (eg, nine), vibrating at frequencies of up to 100 Hz for durations of 5 to 20 seconds, resulting in thousands to tens of thousands of skin piercings per dose.^{377,378} Whether such a potentially painful delivery method would be practical, economical, or esthetically acceptable for human vaccination, as well as advantageous over other methods for cutaneous delivery, remains to be demonstrated.³⁷⁹

Electromagnetic energy

The use of light or electricity, or the heat or radiation they produce, has been pursued to facilitate entry of drug into the skin, either during a brief or constant application of energy, or through the pathways created after a short pulse.

Laser light

Laser light has been used in various ways to breach the stratum corneum. In one technique, a brief pulse ablates this layer, after which drugs are applied directly onto the exposed epidermis, often with an occlusive patch, for the few hours until the stratum regenerates.^{29,35,54,380–383} The LAD (laser-assisted drug delivery) device generates an erbium-doped yttrium-aluminum-garnet (YAG) laser beam whose energy is highly absorbed by skin.^{382,384} It was shown in adult volunteers to facilitate the anesthetic effect of the topical application of lidocaine,³⁸² and it is licensed in the United States and Australia for that purpose.

A new system focuses the laser beam to create 150 pores per activation, with claimed pore diameters of 200 μm and selectable depths of 30, 60, or 90 μm , which should remain in the epidermis, not reaching dermal nerve endings.^{385,386} Another method uses a high-power pulsed laser to create a photomechanical wave that drives particles representing drug

carriers through the stratum corneum.^{387–389} Clinical studies for intended vaccination using all such laser methods have not yet been reported.

Electrophoretics

Iontophoresis—first demonstrated a century ago in rabbits³⁹⁰—involves an electric current to drive charged molecules from an electrode of the same charge toward another of opposite charge located elsewhere on the body.^{31,32,35,54,391–395} Some licensed devices apply this technique for skin anesthesia.^{303,396} A related method is *electro-osmosis*, which induces a flow of solvent to carry uncharged molecules.^{245,397} Voltages greater than 1 volt in themselves increase skin permeability, perhaps by opening up pathways along hair follicles. But these techniques do not work well with larger molecules, which characterize many vaccine antigen proteins.

Thermoporation and electroporation

Thermoporation, also termed *microporation*, uses heat to vaporize tiny openings in the stratum corneum.^{31,32,54,398,399} In the PassPort system,^{26,400} this heat is generated by a disposable array of metallic filaments held momentarily against the skin by a device the size of a computer mouse (see Figure 61-4D). At activation, electric pulses are induced to heat the filaments. An adhesive patch containing vaccine or therapeutic agent is then applied over the micropores just created. In a hairless mouse model, this technique elicited 10 to 100-fold greater cellular and humoral responses to an adenovirus vaccine than intact skin, as well as 100% protection to surrogate tumor challenge (27% for intact skin).³⁹⁸ In the same model, adenovirus-vectored melanoma antigen applied to the micropores roughly doubled the average onset time of tumors after challenge, and it protected one of six mice, compared with none of eight vaccinated controls with intact skin. Microporated recombinant influenza H5 hemagglutinin protected BALB/c mice from challenge with a lethal H5N1 strain.⁴⁰¹ Skin micropores also permitted the passage of insulin in pharmacokinetic human trials with historical controls,^{402,403} and in the other direction allowed interstitial fluid to be extracted for potential glucose monitoring.⁴⁰⁴

Another device similarly generates micropores with heat induced by radiofrequency waves (ViaDerm).^{26,405} A different technique uses short, 100- μsec pulses of superheated steam in microliter amounts to remove the stratum corneum.⁴⁰⁶ Without apparent effect on deeper skin elements in a human cadaver model, this resulted in 1,000-fold in vitro increases in permeability to sulforhodamine B and bovine serum albumin as surrogate molecules for drug or antigen.

Electroporation uses very short electrical pulses to produce in the intercellular lipid matrix of the stratum corneum temporary pores of nanometer-range diameters, which remain open and permeable for hours.^{31,407–412} In vitro and in vivo preclinical studies of this technique demonstrated skin entry of larger molecules, such as heparin (12 kDa), peptides, proteins (such as luteinizing-hormone-releasing hormone), and nucleic acids,^{392,398,413–415} with potentially extensive use for investigational DNA vaccines in animals and humans.⁴¹⁶

The Easy Vax⁴¹⁷ and related Derma Vax epidermal electroporation systems combine the application of antigen or drug-coated 2-mm-long mini-needles, followed by electroporation. Smallpox antigen in plasmid DNA was dried onto the tips of arrays and inserted into the skin of mice, and when followed by six electric pulses, it induced protection from smallpox challenge.⁴¹⁸ A prostate cancer DNA vaccine was similarly administered.⁴¹⁹ Electroporation by the IM route is also pursued to enhance vaccination with DNA antigens.^{412,420,421} A hollow needle injects the drug conventionally into muscle, while parallel solid needles surrounding the injected dose create the current to generate pores in the target muscle tissue. Investigational or marketed products are CythorLab,⁴²² Easy Vax,⁴¹⁷ Electrokinetic Device,⁴²³ ECM,⁴²⁴ MedPulser,^{420,421,425} and TriGrid,^{426,427} among others.

Sound energy

To facilitate drug or antigen delivery, the connection between keratinocytes can be solubilized by ultrasonic waves and short-duration shock waves.^{29,31,32,35,245,428–430} These are theorized to induce cavitation—the formation and collapse of microbubbles—which disrupts the intercellular bilayers in the stratum corneum. Low frequencies (< 100 kHz) appear to work better than the higher frequencies used in therapeutic ultrasound (> 1 MHz). Transdermal tetanus toxoid immunization of mice was enhanced 10-fold compared with the SC route when subjected to ultrasound at 20 kHz.⁴³¹ High-molecular-weight molecules delivered include insulin, erythropoietin, interferon, and low-molecular-weight heparin.^{31,429,432,433} Various groups are pursuing ultrasound for enhanced drug delivery.^{384,434,435}

Kinetic deposition

The transfection of cells by kinetic methods to deposit DNA-coated gold particles into them was pioneered in the 1980s.⁴³⁶ The Helios or PDS 1000/HE gene guns³³⁶ and the Accell injector³³⁷ have become standard bench tools for biolistic delivery of nucleic acid plasmids into a wide variety of plants and animals to transfect them to express the coded genes.^{42,437,438} Delivery of DNA into the skin overcomes the usual polarized T-helper cell type 1 (Th1) response when DNA is delivered into muscle.^{30,439,440} These devices are unavailable for human vaccination (patent rights are held by PowderMed⁴⁴¹). Documenting the safety of DNA as an antigen by any route remains a major regulatory obstacle for such a paradigm shift in human vaccination.³⁰

Powder or particle technology

The proprietary terms *epidermal powder immunization* (EPI) and *particle-mediated epidermal delivery* (PMED) refer to the use of helium gas to blow powdered proteins, polysaccharides, or inactivated pathogens (EPI) or DNA-coated particles (PMED) into the epidermis at supersonic speeds.⁴⁴² This unique method of vaccination was developed in the early 1990s by Oxford BioSciences, which over the years was renamed PowderJect, acquired by Chiron,⁴⁴³ spun off as PowderMed,⁴⁴¹ and finally acquired by Pfizer⁴⁴⁴ in 2006. Delivery is by either reusable (XR series) or single-use disposable (ND series) devices (see Figure 61-4F), with the latter targeted for commercialization.

Conventional protein antigens for delivery by EPI are spray-dried into powders of suitable density and size (20 to 70 μm),^{445,446} but the economics of manufacturing such formulations may be an obstacle.³⁰ For DNA vaccines delivered by PMED, plasmids coding for desired antigens are coated onto gold beads (1 to 3 μm in diameter) and, when deposited into epidermal antigen-presenting cells, they are eluted and transcribed.⁴⁴⁷ A number of preclinical studies in various animal models have been conducted.^{442,445,448,449}

Human trials of DNA vaccines containing up to one order of magnitude less antigen than the amount used for IM routes have induced humoral and cellular immune responses for hepatitis B in subjects both naïve and previously vaccinated with conventional vaccine.^{450–453} PMED vaccination has also been studied for DNA priming in trials of malaria vaccine,^{454,455} has produced seroprotective immune responses by DNA vaccine for seasonal strains of influenza,^{83,456} and has reduced influenza symptoms and viral shedding after human challenge.⁴⁵⁷ Clinical trials still ongoing or unpublished are studying antigens for H5 avian influenza (DNA),⁴⁵⁸ herpes simplex virus 2,⁴⁵⁹ HIV and non-small cell lung cancer.^{460,461}

In the hepatitis B and influenza trials cited earlier, there were no severe local reactions, but erythema, swelling, and flaking or crust formation occurred in nearly all subjects, albeit resolving by day 28. Skin discoloration, however, persisted through day 56 in 29 (97%) of 30 subjects,⁴⁵³ through day 180 in 21 (25%) of 84 injection sites,²³⁶ and beyond 12 months in 5 (25%) of 20

patients with long-term follow-up.⁴⁵³ No anti-double-stranded DNA antibodies were detected. The deposition of the gold particles was studied in pigs, in which most were deposited in the stratum corneum and epidermis and were eventually sloughed by exfoliation by 28 days.⁴⁶² At days 56 and 141 after administration, a few particles remained in the basal epidermal layer and in macrophages in the dermis and regional lymph nodes. Six clinical trials of PMED were initiated in 2006 and reported complete by 2007 or 2008 for delivery of investigational herpes simplex type 2 vaccine; seasonal, pandemic, and trivalent DNA influenza vaccines; and hepatitis B vaccine.⁴⁶³ Results were not yet published as of January 2012.

Preclinical studies of EPI or PMED in murine, porcine, and primate models have shown immunogenicity or protection for either powdered or DNA plasmid antigens for various other pathogens, including Eurasian encephalitic viruses,⁴⁶⁴ hantaviruses,⁴⁶⁵ HIV,^{466,467} influenza H5N1,⁴⁴⁸ malaria,⁴⁶⁸ SARS coronavirus,⁴⁶⁹ smallpox,⁴⁷⁰ and Venezuelan equine encephalitis.⁴⁷¹

Other kinetic and thermal methods

Another delivery method, termed needle-free *solid dose injector* (SDI), is from a British firm, Glide Pharma (see Figure 61-4G).^{26,472–474} It uses a spring-loaded device to quickly push into SC tissue a sharp, pointed, biodegradable “pioneer tip” and the solid or semisolid medication behind it in the chamber—both about the width of a grain of rice.

Microscission involves a stream of gas containing tiny crystals of inert aluminum oxide to bombard small areas of the skin. A mask on the skin limits the sandblasting effect to narrow areas, so channels are created in the stratum corneum to which drug is then applied.⁴⁷⁵ Another method uses a fast and powerful contractile fiber-activated pump to fire drug at the skin with sufficient velocity to penetrate the epidermis.³⁸⁴ A miniaturized form of traditional jet injection uses piezoelectric transducers to propel liquid microjets into the skin.⁴⁷⁶

Adjuvants and enhancers for cutaneous vaccination

As bathers notice in their fingertips, prolonged wetting of the skin, or occluding it to hold in body moisture, produces fluid accumulation in intercellular spaces and swelling of the keratinocytes, which permits enhanced passage of applied agents.²⁵⁶ Rubbing the skin with acetone also enhances antigen passage by extracting epidermal lipids.²⁵¹

Bacterial exotoxins

Discovery of the remarkable adjuvant effect of bacterial ADP-ribosylating exotoxins, such as the B (binding) subunits of cholera toxin and the structurally similar, heat-labile toxin of enterotoxigenic *E. coli*, has prompted much interest in using these to enhance cutaneous delivery.^{39,244,477–484} The group that has progressed the furthest in clinical trials is Intercell,²⁵⁸ the successor to pioneering work begun by the US Army and then by Iomai (see “Skin preparation system and transcutaneous immunization”, earlier). Another group used cholera toxin as an adjuvant when administering influenza vaccine to mice with skin pretreated with microneedles.⁴⁸⁵

For safety reasons, these toxins have been engineered, or mutants selected, to reduce toxicity while retaining adjuvanticity.^{482,486–488} Nevertheless, one such use as adjuvant in a licensed, Swiss-made intranasal influenza vaccine was hypothesized as the cause of temporary paralysis of the seventh cranial nerve (Bell's palsy), prompting market withdrawal.⁴⁶

Chemical, protein and colloidal enhancers

Chemical penetration enhancers under consideration as skin adjuvants, alone or in conjunction with iontophoresis, ultrasound, or electroporation methods, include oleic and retinoic acids,²⁵⁵ dimethylsulfoxide (DMSO), ethanol, limonene, polysorbate, and others.³¹ Flagellin, a bacterial surface component

protein, was engineered to express influenza nucleoprotein epitope and applied to the bare skin of mice, inducing virus-specific interferon-gamma T cells.²⁴⁴ Certain colloids may serve as antigen carriers.³³ Deformable lipid vesicles ("transfersomes") containing tetanus toxoid applied to animal skin yielded comparable immune responses with alum-adsorbed tetanus toxoid given by the IM route.⁴⁸⁹

Combination methods

Other novel methods of delivery include the use of short needles to poke an initial opening into the skin, followed immediately by SC or IM jet injection with much lower pressures than otherwise would be needed.^{490,491}

Jet injection

History and applications

Jet injectors (JIs) squirt liquid under high pressure to deliver medication without needles into targeted tissues (Table 61-1).^{22,492-521} The technology was invented in France in the 1860s (Figure 61-5A),^{492,522,523} a patent was filed for in 1936,⁵²⁴ and it was reintroduced in the 1940s as the Hypospray⁵²⁵⁻⁵²⁷ for patient self-injection with insulin (see Figure 61-5B). In the 1950s, the US military developed a high-speed system (see Figure 61-5C), which was imitated by others (see Figure 61-5D,E,F,G,I), and the units were once referred to as jet guns for mass vaccination programs.⁵²⁸⁻⁵³² Over the past half-century, JIs have been used to administer hundreds of millions, if not billions, of vaccine doses for mass campaigns in humans against smallpox,^{1,533-538} measles,^{533,535,538-541} polio,^{531,542} meningitis,⁵⁴³⁻⁵⁴⁵ influenza,^{546,547} yellow fever,^{533,538,548,549} cholera⁵⁵⁰ and other diseases.^{21,542,543,551-554} During the swine influenza mass campaign of 1976-77 in the United States, a substantial proportion of the approximately 43 million doses administered that season⁵⁵⁵ were by JIs (CDC, unpublished data).^{555,556}

JIs have also been used for a wide variety of therapeutic drugs, including local^{557,558} and pre-general^{559,560} anesthetics,⁵⁶¹ antibiotics,^{562,563} anticoagulants,^{564,565} antivirals,^{566,567} corticosteroids,^{568,569} cytotoxics,⁵⁷⁰ immunomodulators,^{241,571} insulin^{526,572,573} and other hormones,⁵⁷⁴⁻⁵⁷⁶ and vitamins.⁵⁷⁷ Veterinary models for agricultural use are widespread.⁵⁷⁸ In recent years, the devices have been used to administer various antigens to both humans and animal models for a variety of investigational vaccines, including dengue,^{230,579-581} herpes simplex type 2,⁵⁸² HIV/AIDS,^{231,583,584} Japanese encephalitis,⁵⁸⁵ malaria,²³² and melanoma.⁵⁸⁶

Occupational and patient safety, economics

Increasing concern for needle-stick injuries and possible transmission of bloodborne pathogens to health workers, as well as the more expensive needle-shielding syringes that occupational health regulations now require to reduce the risk of injury,⁵⁸⁷ have boosted interest in JIs in developed countries.⁵⁸⁸ Another economic factor is the high cost of proper disposal of highly regulated sharps waste, which is not required for used JI syringes (see "Disposable-syringe jet injectors", later). As the latter may be soiled with blood or tissue fluid, they should be discarded with conventional red-bag medical waste, along with used bandages and similar materials.⁵⁸⁷

For many developing countries, where inadvertent or intentional reuse of nonsterile needle-syringes is a serious problem,^{10,11} modeling indicated significant cost savings for the use of needle-free JIs compared with needle-syringes, especially when the indirect costs of iatrogenic disease resulting from the latter were included.^{589,590} (Current best practice aims for all vaccination syringes in these countries to be auto-disabling to prevent reuse,⁵⁹¹ but this goal is far from achieved.⁵⁹²)

Mechanical and clinical aspects

Designs, power supplies, types

Common features of all JIs include a dose chamber of sufficient strength to hold the liquid when pressurized, a moving piston at the proximal end to compress the liquid, and a tiny orifice (commonly ~ 0.12 mm in diameter, ranging from 0.05 to 0.36 mm)^{495,593} at the distal end to focus the exiting stream for delivery into the patient. The pistons of the majority of modern JIs are pushed by the sudden release of energy stored in a compressed metal spring, and a few use compressed gas such as CO₂ or N₂ (see Table 61-1). Investigational JIs are powered by the expanding pressure of chemical combustion, a technology similar to that found in automotive safety air bags,^{84d,302,594,595} as well as by Lorentz-force electromagnetic induction.^{595a}

The source of energy to compress the spring is usually supplied manually or pedally through an integral or separate tool to apply mechanical advantage or hydraulic pressure. A few use electrical power from batteries or wall (main) electrical current. An experimental JI system controlled by electronic microprocessors has been proposed,⁵⁹⁶ but its cost and practicality for routine immunization remain unknown.

Although devices vary, peak pressures in the dose chambers range from 14 to 35 megapascals (~ 2,000 to 5,000 psi) and occur quite early so that the stream can puncture the skin. After the peak, pressures drop about one third to two thirds during a descending plateau phase until rapid tail-off at the end of the piston's stroke. The velocity of the jet stream exceeds 100 m/sec.⁵⁹⁷ Complete injection lasts about 1/3 to 1/2 a second, depending on the volume delivered, the orifice cross-section, and other variables.

JIs can be classified in many ways: by their energy storage and sources, by intended market (human versus veterinary), by intended usage (eg, repeated self-administration of insulin by the same patient versus vaccination of consecutive patients), by how the dose chamber is filled (medication vial attached "on tool" versus filled "off tool"), by reusability of the entire device (single-use disposable versus reusable), and by reusability of the fluid pathway and patient-contact components (multiuse versus disposable). This last criterion results in a key distinction between multiuse-nozzle jet injectors (MUNJIs) and disposable-syringe jet injectors (DSJIs; once called disposable-cartridge jet injectors), with major implications for immunization safety (see "Safety of multiuse-nozzle jet injectors" and "Disposable-syringe jet injectors", later).

Deposition in target tissues

In vivo imaging indicates that jet-injected medication tends to spread along paths of least resistance in a generally conical distribution.⁵⁹⁸⁻⁶⁰⁴ The depth achieved depends primarily on the power imparted to the liquid, and on variables such as orifice diameter, viscosity of the dose, tautness and thickness of the skin and fat layer, and angle of injection.^{495,496,525,597,599,605,606} Only the SC compartment is reached by many DSJIs designed for self-administration by patients of insulin, hormones, and other drugs, as well as some MUNJIs used in dental anesthesia (eg, Fig. 61-5H).^{607,608}

Most MUNJIs developed for mass vaccination campaigns are powered to reach IM tissues—for example, the Ped-O-Jet⁶⁰⁹ (see Figure 61-5C) and Med-E-Jet⁶¹⁰ (see Figure 61-5E), as well as several new-generation DSJIs. The Biojector 2000 varies the orifice of different cartridges on the same injector to deliver either by the IM or the SC route (Figure 61-6I).^{83,611,612} The PharmaJet⁸² (not shown) varies spring strength of color-coded injectors for IM delivery to different-size patients. For its newer Stratis model, SC delivery is by operator technique to pinch up and inject into the fat layer (see Figure 61-6C). The LectraJet⁶¹³ can also vary spring strength between models (see Figure 61-6A,B). Given

Table 61-1 Historical, Currently Marketed, and Investigational Jet Injectors Used, Studied, or Considered for Vaccination (see footnotes for explanation of abbreviations)

Current/last manufacturer	Device trade name(s)	Year(s)*	Market/primary uses	Energy source/storage	Type	Filling	Target tissue	References
Activa Brand Products ⁵⁰⁰	Preci-Jet, [†] Preci-Jet 50, [†] AdvantaJet GentleJet, Freedom Jet	1984	Hu/In	Ma/Sp	MUNJI	On-F	SC	394, 575
American Jet Injector ⁵⁰¹	Am-O-Jet	1995	Hu/Va	Pe/Sp	MUNJI	On-I	ID, IM	715
Antares Pharma ⁷³¹	Medi-Jector [†]	1972	Hu/Va	Ma/Sp	MUNJI	On-I	M, SC	532, 569
	Medi-Jectors II, [†] III, [†] IV [†]	1980s–90s	Hu/In	Ma/Sp	MUNJI	On-F	SC	604
	Medi-Jector Choice (MJ 6) [†]	1997	Hu/In	Ma/Sp	DSJI	On-F	SC	—
	Medi-Jector Vision [‡] (MJ 7, ZomaJet, SciToJet, Twin-Jector EZ II, Tjet)	1999	Hu/In, Gh	Ma/Sp	DSJI	On-F	SC	572, Fig. 61-6D
	Valeo (MJ 8) [§]	2000s	Hu/In, Gh	Ma/Sp	DSJI	Md, Sd	SC	495
	Medi-Jector MJ 10 [§]	1997	Hu	Ga/Ga	SUDJI	Mf	SC	—
	Vibex [§]	2001	Hu/Va	Ma/Sp	Mini-needle DSJI, SUDJI	Mf, Off	ID, SC	495
	Vaccijet électrique, Avijet	—	Ve/Va	Ba/Sp	MUNJI	On-I, via tube	ID, IM, SC	—
	Vaccijet manuel	—	Ve/Va	Ma/Sp	MUNJI	On-I	ID, IM SC	—
Avant Medical ⁵⁰²	Guardian101 [§]	2002	Hu/Un, Va	Ma/Sp	DSJI	Off	SC	—
Becton, Dickinson ⁸⁴	Velodermic ^{†,§}	1940s	Hu	Ga/Ga (N ₂)	DSJI	—	—	492, 542, 572, 598
Beijing QS Medical Technology Co., Ltd. ^{84a}	QS Jet	2010s	Hu/In	Ma/Sp	DSJI	Off, Md	SC	—
Bio-Curve Beauty & Health Equipment Factory ^{84b}	BC-M7 SMART JET	2010s	Hu/Un	Ma/Sp	DSJI	Off	SC	—
Bioject ⁸³	Biojector 2000	1993	Hu/Va, Av	Ga/Ga (CO ₂)	DSJI	Off	ID [§] , IM, SC	34, 166, 167, 231, 414, 559, 566, 567, 581-584, 586, 611, 612, 614, 638-640, 642, 651, 653, 658, 677, 681-684, 696, Fig. 61-6I
	Vitajet, [†] VitajetII [†]	1984	Hu/In	Ma/Sp	MUNJI	On-F	SC	—
	Vitajet 3 (cool.click, [~] SeroJet, [~] mhi-500, [§] Canine Transdermal Device ^{**})	1996	Hu, Ve/In Gh, Va	Ma/Sp	DSJI	On-F	SC	34, 597, 657
	Iject [§]	2000s	Hu/Un	Ga/Ga (N ₂)	SUDJI	Mf	SC	730, Fig. 61-6K
	Iject R [§]	2000s	Hu/Un	Ga/Ga (N ₂)	DSJI	Mf	SC	730

Table 61-1 Historical, Currently Marketed, and Investigational Jet Injectors Used, Studied, or Considered for Vaccination (see footnotes for explanation of abbreviations)—cont'd

Current/last manufacturer	Device trade name(s)	Year(s)*	Market/primary uses	Energy source/storage	Type	Filling	Target tissue	References
	Vitavax [§]	2004	Hu/Va	Ma/Sp	DSJI	On-F	SC	—
	Vetjet ^{††}	—	Ve/Va	Ma/Sp	DSJI	On-F	SC	503
	Mhi-500 [†]	2000s	Hu/In	Ma/Sp	DSJI	On-F	SC	510
	Bioject Zetajet (once known as Vitavax)	2009	Hu/Va	Ma/Sp	DSJI	On-F	ID [§] , IM, SC	740, Fig. 61-6J
	Bioject ID Pen	2011	Hu/Va	Ma/Sp	DSJI	Off	ID	Fig. 61-2K
Chemical Automatics Design Bureau (CADB) ⁵⁰⁴	BI-1, BI-1M, BI-2, BI-3, BI-3M, BIP-4, BI-8, BI-19, ISI-1, SShA	1960s	Hu/Va	Ma/Sp	MUNJI	On-I	SC, IM	493, 547, 617, 643-646, 668, 689, 690, 714, 729
Consort Medical plc, Bepak division ⁵¹⁰	mhi-500 [†] (InsulinJet [†])	2001	Hu/In	Ma/Sp	DSJI	On-F	SC	—
	SQ-PEN	2002	Hu/In	Ma/Sp	DSJI	On-F	SC	—
	SQ-X	2002	Hu/In	Ma/Sp	DSJI	On-F	SC	—
	MHP-1	2010s	Hu/In	Ma/Sp	DSJI	On-F	SC	—
	cool.click II	2010s	Hu/Ho	Ma/Sp	DSJI	On-F	SC	—
Crossject ⁵⁹⁴	Crossject [§]	2001	Hu/Un	Ch/Ch	SUDJI	Mf	SC, IM, ID	—
	Zeneo [§]	2010s	Hu/Mu	Ch/Ch	SUDJI	Mf	ID, M, SC	84e
D'Antonio Consultants, International (DCI) ⁶¹³	LectraJet HS [§]	1980s	Hu/Va	Ba/Sp	DSJI	Off	ID, M, SC	34, 679, Fig. 61-6A
	LectraVet	1980s	Ve/Va, Mu	Ba/Sp	MUNJI	On-I	IM, SC	—
	LectraJet M3 RA	2011	Hu/Va	Ma/Sp	DSJI	Off	ID, M, SC	34, 654, Fig. 61-6B
EMS Electro Medical Systems ⁵⁰⁵	Swiss Injector [§] , EMS/RPM [§]	1990s	Un/Un	—	MUNJI	On-F	IM	611
	EMS/MPM [§]	1990s	Un/Un	—	MUNJI	Md	IM	611
EuroJet Medical ⁵⁰⁶	E-Jet 500	2003	Hu, Ve/Ho, In, St, Va	Ma/Sp	DSJI	Off	SC	—
	E-Jet 50	2003	Hu/Va	Ma/Sp	DSJI	Off	SC	—
Felton ⁷²¹	BI-100, [§] HSI-500 [§]	1990s	Hu/Va	Pe/Sp	MUNJI	On-I	IM, SC	34, 722
	Pulse 200, 250	1990s	Ve/Mu	Ga/Ga	MUNJI	On-I	IM, SC	—
H. Galante et Compagnie ⁵⁰⁸	Device for l'Aquapuncture [†]	1865	Hu/Mu	Ma/Ma	MUNJI	ON-I	—	523, Fig. 61-5A
Genesis Medical ⁵⁰⁷	Sensa-Jet ^{†,§}	1990s	Hu/Va	Ma/Sp	DSJI	Off	SC	—
Heng Yang Weida Science Technology ⁵⁰⁹	Pro-Jeey 2000	—	Hu/Un	—	—	—	—	—
INJEX – Equidyne Systems ⁷³²	INJEX 30 and 50 [§] models, ZipTip ^{††}	2000	Hu/In, Gh	Ma/Sp	DSJI	Off	SC	34, 576, 665, Fig. 61-6G
	Jet Syringe, [§] ROJEX [§]	2000s	Hu/In, Gh	Ma/Sp	SUDJI	Mf or Off	SC	—

Keystone Industries ^{607,609}	Ped-O-Jet [†]	1950s	Hu/Va	Pe, Ei/Sp	MUNJI	On-I	ID, M, SC	1, 80, 81, 172, 529, 532-535, 538, 547-549, 554, 556, 570, 574, 620, 621, 636, 645, 649, 657, 661, 663, 667, 672, 679, 687, 711, 712, 715, 720, Figs. 61-2E, 61-5C
MADA Medical Products ⁶⁰⁸	Syrijet	1960s	De, Hu/An, St	Ma/Sp	MUNJI	Md, Sd	ID, SC	574, 603, 607, 685, 712
	MadaJet, MadaJet XL	1980s	De, Hu/An, St	Ma/Sp	MUNJI	Md	ID, SC	241, 557, 716, Fig. 61-5H
Med-E-Jet D ⁶¹⁰	Med-E-Jet	Early 1970s	Hu/Va	Ga/Ga (CO ₂ , air)	MUNJI	On-I	ID, M, SC	532, 560, 564, 637, 705-708, 714, Fig. 61-5E
Medical International Technologies ⁷²⁵	MED-JET	1990s	Hu/An, Va	Ga/Ga (CO ₂)	MUNJI	ON-I	IM, SC	726, 727
	MED-JET H-III	2010s	Hu/Va, St, Mu	Ga/Ga	MUNJI	ON-I	IM, SC	726, 727
	MED-JET MBX	2010s	Hu/Mu, St	Ga/Ga	MUNJI	ON-I	ID, M, SC	726, 727, Fig. 61-5F
	MED-JET H-IV [§]	2010s	Hu/Va, Mu	Ga/Ga	DSJI	Off	ID, M, SC	—
	Agro-Jet, MIT-II, MIT-IIP, MIT-III, MIT-V, MIT-VI, MIT-X, MIT-XIV	1990s, 2000s	Ve/Va, Mu	Ga/Ga (CO ₂)	MUNJI	ON-I	IM, SC	—
Microbiological Research Establishment ⁵¹¹	Porton Needleless Injector, [†] Port-O-Jet [†]	1962	Hu/Va	Pe/Sp	MUNJI	ON-I	ID, SC	602, 655, 702
National Medical Products ⁷³³	J-Tip	1990s	Hu/In	Ga/Ga (CO ₂)	SUDJI	On-F	SC	558, Fig. 61-6F
Nidec Tosok Corporation ⁵¹²	Hyjetor [†]	1970s	Hu/Un	Pe/Hy	MUNJI	On-I	ID, M, SC	—
PATEV GmbH & Co KG	Pyrofast [§]	2009	Hu/Un	Ch/Ch	SUDJI	Off	ID, M, SC	595
PATH ¹⁰⁶	MEDIVAX ^{†,§}	1990s	Hu/Va	Pe/Ga (air)	DSJI	On-I	SC, IM	715
PenJet Corporation ⁵¹³	PenJet [§]	1990s	Hu/Va	Ga/Ga (N ₂)	SUDJI	Mf	SC	—
PharmaJet, Inc. ⁸²	PharmaJet	2000s	Hu, Ve/Va	Ma/Sp	DSJI	Off	ID, M, SC	641, 738, 739
	Stratis	2011	Hu	Ma/Sp	DSJI	Off	IM, SC	641, Fig. 61-6C
	Tropis	2011	Hu/Va	Ma/Sp	DSJI	Off	ID	169, 579, 580, 641, Fig. 61-2J
Prolitec SA ⁵¹⁴	IsaJet, [†] Isa40 Isa10	1990s	Hu, Ve/Un	Ma/Sp	MUNJI	On-I	IDm	—
	Mesoflash M10 [†]	1980s	Ve/Un	Ma/Sp	MUNJI	On-I	IDm	—
	Mesoflash M30 [†] and M40 [†]	1980s	Hu/Un	Ma/Sp	MUNJI	On-I	IDm	—
Sanofi Pasteur ⁷³⁶ (manufactured under former Institut Mérieux and Pasteur Mérieux Sérums & Vaccins entities)	Im-O-Jet [†]	1980s	Hu/Va	Pe/Sp	MUNJI	On-I	SC	194, 671, 674, 737, Fig. 61-5G
	Mini-Imojet, ^{†,§} PM 3C ^{†,§}	1980s	Hu/Va	Ma/Sp	DSJI	Mf	SC	34, 134, 648, 650, 660, 737, Fig. 61-6H

Table 61-1 Historical, Currently Marketed, and Investigational Jet Injectors Used, Studied, or Considered for Vaccination (see footnotes for explanation of abbreviations)—cont'd

Current/last manufacturer	Device trade name(s)	Year(s)*	Market/primary uses	Energy source/storage	Type	Filling	Target tissue	References
Robert P. Scherer Co. ⁵²⁷	Hypospray [†]	1940s	Hu/In	Ma/Sp	DSJI	Off	ID, SC	492, 525, 526, 562, 563, 568, 574, 577, 599, 605, Fig. 61-5B
	Hypospray Professional [†]	1950s	Hu/Va	Ma/Sp	MUNJI	On-I	ID, M, SC	220, 624, Fig. 61-5I
	Hypospray Multidose Jet Injector, [†] K, [†] K-2, [†] K-3 [†] models	1952	Hu/Va	El/Sp	MUNJI	On-I	ID, M, SC	130, 140, 542, 550, 551, 601, 624, 628, 688, 691, 699, Fig. 61-5D
Schuco International ⁵¹⁵	Panjet multiple models, [†] Intrajet, [†] SchucoJet [†]	1960s	Hu/Va	Ma/Sp	MUNJI	On-F, Md	ID, SC	194, 208, 622, 623
Shimadzu Corporation ⁵¹⁶	ShimaJET	—	Hu/In, Va	Ma/Sp	DSJI	On-F	SC	585, 680, 686
SIC M ⁵¹⁷	JET2000	—	Hu/Va	Ma/Sp	MUNJI	On-I	—	715
	DG-77	—	Hu/Va	Ma/Sp	MUNJI	On-I	—	573
Sino Goldbuilder Med Tech (Beijing) Co., Ltd. ^{84c}	Goldbuilder Ruisu GB-03	2010s	Hu/In	Ma/Sp	DSJI	Off	SC	—
Soci��t�� AKRA DermoJet ⁵¹⁸	DermoJet Standard, Dermojet type HR, Dermojet model G	1960s	Hu/Va	Ma/Sp	MUNJI	On-I, Md	ID, IDm, SC	74, 217, 218, 226, 207, 209, 211-213, 553, 571, 574, 632, 661
	Dermojet Automatic, Vacci-Jet	—	Hu/Un	Ma/Sp	MUNJI	On-I	SC	—
Team Consulting ^{84d}	Chemomotor [�]	2000s	Hu/Va	Ch/Ch(butane)	DSJI	Off	ID, SC, IM	—
Valeritas ³⁰²	Mini-Ject [�]	2000s	Hu/Mu Va	Ch/Ch	SUDJI	Mf	ID, M, SC	678
Z & W Manufacturing ⁵¹⁹	Press-O-Jet [†]	1950s	Hu/Va	Ma/Sp	MUNJI	On-F	SC/IM	528, 542, 546, 574, 659, 666
Zogenix ⁷³⁴	IntraJect [�]	1990s	Hu/Ho	Ga/Ga (N ₂)	SUDJI	Mf	SC	593, Fig. 61-6E
	Sumavel DosePro ^{��}	2010	Hu	Ga/Ga (N ₂)	SUDJI	Mf	SC	735

Market/primary uses: An, anesthetic; Av, antiviral; De, dentistry; Gh, growth hormone; Ho, hormone; Hu, human medicine; In, insulin; Mu, multiple; St, steroids; Un, unspecified; Va, vaccine; Ve, veterinary.

Energy source/storage: Ba, battery; Ch, chemical (via expanding gases of reaction or combustion); El, wall (mains) electricity; Ga, compressed gas (cylinder or electrical compressor); Hy, hydraulic fluid pressurized in foot-pump accumulator; Ma, manual muscle; Pe, pedal muscle; Sp, metal spring.

Type: DSJI, disposable-syringe jet injector; MUNJI, multiuse-nozzle jet injector; SUDJI, single-use disposable jet injector (entire unit discarded after use).

Filling: Md, multiple doses possible from dose chamber before refilling required; Mf, manufacturer prefilled only; Off, off tool (dose chamber [syringe] is filled from vial before insertion into injector); On-F, on tool (primary container [vial] attaches temporarily to injector to fill dose chamber during filling but is removed before injection); On-I, on tool (primary container [vial] remains attached to injector to fill dose chambers repeatedly but stays attached during injections); Sd, dose chamber is a prefilled, standard drug cartridge (primary container).

Target tissue: ID, intradermal; IDm, intradermal with multiple orifices for simultaneous injection; IM, intramuscular; SC, subcutaneous.

*Approximate year(s) first introduced to market; or if not, year(s) investigational development initiated; or if not, year patent filed.

[†]Device withdrawn from market, no longer manufactured, or abandoned in development.

[ ]Vision injector versions are licensed to Ferring Pharmaceuticals BV (ZomaJet), SciGen Ltd (SciTojet), JCR Pharmaceuticals (Twin-Jector EZ II), and Teva (Tjet for TEV-TROPIN human growth hormone).

^{  }Investigational device, or not yet sold commercially for routine use in humans or animals.

^{   }The cool.click and SeroJet devices are the Vitajet 3 design licensed by Bioject to EMD Serono⁵²⁰ for delivery of the Saizen and Serostim brands of somatropin (recombinant human growth hormone) for treatment of growth hormone deficiency and AIDS-wasting diseases, respectively.

[ ]The mhi-500 (by The Medical House, acquired by Bespak⁵¹⁰) device contains Vitajet 3 technology licensed by Bioject.

^{  }Canine Transdermal Device is an adaptation of the Bioject Vitajet3 jet injector licensed to Merial (Sanofi group) for delivery of its Oncept DNA vaccine for treatment of oral melanoma in dogs, licensed in the United States in 2010.

^{   }The Vetjet (by Merial521) device is the Vitajet 3 design licensed by Bioject to Merial for delivery to cats of PureVax brand of feline leukemia virus vaccine.

^{    }The ZipTip (by Pfizer) is the INJEX design licensed to Pfizer for delivery of Genotropin recombinant human growth hormone.

^{     }Zogenix SUMAVEL DosePro delivers sumatriptan indicated for acute migraine and cluster headache. Novel borosilicate glass dose chamber prefilled by drug manufacturer.

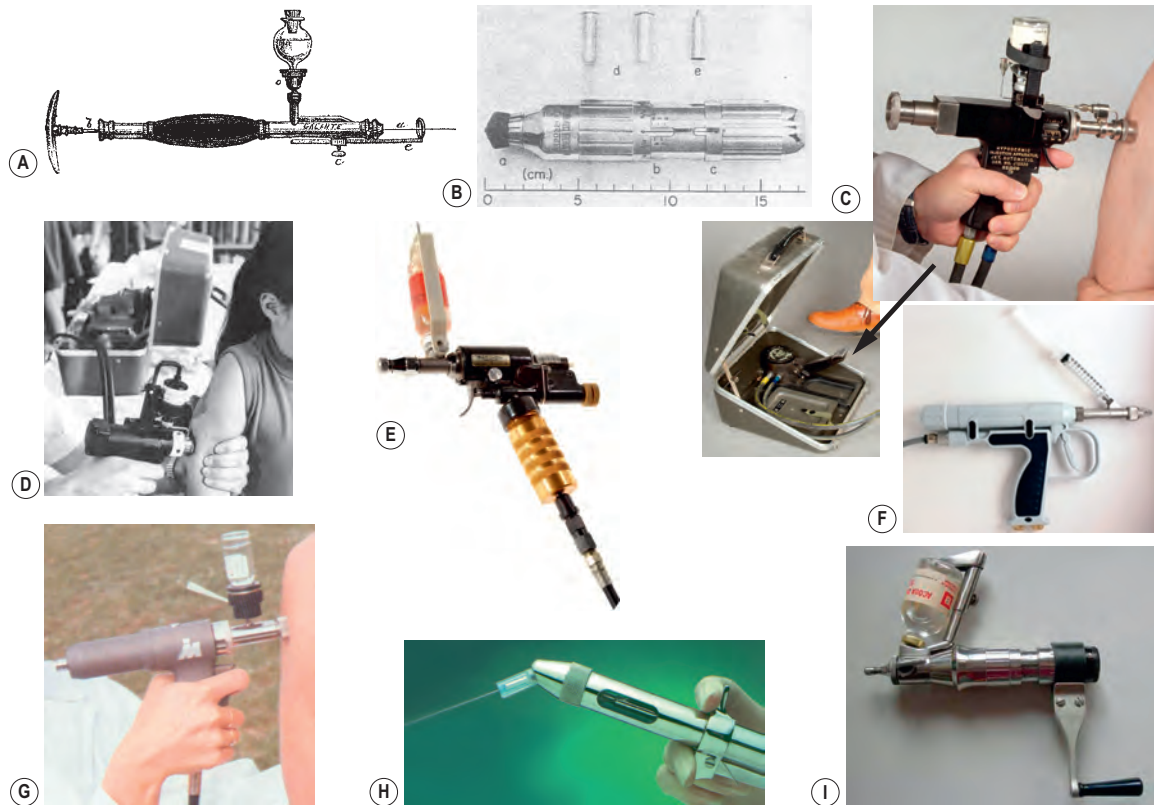


Figure 61-5 Selected Multiuse Nozzle Jet Injectors (MUNJIs). (A) Aqua-puncture device of Galante et Compagnie,⁵²³ circa 1866, of historical interest as first known jet injector. (From Béclard F. Présentation de l'injecteur de Galante, Séance du 18 Décembre, 1866, Prés. Bouchardat. Bulletin de l'Académie Impériale de Médecine (France) 32:321-327, 1866.) (B) Hypospray⁵²⁷ manual MUNJI (Robert P. Scherer Company) for individual patient or caregiver administration; the first modern-era, commercial jet injector, introduced in the 1940s, with reusable, resterilizable MetaPule dose-chamber cartridges. (From Perkin FS, Todd GM, Brown TM, et al. Jet injection of insulin in treatment of diabetes mellitus. Proc Am Diabetes Assoc 10:185-199, 1950.) (C) Ped-O-Jet⁶⁰⁹ (Keystone Industries), the most widely used MUNJI worldwide, before withdrawal from public health use by the 1990s for cross-contamination risk. Its metal spring is compressed by hydraulic fluid pumped by (C, inset) a foot pedal in its carrying case, or by electric pump (not shown). Depth of delivery determined by removable nozzle used, either a subcutaneous/intramuscular (SC/IM) nozzle (shown here) or an intradermal (ID) nozzle (see Figure 61-2E). (D) Hypospray⁵²⁷ motorized high-speed MUNJI (Robert P. Scherer Company), once used for mass campaigns. Power to cock its metal spring was supplied by the hydraulic tubes from the electrical pump in its carrying case (shown in background). (E) Med-E-Jet⁶¹⁰ MUNJI, powered by metal springs compressed either by a CO₂ gas cartridge in the handle, capable of about a dozen injections, or by pneumatic hose connection to a separate tank or electric compressor pump. Capable of intradermal injections using a nozzle spacer. A device of this type was confirmed to be responsible for a hepatitis B outbreak in a California clinic.⁷⁰⁷⁻⁷⁰⁹ Not known to be in current use in the United States. (F) Med-Jet MBX MUNJI (Medical International Technology⁷²⁵), made in Canada and licensed in 2011 in China⁷²⁶ and Russia⁷²⁷ for use in humans. (G) ImoJet spring-powered MUNJI (Courtesy of Sanofi Pasteur,⁷³⁶) with remote power source (not shown), once used in mass campaigns. (H) MadaJet (Mada International⁶⁰⁸), a MUNJI still used for injections in dentistry, podiatry, and perhaps other medical specialties (no known use for vaccination). The teflon sheath over the nozzle is designed to deliver the anesthetic in a spray pattern that penetrates 2-3 mm below the epithelium, producing a wheal that is 3-5 mm in diameter. (I) Hypospray⁵²⁷ professional model MUNJI (Robert P. Scherer Company) uses manual hand crank to cock metal spring. Once used in routine immunization in medical clinics, and in mass campaigns. (Figure 61-5A,⁵²³; 61-5B,⁵²⁶; 61-5C, C inset, E, courtesy of James Gathany, Greg Knobloch [CDC Photographic Services]; 61-5D, courtesy of Public Health Image Library, CDC; 5F, courtesy of Medical International Technology;⁷²⁵ 61-5G, courtesy of Sanofi Pasteur;⁷³⁶ 61-5H, courtesy of Mada International⁶⁰⁸ [Robert Sorbello]; 61-5I, courtesy of Catalent Pharma Solutions, Somerset NJ.⁵²⁷)

great patient variation, it is no surprise that imaging data suggest that JIs often miss the intended IM or SC compartment.⁶¹⁴ However, this may have little clinical relevance and may be no different from needle injections, for which fat-pad thickness is often underestimated when selecting needle length, or when the needle is not fully inserted.^{615,616} In Russia, an unconventional target tissue—the lung—was reached by “intrapulmonary” jet injections [between the ribs] of antibiotics, bronchodilators, and steroids.⁶¹⁷

Cutaneous delivery

As mentioned (see “Cutaneous vaccination”, earlier), there has been a resurgence of interest in skin vaccination because of its potential dosage-sparing capability and minimal invasiveness. Jet injectors for classic ID delivery offer the additional

advantage of simplicity over the tedious and difficult classic Mantoux injection, as well as the ability to use existing off-the-shelf vaccines without reformulation. Older MUNJI models, such as the Ped-O-Jet, used specialized nozzles with recessed orifices offset by 45 degrees from perpendicular to the skin, creating an air gap that weakened its jet stream so as to leave the dose in the skin (see Figure 61-2E).

The Ped-O-Jet (and to a much lesser extent other MUNJIs [see Figure 61-5D]) administered tens of millions of smallpox vaccine doses for the first half of the WHO Smallpox Eradication Programme in South America and West Africa in the late 1960s to early 1970s, until invention of the simpler and swifter bifurcated needle.^{1,81,538} JIs also delivered yellow fever^{172,173,534,548} and BCG vaccines⁶¹⁸⁻⁶²³ by the ID route, as well as various tuberculosis skin testing (TST) antigens.⁶²⁴⁻⁶³² However, variations in



Figure 61-6 Selected Disposable-Syringe Jet Injectors (DSJIs), Licensed or Investigational (as noted). (A) Investigational LectraJet HS (high-speed) motorized DSJI (D'Antonio Consultants International⁶¹³) features built-in motor and rechargeable battery for rapidly compressing metal spring between injections at rates exceeding 600 per hour, with battery capacity of > 3,000 injections per charge. Capable of rapid, fingers-free loading and unloading of single-use syringes from (A, inset) a sterile-packaged, 30-unit magazine for mass vaccination. Magazine may also be mounted on nondominant forearm for vaccinator mobility. Capable of backup manual spring-cocking if batteries are depleted. (B) LectraJet M3 (manual) model DSJI,⁶¹³ sharing the same common syringe as the adjacent HS model for rapid, fingers-free loading and unloading of syringes. IM or SC delivery set by varying syringe orifice diameter. Cocked using off-tool carrying case (not shown). Cleared for US marketing by FDA in 2009. Syringes and needle-free vial adaptors also supplied in individually wrapped sterile envelopes for routine immunization (not shown). (C) PharmaJet Stratis⁶² DSJI, for 0.5-mL dose delivery. Delivery IM or SC set by vaccinator technique (fat layer pinched up for SC). Cocked using off-tool carrying case (not shown). (C, inset) Syringe is filled by pulling back and breaking off its blue shaft and thumb tab from conventional single-dose and multidose vials using needle-free vial adaptor (not shown). On insertion into device, any excess liquid is returned to vial to minimize wastage of overfill. Cleared for US marketing by the FDA in 2011. See Figure 61-2J for intradermal DSJI from same device manufacturer. (D) Medi-Jector Vision DSJI,⁷³¹ used primarily for self-administration by patients of insulin and other medications. (E) Sumavel DosePro single-use DSJI (Zogenix⁷³⁴), licensed as drug-device combination product for subcutaneous delivery of prefilled sumatriptan for treatment of migraine and cluster headaches.⁷³⁵ Uses novel, borosilicate-glass dose chamber prefilled by drug manufacturer. (F) J-Tip, single-use DSJI,⁷³³ powered by compressed nitrogen gas. (G) Injex DSJI,⁷³² metal spring compressed by separate cocking device. (H) Imule manufacturer-prefilled DSJI syringe^{660,737} for Vaxigrip influenza vaccine (Institut Mérieux/Pasteur Mérieux Sérums & Vaccins⁷³⁶). The syringe served as both primary vaccine packaging in a presentation smaller than conventional single-dose glass vial (millimeter scale on left), as well as the single-use disposable syringe for jet injection. Upon removing the label (H, center), inserting into the Mini-Imojet DSJI (not shown), and removing the rubber cap (H, right), the dose was ready for injection. Studied in human trials for five vaccines^{134,648,650} and found successful in immune responses and safety. Subsequently abandoned by the manufacturer. (I) Biojector 2000 DSJI (Bioject Medical Technologies⁸³), capable of subcutaneous and intramuscular injections using syringes of differing orifice diameters.⁶¹⁴ Cleared for US marketing by FDA in 1990s. Powered by compressed CO₂ cartridge, or by connection to separate compressed gas source. An investigational spacer for intradermal delivery (illustrated elsewhere²²) creates a 2-cm air gap to weaken the jet stream, leaving the injectate in the skin. Used by US Navy and Coast Guard for approximately one-third million vaccinations per year of sailors and dependents from 1997 through 2011. (J) ZetaJet metal-spring-powered DSJI,⁸³ features built-in crank for manual re-cocking of metal spring (Bioject Medical Technologies⁸³). Uses different auto-disabling cartridges for SC, IM, and ID injections (licensed by US FDA in 2009). See Figure 61-2K for intradermal DSJI from same device manufacturer. (K) Investigational Iject DSJI,^{83,730} designed for either single-use or reuse upon refitting with its manufacturer-prefilled borosilicate glass dose chamber. (Figure 61-6A, A inset, B, courtesy of D'Antonio Consultants International, Inc.⁶¹³; 6C, C inset, courtesy of PharmaJet, Inc.⁶²; 6D, courtesy of Antares Pharma⁷³¹; 6E, courtesy of Zogenix⁷³⁴; 6F, courtesy of National Medical Products⁷³³; 6G, courtesy of INJEX-Equidyne Systems⁷³²; 6H, courtesy of Bruce G. Weniger; 6I, courtesy of James Gathany (CDC Photographic Services); 6J, K, courtesy of Bioject Medical Technologies.⁸³)

consequent TST reaction sizes^{74,633} led WHO to discourage JI use for BCG and TST.^{634,635}

For devices without a specialized ID nozzle, some vaccinators attach spacers or tubing to a regular nozzle, creating a gap between orifice and skin, which weakens the jet and provides space for a bleb that leaves the dose in the skin.^{22,222,534,535,629,636}

This ID technique was pursued investigational for local anesthesia⁶³⁷ and DNA vaccines.^{638–640}

As described earlier (see “Poliomyelitis”), WHO and others involved in polio eradication are pursuing the use of DSJIs for needle-free, dosage-sparing ID delivery of IPV once OPV is discontinued for both technical reasons and cost (full-dosage

IPV is 20 times more costly than OPV).^{166–169,641} Other vaccines that have been studied for ID delivery by DSJIs include HIV/AIDS^{584,639} and influenza,⁶⁴² and studies are underway or planned for dengue,^{230,579,580} human papillomavirus, and rabies. The PharmaJet Tropis (see Figure 61-2J) and the Bioject ID Pen (see Figure 61-2K) are two new spring-powered DSJIs designed solely for ID delivery of 0.05 and 0.1 mL volumes. The former was licensed in the United States in 2011, and clearance for the latter is expected in 2012.

Immune response

A large body of clinical literature shows the immunogenicity of JIs to be usually equal to, and sometimes better than, that induced by conventional needle and syringe for a wide variety of vaccines.^{493,494,496} Among inactivated and toxoid vaccines, this includes anthrax,^{643–646} cholera,⁶⁴⁷ whole-cell diphtheria-tetanus-pertussis,^{207,208,538,648} diphtheria-tetanus,²¹¹ hepatitis A,^{648–651} hepatitis B,^{194,652,653} influenza,^{130,134,140,546,648,654–658} plague,^{643,644} polio,⁶⁵⁹ tetanus,^{554,648,660,661} and typhoid.^{648,662} With the exception of the variable delayed-hypersensitivity responses to BCG discussed earlier, other live vaccines inducing suitable immune responses when administered by JI into their usual tissue compartment are measles,^{218,220,226,534,538–540,548,636,663,664} measles-mumps-rubella,⁶⁶⁵ measles-smallpox,^{534,535,548} measles-smallpox–yellow fever,^{534,548} smallpox,^{1,80,81,534,538,636,663,666–668} BCG–yellow fever,¹⁷² and yellow fever.^{171–173,534,538,549}

The immunogenicity or efficacy of traditional meningococcal polysaccharide vaccines administered by JIs have been demonstrated for serogroup A in the clinic^{202,669} and in outbreaks in the meningitis belt of western sub-Saharan Africa,^{543,670–674} as well as for serogroup C in South America^{675–677} and Africa.^{543,674} Jet injection of the newer Vi capsular polysaccharide typhoid vaccine resulted in 87% seroconversion, versus 69% by needle-syringe ($P < .05$).⁶⁴⁸ Clinical studies have not yet been published of JI for modern protein-conjugated polysaccharide vaccines for *H. influenzae* type b, pneumococcus, or meningococcus.

A wide variety of investigational recombinant nucleic acid vaccines are being delivered in preclinical and clinical trials using various JIs.^{638,640,678–686}

Reactogenicity

When JIs and needles used to deliver IM and SC injections are compared in terms of immediate pain, the results depend on the medication involved. Insulin, other nonirritating drugs, and nonadjuvanted vaccines are usually reported to result in either reduced or equivalent pain compared with needles,^{525,534,546,559,576,577,605,665} but not always.⁶⁵⁸ True double-blinded, needle-controlled studies for such subjective criteria are difficult to implement and are thus rare. In an exception,⁶⁵⁴ one group applied earphones to all volunteers and played music loud enough to mask the mechanical noise for the half receiving the DSJI injection. All volunteers inserted their arms through a screen to block their view, and the injection of those randomized to the needle-syringe group occurred through the center of a plastic ring the same size as the jet injector nozzle, so that both groups experienced the same skin-contact sensation just prior to injection by a nurse not involved in study assessment. Mild or moderate erythema was measured in 97% of DSJI vaccinees, but only 73% of the N-S group ($P = .03$). Mild or moderate induration occurred in 93% and 27% of DSJI and N-S groups, respectively ($P < .0001$).

Vaccines with alum adjuvants or other irritating components tend to result in higher frequencies of delayed local reactions (eg, soreness, edema, erythema) when jet-injected, probably because small amounts remain in the track left through skin and superficial tissue. These include vaccines for diphtheria-tetanus-pertussis (whole cell),^{208,538,551,658} hepatitis A,^{648,649,651,687} hepatitis B,^{194,652,653} tetanus,^{552,554,648,660,661,688} tetanus-diphtheria,²¹¹

tetanus-diphtheria-polio,⁵⁵¹ and typhoid.^{648,662,689,690} In most cases, local reactions were mild, resolved within days, and were not reported to compromise clinical tolerance and safety. A chronic granuloma was reported after JI vaccination with tetanus toxoid adsorbed to alum,⁶⁹¹ and pigmented macules persisted in a few hepatitis B vaccinees.⁶⁵²

Other adverse events

Bleeding and, less often, ecchymosis are reported to occur at the jet injection site more frequently than with needle injections.^{134,525,528,530,531,542,546,559,564,572,575,577,605,633,648,659,692–694} Rarely, the jet stream may cause a laceration if the health care worker has not properly immobilized the limb and injector in relation to each other during injection.^{525,530,546,577,648} Rare case reports of other adverse events include transient neuropathy,^{695,696} hematoma,^{569,697} and eye penetration when used to deliver anesthetic for lower eyelid surgery.⁶⁹⁸

Safety of multiuse-nozzle jet injectors (MUNJIs)

Beginning in the 1960s, concerns arose for potential iatrogenic transmission of bloodborne pathogens by MUNJIs, which use the same nozzle to inject consecutive patients without intervening sterilization.^{688,693,694,699} Unpublished bench and chimpanzee studies indicated hepatitis B contamination could occur because blood or HBsAg remained in nozzle orifices despite recommended alcohol swabbing between injections.^{700,701} Others, however, reported negative results in bench or animal testing when they tried to detect contamination,^{529,564,702,703} or they pointed to the lack of epidemiologic evidence of a problem.^{551,702,704,705} Then in 1985, Brink and colleagues described a careful animal model in which a Med-E-Jet transmitted lactate dehydrogenase elevating virus (LDV) between mice in 16 (33%) of 49 animals.⁷⁰⁶

A few months later, fact superseded theory when a Med-E-Jet caused an outbreak of several dozen cases of hepatitis B among patients in a California clinic.^{707–709} Subsequent clinical,⁷¹⁰ field,^{711,712} bench,⁷¹³ animal,^{714,715} and epidemiologic,^{716,717} studies added more evidence that MUNJIs could transmit pathogens between patients. This led to warnings and discontinuation of their use by public health authorities,^{718,719} and to market withdrawal of the Ped-O-Jet and discontinuation of its US military use in 1997.^{497,720}

In the mid 2000s, a MUNJI was reengineered with disposable caps to try to prevent contaminating blood or tissue fluid from splashing back onto the reusable nozzle, potentially to infect the next patient.⁷²¹ The cap contained three plastic washers with axially aligned central holes of about 1 mm in diameter for the jet stream to pass in one direction along the centimeter-wide gap between orifice and skin. However, after injections with saline of volunteers in China who carried hepatitis B virus, 8% of subsequent ejectates into vials—representing the next vaccinees in a clinic or mass campaign—were found by polymerase chain reaction assay to contain hepatitis B antigen.⁷²² High-speed microcinematography also revealed extensive splashback from the skin during injection with MUNJIs.⁴⁹⁶

This body of evidence supports the conclusion that the design of MUNJIs is inherently unsafe, and any reuse of fluid pathways or unsterile components that are in direct or indirect contact with consecutive patients should be abandoned. Even if contamination could be shown to be extremely rare, it is unlikely that policymakers could be convinced to set any level of acceptable risk.

Despite the recommendations against MUNJI use for vaccination by public health authorities,^{723,724} and their withdrawal by the US military,⁷²⁰ models such as the MadaJet⁶⁰⁸ (see Figure 61-5H) and SyriJet⁶⁰⁷ continue to be used in the United States in dentistry and podiatry and perhaps other specialties. Also, despite the Chinese venue for the definitive study documenting MUNJI cross-contamination,⁷²² the Chinese Food and Drug Administration was reported in February of 2011 to

have licensed the Med-Jet⁷²⁵ line of MUNJIs in that country for human applications,⁷²⁶ as did Russian regulators in April, 2011,⁷²⁷ for vaccination, psychiatrics, dermatology, and meso-therapy indications.

MUNJIs allowed a single health worker to vaccinate 600 or more patients per hour.^{494,530,532,546} The withdrawal of the device posed challenges for conducting mass immunization campaigns for disease control programs and in response to pandemic or bioterrorism threat. Indeed, while the Soviet biological warfare effort was underway in secret,⁷²⁸ numerous clinical trials were published of high-speed Russian MUNJIs capable of rapidly protecting soldiers or civilians against potential biowarfare agents such as anthrax, botulism, plague, smallpox, and tularemia.^{493,643–646,668,689,690,729}

Disposable-syringe jet injectors (DSJIs)

To overcome concerns over MUNJIs and their withdrawal, a new generation of safer, disposable-syringe JIs have appeared since the early 1990s (see Table 61-1 and Figure 61-6).^{22,494–499,730} Each sterile syringe (cartridge) has its own orifice and nozzle and is discarded between patients. Although many are used for self-administration of insulin,^{731–733} other hormones, and drugs^{734,735} (eg, see Figure 61-6D,E,F,G), a few are targeted for vaccine administration (see Figure 61-6A,B,C,I,J). Newer systems feature, for example, auto-disabling designs to prevent refilling and reuse on consecutive patients, in contemplation of developing-world markets.

One unique and revolutionary system, developed by Charles Mérieux and colleagues at Institut Mérieux and Pasteur Mérieux Sérums et Vaccins, predecessor companies of Sanofi Pasteur,⁷³⁶ was the manufacturer-prefilled Imule syringe (see Figure 61-6H) for use in the Mini-Imojet DSJI (not shown). The Imule served as both the primary container for shipment from the vaccine manufacturer and for cold-chain storage, as well as the syringe (with rubber-stoppered bottom) for DSJI delivery, obviating the need for end-users to purchase any disposables.^{34,660,737} Although demonstrated in the clinic and field to be immunogenic and safe for diphtheria-tetanus-pertussis (whole cell),⁶⁴⁸ hepatitis A,^{648,650} influenza,^{134,648} tetanus,^{648,660} and typhoid vaccines,⁶⁴⁸ the system was eventually abandoned upon corporate merger.

The pioneering DSJI for the vaccine market was the Biojector 2000 (see Figure 61-6I), introduced in the United States in the 1990s.^{34,83,166,167,231,414,559,566,567,581–584,586,611,612,614,638–641,651,653,658,678,681–684,696} Through the 2000s, it was delivering approximately 1 million IM and SC vaccine doses per year at private, public, and US Navy and Coast Guard immunization clinics in the United States, and it was used in many studies of investigational vaccines (see Table 61-1). Another US company, PharmaJet, entered the market in 2009 with licensure of its eponymous device for IM and SC injections, subsequently upgraded as the Stratis (see Figure 61-6C). Various models have been studied for investigational veterinary^{738,739} and human^{169,579,580,641} applications. By the 2011–12 influenza season, it had shipped several hundred thousand syringes to public health agencies and supermarket and drug chains, until the market collapsed on October 26, 2011, with a surprise FDA announcement affecting all DSJI companies (see “Regulatory matters”, later).

Since the 1990s, to meet developing world needs for needle-free vaccination systems that are economical, auto-disabling to prevent reuse, and suitable for both mass campaigns and routine immunization, the US Government (through both the CDC and the US Agency for International Development), the nonprofit organization PATH,¹⁰⁶ and WHO have promoted the research and development (R&D) and utilization of DSJI technologies. Between 1995 and 2010, the CDC awarded Small Business Innovation Research contracts totaling approximately \$10 million to three competing companies.

One project helped develop the high-speed LectraJet (see Figure 61-6A), with its unique system for fingers-free loading and unloading of cartridges that permits vaccinating at least 600 persons per hour for mass campaigns,^{34,613} as well as a manual model sharing the same syringes for routine immunization that was found safe and immunogenic for influenza vaccination and was licensed in the United States in 2009 (see Figure 61-6B).⁶⁵⁴ Another set of contracts assisted in adapting PharmaJet technology for ID delivery, which was licensed in 2011 (see Figure 61-2J).⁸² A third set of Small Business Innovation Research contracts supported R&D of the ZetaJet (see Figure 61-6J), which was licensed in 2009 and once called the Vitavax.⁷⁴⁰ Its built-in hand-crank to wind its spring was a feature targeted for developing-country value.⁸³

PATH¹⁰⁶ has also been a major player in this field, conducting its own R&D as well as assisting the companies developing the DSJIs mentioned here.^{170,741} In 2008, the Bill and Melinda Gates Foundation awarded PATH \$9.8 million to enhance its close collaboration with industry to determine the value, appropriateness, extent of application, and regulatory pathways for DSJIs to deliver vaccines in developing-country immunization programs.⁷⁴² The PATH initiative for DSJIs has included sponsoring and coordinating key policy analyses on ID delivery (including by non-DSJI means), economic modeling, and clinical trials on multiple continents.^{23,24,165,590}

Regulatory matters

In 2009, to prompt public comment before formal promulgation, the FDA published a draft guidance document on pen, jet, and related injectors intended for use with drugs and biological products.^{743,744} No similar prior effort to clarify the regulatory landscape for these devices had ever been published. Many existing devices, including all the MUNJIs, either had been grandfathered onto the market by virtue of preceding the 1976 cutoff date for medical device regulation, or were cleared for sale on the basis of “substantial equivalence” to such injectors (or to other “predicates” that had themselves been linked back to earlier devices). The draft document covered design and construction features, bench testing aspects, sterility and labeling issues, and most importantly, clinical testing.

Among the many docket submissions commenting on the draft,⁷⁴⁵ the most common observation was that the proposed guidance document did not distinguish sufficiently between the broad types of devices it covered—including DSJIs, MUNJIs, and pen (needle) injectors—and that their differences deserved distinctions in how they should be regulated in accordance with the “least burdensome” principle. For example, the same level of stringency for demonstrating the safety of MUNJIs because of their cross-contamination risk (as summarized in “Safety of multiuse-nozzle jet injectors”, earlier) was to apply also to DSJIs.

Another major critique of the draft guidance was its proposal that before licensure, device manufacturers should identify “the drugs/biologic products that are currently approved and marketed for the dosage, rate, and route of administration proposed for the general use injector”. As pointed out in PATH’s comprehensive docket submission,⁷⁴⁶ this might necessarily require them to conduct clinical trials for every drug or vaccine that a physician may decide to administer. It would thus pose a major obstacle for innovation and development of “general use” devices that are sold empty, that are not labeled or promoted for use with any particular drug, and that rely on the clinical judgment of the physician in practicing medicine in accordance with evidence in the scientific literature and any standards of care (as is the case for needles and syringes). This would apply even for off-label uses not specifically approved by the FDA for the drug involved, as the FDA itself has elegantly stated.⁷⁴⁷ As of June 2012, no formal promulgation of the guidance document has occurred.

On October 21, 2011, at the peak of the US influenza vaccination season, the FDA issued an unusual and surprising warning to physicians, without the usual advance notice and consultation with affected parties and agencies. It advised against the use of jet injectors to deliver influenza vaccines because there were “no data” substantiating such use.⁷⁴⁸ The effect was dramatic. Drugstore and grocery chains immediately cancelled orders for what was expected to be several million syringes and thousands of accompanying devices, putting at risk the survival of the small companies involved.

Within days, the FDA replaced the categorical statement on its website with a more nuanced one pointing out that it had not been provided any data from the manufacturers of the six then-current US-marketed influenza vaccines for delivery by JIs. It also cited “limited data”^{654,658} from two JI studies (both conducted with CDC involvement) demonstrating similar immune responses to influenza vaccines administered by jet injectors and by needles, and therefore that “FDA and CDC believe that people who got their influenza vaccine via jet injector do not need to be re-vaccinated.”⁷⁴⁹

The CDC's Advisory Committee on Immunization Practices has for many years recognized jet injection as an effective method of vaccination,^{723,750,751} based on the substantial literature and experience reviewed in this chapter. It remains unknown whether such a standard of care for accepted public health and medical practice⁷⁴⁷ can restore a market for such off-label use, or whether vaccine manufacturers will undertake new studies and petition the FDA to add jet injection to their product labels. Thus, the future remains uncertain for the small businesses that constitute the global industry for the manufacture of safe, modern jet injector systems for vaccination.

Respiratory vaccination

Since the very early history of immunization, the respiratory tract has been a promising route for vaccine delivery.¹ However, only in 2004 did respiratory vaccines first become a part of routine modern immunization practice, with the licensure of an intranasal (IN), live attenuated influenza vaccine (FluMist) in the United States (see Chapter 18). The major potential advantages of respiratory immunization are that it avoids the risks and concerns associated with parenteral injection, and it generally provides stronger mucosal immunity than vaccination by that route. However, multiple obstacles (see “Challenges for respiratory delivery of vaccines”, later) have restricted wider application. As of 2011, FluMist was the only respiratory vaccine in general use. In contrast, the respiratory route is used to deliver a wide and expanding variety of pharmaceutical products.^{752,753}

The importance of mucosal immunity is that it prevents infection at the portals of entry for the great majority of human pathogens—the respiratory, gastrointestinal, and genitourinary tracts. In contrast, systemic immunity clears infection only *after* successful invasion, by limiting replication and destroying the pathogens. Ideally, both mucosal and systemic immunity should be raised against targeted pathogens. Strong mucosal immunity may enhance the benefits of immunization for some diseases. For example, by preventing the initial infection, mucosal immunity can reduce the risk of transmission to others, in addition to preventing clinical disease. Prevention of infection at the mucosal surface may be especially important for diseases for which effective systemic immunity has been difficult to achieve, such as tuberculosis and AIDS.

Every mucosal surface available for administering vaccines has been studied with a variety of antigens in animal models, including oral, respiratory, rectal, vaginal, and ocular tissues. Several human vaccines are already licensed and in successful use for delivery by oral ingestion, including those for polio,

cholera, rotavirus, typhoid, and adenovirus (see relevant chapters in this book). Although vaginal and rectal vaccines may work, they would have limited acceptability for social, cultural, and practical reasons. The remainder of this chapter will cover only the upper and lower respiratory tract, focusing on device technologies for deposition into these tissues, optimal presentation of antigen to the respiratory immune system, and adjuvants to enhance its immune response.

Antigen presentation and processing in the respiratory tract

Airborne particle entry and airflow

Like pathogens, respiratory vaccine antigens enter as airborne particles through the nares or mouth into airways designed to foil their entry and passage. Particles inspired through the nose are first filtered by the nasal hairs, and then they must traverse the external nasal valves, slit-like passages that limit airflow from the nares into the internal nasal airways. Djupesland and colleagues showed that only 25% of large, high-speed droplets (average, 43 μm) of a traditional nasal spray reach beyond the external nasal valve.⁷⁵⁴ This nasal filtration system may be bypassed by oral delivery via mask or mouthpiece. However, most large, high-speed particles are stopped in the mouth.

Small particles inhaled via nose or mouth share a common pathway through the oropharynx, larynx, and trachea. The bifurcation of the trachea into the right and left bronchi starts a series of bifurcations, providing further surfaces to trap airborne particles. Only very small, light, and slow-moving particles succeed in navigating the tortuous pulmonary passages to deposit in the lower airways. The smallest particles ($\leq 3 \mu\text{m}$) may reach the alveoli, where they can be rapidly absorbed into systemic circulation. The complex branching of the lung passages also results in an astonishing alveolar surface area, exceeding 100 square meters in a human adult male, compared with an average of only about 150 square centimeters (0.015 m^2) in the nasal airways.⁷⁵⁵ The lower airways in humans do not typically have organized lymphoid tissues, but they do have abundant intraepithelial dendritic cells and alveolar macrophages that process antigens.⁷⁵⁶

Particle deposition, movement, and uptake

In the internal nasal airway, particles deposit on the nasal mucosa covering the turbinates and then join the flow of mucus that is swept by ciliated epithelia toward the pharynx, where it is swallowed. Immune surveillance of antigens in the flow of mucus begins as they are taken up into epithelial cells, intraepithelial dendritic cells, surface macrophages, and microfold (M) cells.^{757,758} M cells are specialized epithelial cells that take up macromolecules, viruses, and bacteria by endocytosis, and then present them to lymphocytes and dendritic cells that congregate in invaginated pockets of the M cells; these pockets communicate with the extracellular space (see Figure 61-1B).⁷⁵⁷⁻⁷⁶⁰

The predominant organized lymphoid tissue of the human respiratory tract is located in the pharynx, where the adenoids and other tonsils (collectively known as Waldeyer's ring) surround the nasal and oral passages. The epithelium overlying these tissues is rich with M cells.⁷⁶¹ Increasing the deposition of vaccine antigen in the posterior nasal passages and nasopharynx near Waldeyer's ring may be desirable to maximize the immune response. Breath actuation of a nasal spray and nasal inhalation of smaller aerosol particles (5 to 20 μm) are two methods to increase nasopharyngeal deposition (see Figure 61-9A,B).^{754,762}

Regional processing

Antigen-presenting cells from the respiratory tract drain to regional lymph nodes, where the B cells preferentially switch to IgA plasmablasts. These plasmablasts “home” back to the

airway epithelium to provide antigen-specific IgA protection.⁷⁶³ T cells also play a major role in mucosal immunologic memory responses. Some lymphocytes exposed to antigen in the respiratory tract migrate to provide protection at remote mucosal sites, such as the vagina. This integrated network of immune cells and tissues is known as the *common mucosal immune system*.^{764,765} Because the respiratory tract is exposed to a myriad of nonpathogenic macromolecules, there are mechanisms for downregulating the immune response to antigenic exposure. This immunologic tolerance must be considered when developing respiratory immunization strategies.⁷⁶⁶

Challenges for respiratory delivery of vaccines

Identifying target tissues

The first challenge in respiratory immunization is determining the appropriate target tissues. Most respiratory drugs traditionally target two areas. For example, the nasal passages are the desired site of action for decongestants, and the lower airways are targeted by asthma medications. The optimal target tissues are not yet understood for most potential respiratory vaccines, and they vary for different antigens. The pharyngeal tonsils are likely candidate targets because of their key role in immunologic priming, but some vaccines may require deposition in the lower airways for uptake by alveolar macrophages and dendritic cells. Scientific methods for evaluating and comparing different target tissues are not yet well developed.

Applying animal models

A second challenge is the difficulty in selecting animal models and extrapolating their results for human respiratory vaccine delivery. Interspecies differences in respiratory immunologic tissue organization limit interpretation of animal target-tissue research results for humans. Moreover, the size and anatomy of the respiratory tracts of common research animals differ greatly from those in humans. For example, in small animals such as rodents, nose drops may deposit to the entire respiratory tract, which would not be the case in humans. Balmelli and colleagues estimated that 30% of 20 μ L of vaccine given to mice as IN drops deposited into the lungs.⁷⁶⁷

Many viruses and bacteria that infect humans do not grow well in animal models. For example, species-specific differences in the distribution of sialic acid receptors on cell surfaces is a crucial factor in tissue and host specificity of influenza A viruses, which limits the number of animal models suitable for influenza research.⁷⁶⁸ Such species-specific differences can make it difficult to use animals to study attenuated live vaccines or vaccine vectors, as well as to challenge animals to assess protection. This impedes the development of safe and effective respiratory vaccines for humans.

Delivering consistent dosages

A third challenge for respiratory immunization is dosage accuracy. The mass or volume of the antigen delivered depends on many factors, including variability in performance by the respiratory delivery device, the behavior and technique of the person administering the vaccine, and differences in the anatomy and physiology between vaccinates (animals) or vaccinees (humans).⁷⁶⁹ Fortunately, for many vaccines there is a wide margin between the dosage necessary to induce protection and the dosage at which the risk of adverse events increases.

The licensure in 2006 in the United States and Europe of the first inhalable insulin (Exubera), a drug for which dosage accuracy and consistency is critical, suggests that this challenge can be overcome for respiratory vaccines.⁷⁷⁰ However, the commercial failure of Exubera poses a cautionary example for developers of potential aerosol vaccines. The product was withdrawn

from the market in 2007 by the manufacturer because of lack of sales, after nearly \$3 billion was invested in development and licensure. The major reasons cited for this market failure were patient and physician concerns about long-term safety, complexity and size of the delivery device, increased cost compared with injection, and the availability of newer injection devices such as insulin pens.⁷⁷¹

Predicting protection from immune response

A fourth major challenge is the lack of accepted correlates of protection of mucosal immunity. In contrast, for many diseases there are laboratory assays to measure well-established criteria for systemic immunity—such as antibody titers above certain cutoffs—that have served for many years to predict protection from disease. In the absence of accepted serologic or cellular correlates of protection induced by mucosal vaccines, clinical trials must use specific disease-prevention endpoints, which can make the studies much larger and more expensive.

Ensuring safety

Several immunization safety issues represent further challenges for respiratory vaccines. One is the risk that vaccine antigen (live or inactivated), adjuvant, or excipients might affect nearby cranial nerves,⁴⁶ or might travel along the olfactory nerve through the cribriform plate into the brain, with resulting adverse central nervous system effects. Vaccines targeting the lower airways may induce or exacerbate bronchospasm or pulmonary inflammation, which can be life threatening. Another risk is cross-contamination: respiratory pathogens from one patient may contaminate the respiratory immunization device and be spread to subsequent patients.⁷⁷² Also, vaccine aerosols may spread beyond the intended vaccinee and affect other persons in the vicinity. Finally, live virus or bacterial vaccines might pose an increased risk to immunocompromised persons if delivered via the respiratory tract.

Designing practical delivery techniques

Remaining challenges relate to the delivery devices. Although many already exist for delivering drugs to the respiratory tract, very few are designed for vaccines. Most respiratory drug devices deliver repetitive doses to a single patient. In contrast, the expected usage for vaccination devices is to deliver single doses to multiple patients, which raises the cross-contamination issue. Although single-use, disposable devices or device components could solve this problem, they must be inexpensive to be cost effective.

Some aerosol-drug delivery devices require patient education to obtain the needed cooperation for adequate dose delivery. This may be difficult in the brief time typically involved in vaccination. In young children, who receive many vaccines, some respiratory delivery methods are not effective.

Although current respiratory drug delivery devices typically target the anterior nasal passages or the lower airway, respiratory vaccination may work best in the quite different target of the pharyngeal tonsils. In theory, ideal nasal delivery devices would prolong effective antigen presentation by depositing over a large surface area in the internal nasal airway, allowing mucus flow to move vaccine gradually across the tonsils.

Advancing the art

New delivery technologies to achieve respiratory immunization are required if this route is to become practical and accepted. As a young field, published research is limited on relevant devices in animals or humans. In most reported animal studies, the delivery device is not mentioned at all, or a laboratory pipette was used for intranasal instillation, which would be unsuitable for humans. For most respiratory devices designed for humans, testing is very difficult or impossible in an animal model.

Last, perhaps the most significant challenges to implementation of respiratory vaccination and other novel vaccine delivery systems in routine immunization practice are the regulatory requirements needed to ensure that the novel systems are safe and effective. The studies and clinical trials needed can be extremely expensive. Vaccine manufacturers are typically reluctant to assume such cost and risk to relicense an existing product already delivering profits, unless the potential benefits and market advantages would be significant. The best opportunity to bring alternative delivery into routine practice may be to use new delivery systems from the start for new vaccines early in their development and licensure process.

Current progress in respiratory tract vaccination

Wet versus dry aerosols

Vaccines can be delivered to the respiratory tract directly as either liquid or dry-powder aerosols. All currently licensed vaccines (for injection, or for oral or nasal delivery) are either stored and administered as a liquid, or stored in dry form and reconstituted to a liquid just prior to administration. Delivery of liquid aerosols is thus closer to usual practice. It is also generally easier to perform animal studies by generating aerosols from existing liquid formulations. Dry aerosols require changes in the formulation and manufacture of the vaccine to achieve and sustain vaccine potency and powder dispersability. If these challenges can be met, dry aerosols have several advantages over liquid aerosols (see "Dry-powder formulations for respiratory delivery", later).

Respiratory vaccination devices

AccuSpray™ nasal sprayer

The only device currently licensed and in use in the United States for respiratory vaccine delivery is the AccuSpray,⁸⁴ which is used for FluMist live attenuated influenza vaccine (LAIV).^{83,84} The device is a sterile, single-patient-use, disposable, prefilled glass syringe fixed with a nonremovable plastic nozzle (Figure 61-7C,D). Its total dose is 0.2 mL, of which 0.1 mL is sprayed consecutively into each nostril. An attachment on the plunger tells the user when to switch nostrils. FluMist vaccination delivered by AccuSpray is highly effective in most populations (see Chapter 18).

Key advantages of AccuSpray delivery are simplicity of use, low cost, disposability outside of sharps waste, and difficulty to refill and reuse. The large particle sizes generated by the sprayer minimize deposition to the lower airways, reducing the risk of adverse pulmonary events. A limitation of the system is that the particle size emitted depends on the speed at which the vacinator depresses the plunger. The median diameters of the particles can range from 200 μm or greater at plunger speeds of up to 33 mm/sec, to 50 μm or less at speeds of 80 mm/sec and greater.^{77,73} Although this wide variability might in theory affect the efficiency of vaccine deposition, LAIV by AccuSpray produces a high rate of protective immunity at the current dosage of 10^7 fluorescent focus units (FFU) for each of the three strains included in the vaccine.

To assess the potential of IN administration of measles vaccine, Simon and coworkers conducted a clinical trial with live attenuated (Moraten Berna) measles vaccine using the



Figure 61-7 Selected Devices for Respiratory Delivery of Liquid Aerosol Vaccines. (A) and (B) Investigational Classic Mexican Device for aerosol vaccine delivery, illustrated by component diagram (A) and use in clinical trials (B). A nonmedical electric compressor (not shown) delivers roughly 9 L of air per minute at a pressure of 30 to 40 psi (207 to 276 kPa) to a jet nebulizer that is kept in crushed ice to maintain vaccine potency. The vaccine aerosol (roughly 0.15 cm^3 of particles averaging $4.3 \mu\text{m}$ in diameter) is delivered through a disposable paper cone held close to the patient's face for 30 seconds.⁷⁷⁶⁻⁷⁷⁹ (From Valdespino-Gómez JL, de Lourdes García-García M, Fernandez-de-Castro J, et al. Measles aerosol vaccination. *Curr Top Microbiol Immunol* 304:165-93, 2006 [Fig. 1, p. 169].) (C) and (D) AccuSpray nasal spray syringe (Becton, Dickinson and Co.⁸⁴) produces an aerosol plume of particles reported from 50 to 200 μm in diameter, depending on plunger speed.^{77,73} (D) AccuSpray used for intranasal delivery of FluMist influenza vaccine (Medimmune, Inc.^{83,84}). Prefilled liquid vaccine is stored refrigerated for single patient use. The total volume is 0.2 mL. A dose separator interrupts delivery at 0.1 mL and, when reset, allows the remaining 0.1 mL to be administered into the opposite nostril. (E) and (F) Investigational AeroVax prototype (AerovectRx, Inc.,⁸⁰⁸ developed by Centers for Disease Control and Prevention and Creare, Inc.⁸⁰⁵). The (E) nebulizer utilizes battery-powered piezoelectric energy to drive an aerosol from a disposable drug cartridge via a microperforated mesh plate through a disposable patient interface, such as (F) nasal prong in patient nostril, oral prong, or mask (not shown). Droplet diameters can be tailored from $< 5 \mu\text{m}$ to 10 to 25 μm for upper or lower airway delivery, respectively. (Figure 61-7A,⁸⁵⁰ 7B, courtesy of José Luis Valdespino (Instituto Nacional de Salud Pública, Mexico); 7C, D, courtesy of Nuphar Rozen-Alder [Becton, Dickinson and Co.]⁸⁴; 7E, F, courtesy of James Gathany [CDC Photographic Services].)

AccuSpray. IN administration produced protective serum antibody titers in only 50% of nonimmune individuals, compared with protective titers in 100% of volunteers who received the vaccine by the SC route.⁷⁷⁴ Notably, IN vaccination resulted in increased production of measles-virus-specific secretory IgA (sIgA) in oral fluid and nasal washes among previously nonimmune individuals, but without evidence of a systemic immune response.⁷⁷⁵

Classic Mexican Device nebulizer

Another respiratory immunization device that has been used in humans is the jet nebulizer system known as the Classic Mexican Device (CMD; see Figure 61-7A,B). With slight modifications, this nebulizer system was used to deliver live attenuated measles vaccines in multiple clinical trials in Mexico and South Africa, and also to vaccinate over 3 million Mexican children against measles in a mass campaign.⁷⁷⁶⁻⁷⁷⁹ The system consists of a general-use (non-medical-grade) compressor that delivers air to a jet nebulizer (from iPi^{780,781}), which holds the vaccine in crushed ice to maintain potency during the vaccination session. The vaccine aerosol is delivered through reusable plastic tubing to a single-use, disposable paper cone (modified from a drinking cup), which is held close to the patient's face for 30 seconds. Typically, the aerosolized dose volume is roughly 0.15 mL, and the mass median aerosol diameter of droplets is 4.3 μm .⁷⁸²

In a recent study by Bennett and colleagues, the dose for each child was delivered from the CMD into a single-use, disposable plastic bag to avoid the risk of cross-contamination of the tubing. This reservoir was then separated from the tubing before the child inhaled its contents via a one-way valved mask. Preschool-age children vaccinated from the aerosol reservoir developed higher antibody GMTs than did subjects vaccinated by the SC route.⁷⁸³ In a study that assessed the distribution of viable vaccine virus across the range of droplet sizes emitted from a CMD, Coates and coworkers estimated that 30% of infective viral particles were contained in droplets with diameters of 5 μm or less, and 23% were in droplets of greater than 10 μm .⁷⁸⁴

Although the CMD has demonstrated a level of safety and immunogenicity, it is heavy, cumbersome, noisy, and requires outlet (mains) electricity and crushed ice. It is thus not practical for routine vaccination.

Measles Aerosol Project nebulizer

Because of the encouraging results of early measles aerosol vaccine trials (see "Classic Mexican Device nebulizer", earlier, and "Live viruses", later), in 2002 the WHO, in partnership with the CDC and the American Red Cross, initiated the Measles Aerosol Project (MAP). Its goal is licensure of at least one live attenuated measles vaccine and its associated aerosol delivery system in the developing world.⁷⁸⁵ The project documented immunogenicity and safety (ie, the lack of local or systemic toxicity) in animal studies.⁷⁸⁶ Three existing therapeutic nebulizers were used for phase 1 clinical trials: the AeroEclipse,⁷⁸⁷ the ComPair,⁷⁸⁸ and the Aeroneb.⁷⁸⁹ The selection criteria were (1) critical performance data, (2) usability under field conditions, (3) vaccine potency during nebulization,⁷⁹⁰ and (4) existing licensure for other uses. Measles vaccine delivery by the three devices, delivered to 145 subjects in India, was reported to be safe, well tolerated, and immunogenic.⁷⁸⁵

A modified version of Aeroneb device was selected for use in the phase 2/3 pivotal trial initiated in 2009 by the MAP.⁷⁹¹ The study was a randomized, open-label, active-control, non-inferiority trial of the measles vaccine in unvaccinated healthy infants from 9 to 11.9 months of age. As of November 2011, study results had not been released or published.⁷⁸⁵

Other aerosol devices studied in vaccine research

The OptiMist is a breath-actuated nasal-spray device that delivers liquid or dry-powder aerosols only during oral exhalation.⁷⁹² Because this raises the soft palate to close the connection between

nose and throat, pulmonary deposition is avoided, and delivery to the posterior nasal segments is increased (see Figure 61-9A,B).⁷⁵⁴ In a human study, inactivated influenza vaccine self-administered using the OptiMist resulted in significant increases in virus-specific IgA in nasal secretions, as well as protective levels of virus-specific serum antibodies, after two doses in more than 80% of subjects.⁷⁹³

A Combitips Plus pipette dispenser⁷⁹⁴ was used to deliver a dry-powder *Neisseria meningitidis* IN vaccine to human subjects. Those vaccinated by the IN route had serum bactericidal antibody titers comparable to that of those vaccinated by conventional injection, and 92% of IN vaccinees had protective titers after the second dose. One third of IN vaccinees reported mild side effects, compared with the two thirds of injection vaccinees who reported mild injection pain.⁷⁹⁵ Another dry-powder inhaler, the single-use, disposable Twincer⁷⁹⁶ (Figure 61-8E), dispersed an inulin-based dry-powder subunit influenza vaccine with an aerodynamic particle size distribution suitable for pulmonary administration.^{797,798}

Two unique dry-powder delivery devices, the PuffHaler⁷⁹⁹⁻⁸⁰¹ (see Figure 61-8A,B) and the Becton, Dickinson (BD) Solovent⁸⁴ (see Figure 61-8C,D), were developed and tested as part of an initiative to develop a measles vaccine dry powder (MVDP). The project is led by Aktiv-Dry, LLC⁷⁹⁹ (see "Dry-powder formulations for respiratory delivery", later). Each device disperses MVDP into an inexpensive, single-use, disposable reservoir from which the patient inhales, eliminating the risk of cross-contamination. After successful demonstration in the cotton-rat model,⁸⁰² MVDP was evaluated in rhesus macaques using PuffHaler and BD Solovent via mask and via the direct IN route from the devices.⁸⁰³ Respiratory delivery induced robust, significant measles-specific humoral and T-cell responses with no adverse effects. When challenged more than 1 year later, the MVDP-vaccinated macaques were protected from infection with wild-type measles virus.⁸⁰³ In other studies, the BD Solovent was effective for direct nasal delivery of influenza vaccine to rats, and of anthrax vaccine to rabbits.^{101,804}

The CDC developed the AeroVax nebulizer (see Figure 61-7E,F), in collaboration with Creare, Inc.^{22,805} It utilizes a disposable patient interface (nasal prong, oral prong, or mask) and a disposable drug cartridge to prevent cross-contamination. Disposable drug cartridges can be manufactured to generate custom particle size distributions (eg, 10- to 25- μm droplets for upper-airway delivery, or droplets of 5 μm or less to reach the lower airway). Delivery of live attenuated measles vaccine via nasal prong was shown to be safe and immunogenic in macaques.⁷⁸⁶

A 15-second aerosol delivery by the AeroVax device of influenza virus X31 induced a robust immune response in mice, which protected them against homologous (X31) and heterologous (PR8) influenza challenge.⁸⁰⁶ Nasal aerosol delivery of LAIV to ferrets elicited high levels of serum neutralizing antibodies and protected them from homologous virus challenge at conventional (median tissue culture infective dose [TCID₅₀], 10⁷) and significantly reduced (TCID₅₀, 10³) dosages, and provided a significant level of subtype-specific cross-protection.⁸⁰⁷ AerovectRx, Inc.,⁸⁰⁸ acquired the rights to manufacture and distribute this technology.

An investigational device for nasal delivery of dry-powder vaccine to nasopharyngeal tissues only was developed by the CDC and Creare, Inc.⁸⁰⁵ (see Figure 61-8F). It operates by patient exhalation through the mouth, blowing the powder into the nose while simultaneously generating air flow that limits entry to the lower respiratory tract. Its deposition pattern to targeted nasal tissues was documented in three-dimensional plastic models developed by CFDRC, Inc.,⁸⁰⁹ from in vivo computerized tomography of a child's head (Figure 61-9C,D, and see Figure 61-8F).

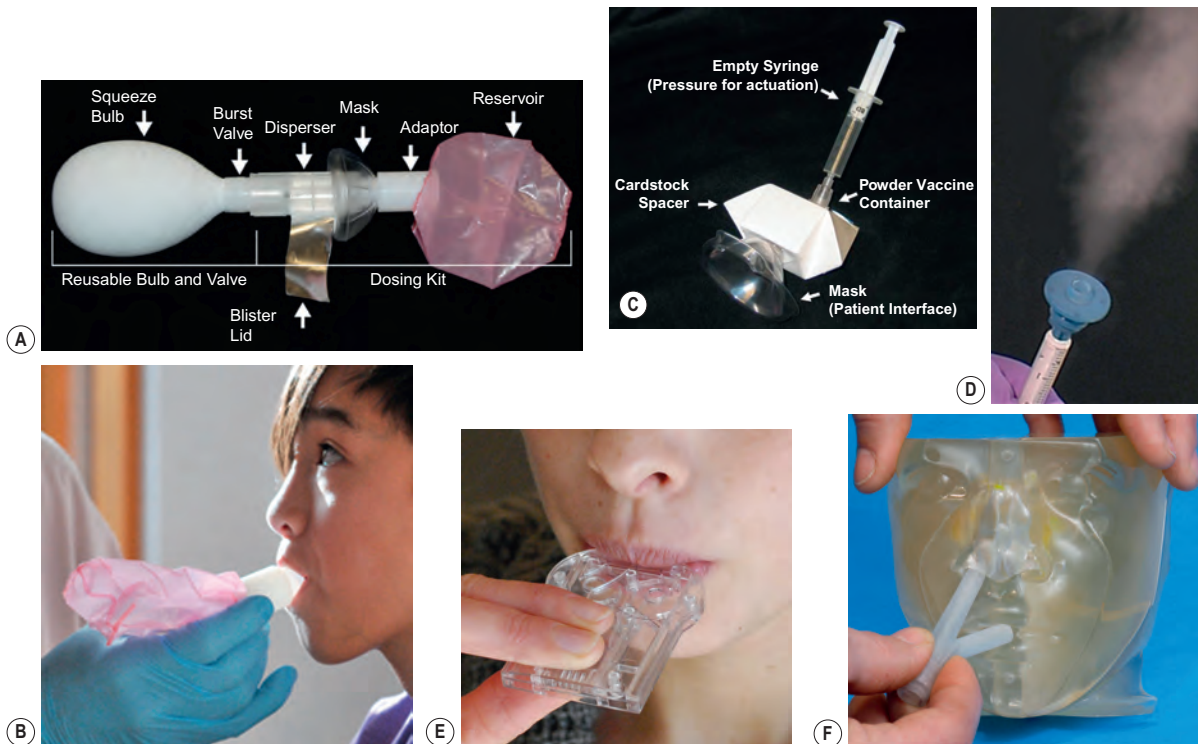


Figure 61-8 Selected Devices for Respiratory Delivery of Dry-Powder Aerosol Vaccines. (A) Investigational PuffHaler dry-powder inhaler (Aktivdry, LLC⁷⁹⁹). Air from the *squeeze bulb* lofts vaccine powder from the *dispenser* into the *reservoir* once the pressure threshold of the *burst valve* is exceeded. (From Kisich KO, Higgins MP, Park I, et al. Dry powder measles vaccine: particle deposition, virus replication, and immune response in cotton rats following inhalation. *Vaccine* 29:905-912, 2011 [Fig. 1, p. 907].) (B) Patient inhales from the single-use-only *reservoir* of PuffHaler after detachment from the device, either directly (as shown) or via a disposable mask (not shown). (From Lay J. The man fighting measles one breath at a time. *Coloradan Magazine* [University of Colorado, Boulder], March 2011, [photo, p. 10, by Glenn Arakawa].) (C) Investigational Solovent dry-powder inhaler prototype (Becton, Dickinson and Co.⁸⁴). Air from the *empty syringe* ruptures the membranes of the *vaccine container*, releasing into the *cardstock spacer* a plume of dry powder. Patient inhales from the *cardstock spacer* directly, or via a *mask*. The *vaccine container* (capsule), *spacer* and *mask* are single-use disposables. (D) Plume of powder upon release from Solovent in open air for visualization purposes. (E) Investigational Twincer⁷⁹⁶⁻⁷⁹⁸ single-use, disposable, dry-powder inhaler for pulmonary delivery (University of Groningen). The drug formulation is stored in the snap-together plates of the device in an aluminum blister for maximal moisture protection. The powder becomes available for inhalation upon pulling a foil cover that protrudes from the rear of the inhaler (not shown). (F) Investigational nasal dry powder inhaler developed by CDC and Creare, Inc.⁸⁰⁵ A prefilled cup (shown between right thumb and forefinger) containing the powdered vaccine is opened by its attachment to the device. The breath of the patient blowing into the device tube carries the dry powder into the nose. In theory, dispersion during patient exhalation limits pulmonary deposition from the posterior nasal space. The plastic face is a phantom model of the airway of a 5-year-old child constructed according to in vivo computerized tomography (CFDRC, Inc.⁸⁰⁹). (Figure 61-8A, courtesy of Scott Winston [Aktivdry, LLC⁷⁹⁹]; 8B, courtesy of Glenn Asakawa [University of Colorado]⁸⁰¹; 8C, courtesy of Becton, Dickinson and Co.⁸⁴ [Kenneth Powell]; 8D, courtesy of Becton, Dickinson and Co.⁸⁴ [Vincent Sullivan]; 8E, courtesy of University of Groningen [A. H. de Boer]; 8F, courtesy of Darin Knaus [Creare, Inc.⁸⁰⁵].)

Delivery vehicles for vaccination via the respiratory tract

Once the device has delivered vaccine to the appropriate region of the respiratory tract, sufficient quantities of the antigen (and adjuvant if needed) must penetrate mucosal or alveolar barriers to gain access to appropriate cells to activate the immune system. The vehicles or vectors that can be used for this purpose include live attenuated viruses (including those acting as vectors for exogenous antigen), live attenuated bacteria (including vectors), commensal bacterial vectors, virosomes, virus-like particles (VLPs), liposomes, lipopeptides, immune stimulating complexes (ISCOMs), microparticles, and nanoparticles.⁸¹⁰⁻⁸¹⁴

Live viruses

Viruses are prototypical antigen-delivery vehicles because they enter and commandeer cells to replicate themselves, thus multiplying the available antigen that they encode. Also, viruses can induce a natural adjuvant effect through activation of

chemokines and cytokines. The most widely studied respiratory delivery vehicles are live attenuated strains of pathogenic viruses.⁸¹⁵⁻⁸³³ Their major risks are possible reversion to virulence, potential neurotoxicity via the olfactory route, and potential pathogenic effects in immunocompromised persons.

Influenza

Cold-adapted LAIV (FluMist)⁸³⁴ is the only vaccine currently licensed in the United States for delivery by the IN route. Its development, testing, and licensure are reviewed in detail in Chapter 18. LAIV delivered by the IN route demonstrates several potential benefits of this method. It produces both mucosal and systemic immunity, and it provides higher protective efficacy than injected inactivated vaccine in young children.⁸³⁵⁻⁸⁴¹ It provides heterotypic immunity against influenza strains that had antigenically drifted from the vaccine strains.⁸⁴² It may reduce the risk of influenza transmission because it reduces respiratory shedding among immunized children challenged later with a vaccine virus.⁸⁴² Finally, modest coverage with LAIV among school children reduced influenza-related illness rates in unvaccinated adults in a community.⁸⁴³

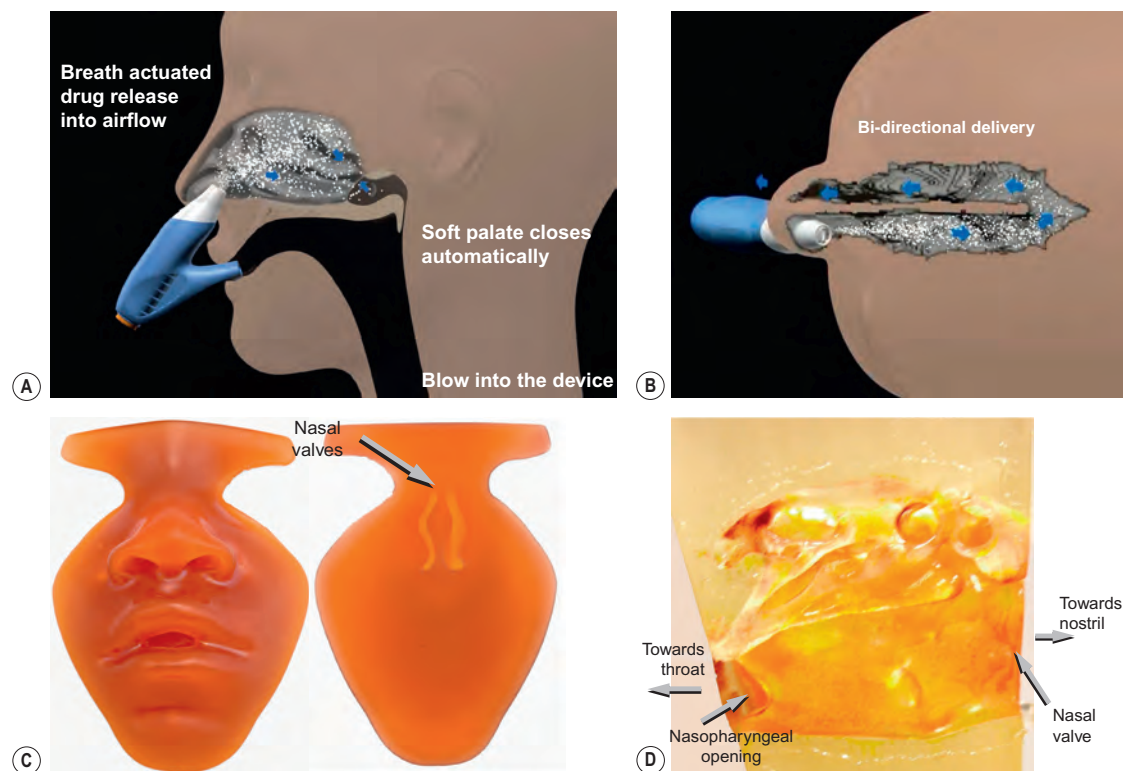


Figure 61-9 Modeling and Evaluation Techniques for Nasal Deposition of Vaccine Aerosols. (A) Sagittal and (B) horizontal sections rendered by computer-assisted imaging to illustrate intranasal delivery by investigational Optimist nasal spray device (OptiNose, Inc.^{754,792}). Exhaling into the device against pressure lifts the soft palate, closing off the nasal cavity and limiting pulmonary deposition. The breath actuates the release of liquid or powder particles, which are carried beyond the nasal valve to target sites. Image (B) shows the air flow passing through the communication posterior to the nasal septum and exiting through the other nasal passage. (C, left) Front and (C, right) rear views of a coronal section of the CFDR⁸⁰⁹ CT-based model shown and described in Figure 61-8F. Its plastic is darkened to highlight the external nasal valves. The rear view (C, right) shows the nasal valves, the transitional area between the squamous epithelia of the nares, and the mucosal tissue lining the internal nasal airway. (D) Medial section of the right internal nasal airway of a plastic, phantom model shown and described in Figure 61-8F (CFDR, Inc.⁸⁰⁹). The external nares and face (not shown) are to the right, proximal to the nasal valve. The pharynx (not shown) is to the left, distal to the nasopharyngeal opening. The red-pigmented powder indicates the deposition pattern from the investigational nasal dry-powder inhaler shown in Figure 61-8F. (Figure 61-9A, B, courtesy of OptiNose⁷⁹² [Per Gisle Djupesland]; 9C, courtesy of James Gathany (CDC Photographic Services); 9D, courtesy of Creare, Inc.⁸⁰⁵ [Darin Knaus].)

Measles

After influenza, measles is the next-most-studied disease for vaccine delivery via the respiratory tract, pioneered by Albert Sabin in the later years of his career.^{844–847} This evidence base prompted the Measles Aerosol Project, described earlier.⁷⁸⁵ Reviews and meta-analyses^{229,848–850} of multiple clinical studies revealed three basic immune-response patterns after measles vaccination.

First, drops or sprays delivered to the conjunctiva, or to the oral or nasal mucosa, produced inconsistent immune responses.^{226,851–859} Second, delivery of small-particle liquid aerosols via pulmonary inhalation to children 10 months of age or older typically produced immune responses in very high proportions of subjects. These responses to aerosol vaccinees were usually equivalent to or greater than the responses to injected vaccinees.^{778,779,783,851,852,856,857,860–870} For example, Dilraj and colleagues found that 96%, 94%, and 86% of schoolchildren who received the aerosol measles vaccine had antibody titers of greater than 300 IU/mL at 1, 2, and 6 years after vaccination, respectively, compared with 91%, 87%, and 73% among injected vaccinees.^{779,867,868}

The third pattern noted was generally lower immune responses for the aerosol route, compared with parenteral injection, among children younger than 10 months.^{776,777,844–847,855,862,865,871,872} For example, Wong-Chew and coworkers found vaccination of 12 and 9 month old infants by injection induced immunity in 100%, but by aerosol route in only 86% and 23%, respectively.^{776,777} One hypothesis was that the very low respiratory minute volume of young infants results in too small a dose of aerosol vaccine in that period of time. A follow-up study by Wong-Chew and colleagues demonstrated that increasing exposure time to aerosol measles vaccine elicits immune responses that are comparable to those seen when an equivalent dose is administered by the SC route in 9 month old infants.⁸⁷³ With regard to vaccine safety, the same reviews and meta-analyses^{229,848–850} noted that no severe adverse events were reported after aerosol measles vaccination in any of the studies. Rates of minor adverse events, when reported, have typically been less than or the same as vaccination by injection.^{776,777,779,864,866,874} Experience in mass campaigns was similar, with de Castro and colleagues reporting no serious adverse events among more than 3.7 million children in Mexico vaccinated by aerosol.⁸⁷⁵

Rubella and mumps

IN delivery of live attenuated rubella vaccine was investigated during the 1970s in many clinical trials.^{876–883} Ganguly and coworkers demonstrated that IN drops or spray of vaccine virus produced mucosal IgA antibody, equivalent serum IgG antibody, and better protection against reinfection compared with SC vaccination.⁸⁷⁸ The subjects who received the IN challenge, however, had higher rates of mild adverse events, usually rhinitis and sore throat.

In more recent studies, Bennett and colleagues found that aerosol vaccination of preschool children with a combination measles-mumps-rubella vaccine produced antibody responses to rubella and mumps equivalent to those produced by injection.⁷⁸³ Sepúlveda and coworkers found that aerosolized measles-rubella combination vaccine in school-age children not previously vaccinated against rubella produced high levels of rubella immunity, equivalent to that seen after SC injection. Fewer adverse events were reported in the aerosol group.⁸⁶⁴ Diaz Ortega and colleagues found that measles-mumps-rubella vaccination by aerosol in college students produced immune responses similar to those produced by injection, with seropositivity retained in all vaccinees 1 year after vaccination.^{869,870}

Live viruses as vaccine vectors

Recombinant viruses acting as vectors by incorporation of a gene expressing a heterologous antigen have advantages similar to those of conventional attenuated live virus vaccines. They deliver the genetic code for the antigen into cells, and it is replicated to activate the immune system. Viruses used as vaccine vectors should, ideally, have very low pathogenic potential, even in immunocompromised people, as well as the capacity to incorporate the necessary foreign genes for desired antigens, promoters, and adjuvants.

Viruses that naturally infect or grow in respiratory tissues are especially well suited as vectors for respiratory immunization. Some studied in animal models include adenoviruses, alpha viruses, poxviruses, baculovirus, vesicular stomatitis virus, and adeno-associated virus.^{884–899} Adenovirus vectors delivered IN in several animal models produced immune responses against many diseases.^{286,288,900–920} For example, defective-complex adenovirus containing Ebola virus genes protected nonhuman primates against aerosol challenge with two Ebola species.⁹¹⁹ Vaccinia strains such as modified vaccinia Ankara (MVA) have also been used effectively as vectors for respiratory immunization.^{921–927} An MVA vector expressing an HIV-1 antigen induced, by the IN route, antigen-specific mucosal CD8⁺ T cells in genital tissue and draining lymph nodes of mice, along with serum and vaginal antibodies.⁹²⁷ One caveat for the use of vectored vaccines is that preexisting immunity, in the population, to the vector virus, either by natural exposure or by previous use in another vaccine, may reduce its effectiveness. However, Song and colleagues reported a series of studies in which adenovirus-vectored vaccines delivered as a fine aerosol to the lungs produced strong immunogenicity even in animals with preexisting anti-adenoviral immunity, suggesting that pulmonary delivery may overcome this limitation to viral vector vaccines.⁹²⁸

Live bacteria

Animal models of respiratory immunization have been used to study attenuated respiratory pathogen vaccines such as *Mycobacterium bovis* (BCG) and attenuated *Bordetella pertussis*, as well as nonrespiratory pathogens such as *Salmonella* and *Shigella* acting as recombinant vectors.^{929–935} Mouse studies also demonstrated an improved immune response to conventional BCG vaccine delivered by the IN route or by aerosol inhalation, compared with injection.^{923,936–942} The studies that included a challenge found that the respiratory route provided better protection than injection. Attenuated *M. tuberculosis* has also been immunogenic by the respiratory route.⁹⁴³

As vectors, bacteria have an advantage over viruses because of their higher capacity for insertion of the heterologous genes expressing antigens, adjuvants, or plasmids for DNA vaccination (see next section).⁸¹¹ Recombinant BCG has been used to express various heterologous antigens, including simian immunodeficiency virus, *Borrelia burgdorferi*, and *Streptococcus pneumoniae*.^{944–947} Live attenuated *Bordetella pertussis* vaccine delivered by the IN route protected mice from *Bordetella* challenge.^{948–951} Similar IN delivery of recombinant *B. pertussis* expressing antigens of *Clostridium tetani*, *H. influenzae*, *N. meningitidis*, and *Schistosoma mansoni* induced strong immune responses in mice.^{952–955}

Attenuated recombinant *Salmonella* vaccines produced strong immune responses against a wide variety of pathogens when delivered by the IN route to rodents.^{935,956–965} Similar results were reported for IN *Shigella* vectors carrying enterotoxigenic *E. coli* and tetanus genes.^{966,967} Commensal bacteria such as food-grade strains of *Lactococcus*, *Lactobacillus*, and *Streptococcus gordonii* have also been explored as vaccine vectors.^{968–972} Bacterial expression of adjuvants such as cholera toxin B, interleukin (IL)-6, and IL-12 has been shown to increase the immune response to respiratory vaccines.^{936,973}

A potential risk of administering live microbes was revealed in mice that developed dosage-dependent granulomatous BCG infiltration of the lungs after IN but not SC vaccination of BCG.⁹³⁷ As with viruses, preexisting immunity to the bacterial vector may diminish the immune response.⁹⁷⁴

DNA vaccines

DNA vaccination involves the delivery of eponymous plasmids directly into host cells to express the desired antigens.⁹⁷⁵ Delivery of naked DNA to the respiratory tract as a vaccine has been studied in animal models for many diseases.^{439,926,976–995} For example, Kuklin and associates found that nasal delivery of a herpes simplex DNA vaccine generated higher levels of vaginal IgA than by the IM route, although the IM vaccine produced stronger serum antibodies and better protection against challenge.⁹⁹⁵ Live attenuated bacteria, especially *Salmonella* and *Shigella*, have been vectored to produce DNA for IN vaccination.^{966,996–999} For example, cotton rats vaccinated with attenuated *Salmonella* vaccine expressing DNA encoding for measles antigens resulted in significant reduction in measles virus titers in lung tissues after challenge.¹⁰⁰⁰ Virosomes, liposomes, and microparticles as carriers of vaccine antigens—discussed next—have also delivered DNA by the respiratory route.^{1001–1005}

Non-replicating vaccine delivery systems

Synthetic constructs, including liposomes, virus-like-particles (VLPs), virosomes, immunostimulating complexes (ISCOMs), microparticles, and nanoparticles, are nonreplicating delivery systems that mimic live viruses in how they appear to the immune system to enhance antigen delivery (they may also carry adjuvant). Their terminology is not mutually exclusive and some terms are used synonymously. The particles are about the same size as viruses, allowing similar uptake by antigen-presenting cells. Many include a lipid component to increase cell membrane permeability, and they may contain unrelated viral or bacterial proteins to activate the immune system.

Liposomes are vesicles composed of a phospholipid bilayer membrane. Antigen can be packaged in its aqueous core, inside the lipid bilayer, or on the outside of the membrane.^{1006–1008} A liposomal HIV-1 vaccine delivered to mice by the IN route resulted in strong IgG and IgA responses in serum and vaginal washes.¹⁰⁰⁹

VLPs are aggregates of viral proteins that may include a lipid component.^{1010,1011} IN vaccination of VLPs with influenza antigens similar to those of the 1918 pandemic strain protected mice and ferrets from lethal 1918 and H5N1 influenza virus challenge.¹⁰¹²

Virosomes have lipid bilayer membranes with embedded viral proteins and resemble viruses except that they lack the genetic material needed to replicate.^{1013,1014} Cusi and coworkers vaccinated mice by the IN route with a reconstituted influenza virosome assembled with plasmids expressing the carcinoembryonic antigen (CEA) gene.¹⁰¹⁵ The intranasally vaccinated mice developed CEA-specific antibodies but were not protected from challenge with CEA-expressing mastocytoma cells. However, when the CEA virosomal vaccine was coadministered by the IN route with reconstituted influenza virosomes with plasmids expressing the CD40L gene as an adjuvant, the level of antibody increased and the mice were protected from tumor-cell challenge.

ISCOMs are cage-like structures roughly 40 nm in size, composed of 12 subunits of saponin (such as Quil A) and cholesterol.¹⁰¹⁶ Several antigens administered in ISCOM-based IN vaccines produced strong systemic and mucosal immune responses.^{1016–1022} For example, IN administration of ISCOMs with a tuberculosis recombinant protein strongly boosted prior BCG immunity and reduced bacterial burden in the lungs compared with nonboosted mice.

Respiratory delivery can also be enhanced by packaging antigens and adjuvants into microparticles or nanoparticles composed of polymers of biodegradable materials such as polylactide (PLA) and polylactide co-glycolide (PLGA), or into biopolymers such as chitin or chitosan.^{1023–1032} Microparticles can be designed to slowly release antigens to increase the duration of antigen presentation. Carcaboso and colleagues reported that mice immunized by the IN route with a synthetic malaria vaccine encapsulated into 1.5- μ m-diameter microparticles of PLGA had significantly higher antigen-specific serum IgG titers than control mice given the vaccine by the SC route with alum adjuvant.¹⁰³⁰ Pulmonary immunization with chitosan microparticles containing diphtheria toxoid resulted in neutralizing antibody titers comparable to or significantly higher than those achieved after SC administration of alum-adsorbed diphtheria toxoid.¹⁰³²

Dry-powder formulations for respiratory delivery

Vaccines based on any of these delivery systems could potentially be formulated into powders for direct delivery in the dry state, a technique for which there is growing interest. For example, a PubMed search in October 2011 using the terms vaccine and powder yielded 33 articles published since 2000, related to respiratory delivery of powder vaccines, compared with only two prior to that year.

A number of obstacles must be overcome to produce successful respirable vaccines as dry particles of the sizes suitable for delivery to the respiratory tract.^{1033–1035} First, formulating powders requires significant and extensive changes in manufacturing methods, even from those used for current lyophilized vaccines. Second, many potential dry formulation ingredients are extremely hygroscopic and gum up when exposed to humidity. Engineering is needed to maintain their structure and dispersability for delivery in the dry state. Third, once the powders are deposited in the respiratory tract, they must be sufficiently hygroscopic to dissolve and release the vaccine for uptake.

Another challenge is that most dry-powder delivery devices require active inhalation by the patient and thus may be impractical for small children. Two potential solutions for this age group, however, are direct nasal delivery, as well as dispensing the powder into a reservoir (see, eg, Figure 61-8A,B) from which the child can breathe normally.⁸⁰³

On the other hand, there are several significant potential advantages to dry-powder vaccination. Doses can be filled into inexpensive, single-use presentations and delivered without on-site aqueous reconstitution, thus avoiding the occasional human error that results in using the wrong or contaminated

diluent. The cost of shipping and storing such diluents would be avoided. Secondary packaging that seals the dose container in an impermeable overwrap, such as metal foil, could maintain low humidity, which may prolong potency and increase shelf life. Recent progress for improving the thermostability of liquid vaccines, and even more so for dry ones,¹⁰³⁶ points to a future in which many vaccines may not require a cold chain.³⁶⁴

Measles vaccine powders

Measles vaccination has been a path-finding application for respiratory delivery of dry powder. Early formulations were finely milled and retained adequate potency, but immune responses were poor when delivered to the respiratory tract of macaques.^{1033,1035} An active developer is Aktiv-Dry,⁷⁹⁹ which is working with partners including the Serum Institute of India (SII), the CDC, and the University of Colorado. In 2005, its MVDP project was awarded over \$19 million in a Grand Challenges in Global Health grant from the Bill and Melinda Gates Foundation to refine a formulation, establish dry-powder measles vaccine production capacity at SII, and complete animal and phase 1 clinical testing.^{1037–1039}

Aktiv-Dry uses a novel spray-drying system to manufacture inhalable MVDP, starting from a bulk liquid of SII-provided, live attenuated antigen containing myo-inositol as a stabilizer. Virus plaque assays demonstrated potency losses in the drying process of 0% to 22%, which is comparable to losses seen with lyophilization.¹⁰⁴⁰ As reported earlier, the end product demonstrated immunogenicity in cotton rats and rhesus macaques.^{802,803} Its licensure-grade toxicology study found no test-article-related effects, or delayed onset of toxicity after inhalation by Sprague-Dawley rats.¹⁰⁴¹ A second toxicology study after administration by mask using either BD Solovent or PuffHaler to measles-seronegative rhesus macaques produced no effects in mortality, clinical observations, respiratory function, clinical pathology, or histopathology.¹⁰⁴²

SII manufactured MVDP, and its clinical trial application was approved by the Drug Controller General of India to conduct a phase 1 safety trial in adults, adolescents, and infants using the PuffHaler or BD Solovent devices. The trial began in May of 2012.

A separate project, reported by Ohtake and coworkers, found that a dry-powder measles vaccine, made by mild spray-drying and with unique stabilizers, was stable for up to 8 weeks at 37°C.¹⁰³⁶

Influenza vaccine powders

Dry-powder vaccines for influenza have been formulated and tested by several groups. A whole, inactivated virus product delivered by the IN route in rats elicited high titers of nasal anti-influenza IgA, as well as serum antibody titers equivalent to those obtained with injected vaccine.⁸⁰⁴ No loss of potency was found when it was stored at 25°C and 25% relative humidity for up to 12 weeks, and at 40°C and 75% relative humidity for 2 weeks.

Another formulation produced by spray-freeze drying, with subunit viral antigen and inulin stabilizer, induced, upon pulmonary delivery, humoral (IgG), cell-mediated (IL-4, interferon gamma), and mucosal (IgA, IgG) immune responses in BALB/c mice.¹⁰⁴³ The pulmonary route for a spray-freeze dried, whole inactivated virus vaccine stabilized with inulin provided protection similar to that provided by IM injection of mice exposed to a lethal dose of live virus.¹⁰⁴⁴

Powder formulations for other vaccines

Other human-disease targets for dry-powder delivery studies include tuberculosis, hepatitis B, norovirus gastroenteritis, anthrax, and plague. A spray-dried formulation of adenovirus-vectored tuberculosis antigen with mannitol-based stabilizers was shown to have characteristics suitable for

pulmonary delivery in terms of thermodynamics, water absorption, particle size distribution and morphology, and virus survival.¹⁰⁴⁵ Nanoparticle-aggregate formulations containing recombinant hepatitis B surface antigen administered to the lungs of guinea pigs produced high IgG and IgA titers.¹⁰⁴⁶ Nasal vaccination with Norwalk-virus VLPs with an inert in situ gelling polysaccharide induced systemic and mucosal antibody titers equal to or greater than those achieved by VLPs plus adjuvant in a liquid formulation delivered by the IN route.¹⁰⁴⁷ Dry-powder vaccines against bioweapons threats have been studied. For example, anthrax vaccine by the IN route provided complete protection against inhalational challenge with roughly 90 times the median lethal dose [LD₅₀], in rabbits, while providing better stability than liquid formulations.^{101,1048,1049} A stable powder vaccine against *Yersinia pestis* administered by the IN route to mice required but an extra dose of vaccine to achieve protection similar to that of IM delivery against plague after lethal challenge.¹⁰⁵⁰

Adjuvants for respiratory delivery

Nonreplicating antigens delivered via the respiratory tract are typically poorly immunogenic and may require adjuvants to stimulate an appropriate immune response. Adjuvants studied for this purpose include bacterial toxins and their derivatives, other bacterial components, bacterial DNA motifs, cytokines and chemokines, plant derivatives, and nanoemulsions.^{1051–1056}

Toxins

Cholera toxin (CT) and *E. coli* heat-labile toxin are potent adjuvants, but in native forms they may be too toxic for some uses in humans (see “Bacterial exotoxins”, earlier).^{1053,1057–1062} LT adjuvant in a commercial Swiss influenza vaccine for IN delivery was suspected as the reason for a many-fold increase in the risk of Bell's palsy after vaccination, leading to market withdrawal of the vaccine in 2001.^{46,47} Although the pathogenesis of the vaccine's effect on the seventh cranial nerve is uncertain, branches of the nerve do run near the nose. Other adverse neurologic effects of CT and LT have been hypothesized, based on their accumulation in the olfactory bulbs of BALB/c mice after nasal administration, sometimes with concurrent inflammation.¹⁰⁶³ As a result, recent adjuvant research has focused on subunits, detoxified versions, and other variants of CT and LT.^{1064–1081} Several of these, such as CTA1-DD, do not accumulate in the olfactory bulb of BALB/c mice.¹⁰⁸²

Structural bacterial components

Other bacterial products that induce potent activation of the innate immune system include lipopolysaccharide and its derivative, monophosphoryl lipid A, as well as outer membrane proteins, flagellins, lipopeptides, filamentous hemagglutinins, and proteosomes.^{815,1083–1093} The last are outer membrane proteins of meningococci, which self-assemble into hydrophobic, proteinaceous nanoparticles.¹⁰⁹⁴ An intranasally delivered, proteosome-based, inactivated influenza vaccine produced serum and mucosal antibodies in human subjects.¹⁰⁸⁴ *N. meningitidis* B proteoliposome-derived cochleate was demonstrated to be a potent mucosal adjuvant.¹⁰⁹⁵ Three doses of tetanus toxoid vaccine with this adjuvant administered by the IN route to mice promoted IgG serum titers and IgA titers in saliva and vaginal washes that were significantly higher than to tetanus toxoid alone.

Nucleotide stimulators of innate immunity

Oligodeoxynucleotides of cytosine and guanine with phosphodiester backbone (CpG ODNs) mimic motifs found in bacterial DNA. They are potent adjuvants, as the innate immune system recognizes these as *pathogen-associated molecular*

patterns.^{1096–1100} Abe and colleagues found that a nontypeable *H. influenzae* (NTHi) vaccine, delivered by the IN route with CpG ODNs, produced mucosal IgA and serum IgG responses similar to those produced by vaccine delivered with CT. Enhanced clearance of NTHi from the nasopharynx after challenge was shown equally in both groups.¹¹⁰⁰ The inclusion of CpG ODNs with four HIV peptide antigens in microparticles delivered by the IN route to mice significantly enhanced peptide-specific IgG and IgA peak titers and prolonged the duration of these antibodies, and it increased the sIgA response in mucosal washes.¹¹⁰¹ However, in another study, daily injections of high-dosage (60 µg) CpG resulted in lymphoid follicle destruction and immunosuppression with liver necrosis after 20 days.¹¹⁰² Therefore, the potential adverse effects of CpG ODNs should be studied.

Protein signalers

Because many adjuvants induce the activation of cytokines and chemokines, investigators have looked at these cellular signaling molecules as adjuvants themselves that might reduce adjuvant toxicity.^{1103–1110} Cytokines have been added directly to vaccine, or encoded for expression by a live vector or DNA vaccines.¹¹⁰⁸ Bracci and colleagues found that, in mice, a single IN dose of an inactivated influenza vaccine provided full protection against virus challenge when the cytokine interferon type I was included as an adjuvant.¹¹⁰⁹ Without it, the same dosage was only partially protective (40%). In mice, IN administration of pneumococcal surface protein A or tetanus toxoid, combined with the cytokine IL-1β, induced protective immunity equivalent to that induced by parenteral delivery.¹¹¹⁰

Natural polymers

Chitin is a natural polysaccharide found in crustaceans. Its partial deacetylation yields chitosan, which is widely used in food products, as an excipient in drugs, and as a nutritional supplement.¹¹¹¹ Chitin and chitosan have mucoadhesive properties and stimulate the innate immune system.¹¹¹² In humans, the addition of chitosan to a detoxified diphtheria toxin based on CRM-197 significantly increased toxin-neutralizing antibody levels upon IN delivery.¹¹¹³ The saponins of the *Quillaja saponaria* tree are potent adjuvants with high toxicity. Quil A, QS-21, and Iscoplep 703 are *Q. saponaria* derivatives with less toxicity.¹⁰⁵⁴ As an adjuvant for an IN HIV-1 DNA vaccine studied in mice, QS-21 consistently increased antigen-specific serum IgG and mucosal IgA compared with vaccine without adjuvant.¹¹¹⁴ Quil A and Iscoplep 703 are commonly used as components of immunostimulating complexes.

Combining adjuvants for respiratory vaccination may synergistically enhance immune protection. For example, IN delivery to mice of an influenza recombinant hemagglutinin (rHA) antigen, along with a combination of proteosomes and lipopolysaccharide adjuvants, enhanced serum IgG and mucosal IgA antibodies up to 250-fold compared with vaccine alone.¹⁰⁸⁸ Also, IN delivery of an influenza vaccine with a combined CTA1-DD/ISCOM adjuvant vector was superior to other vaccine formulations using the ISCOM or CTA1-DD adjuvants alone.¹¹¹⁵

Nanoscale mixtures

Nanoemulsions are another class of adjuvants studied for respiratory vaccination. A soybean oil-in-water nanoemulsion was mixed with either US-licensed Fluzone,⁹⁷ or Fluvirin⁴⁴³ inactivated, influenza vaccine (usually injected by the IM route), and delivered by the IN route to naïve ferrets.¹⁰⁵⁶ Resulting seroconversion rates were 67% to 100% against each of the three viral strains present in the vaccine. There was also protection against homologous

viral challenge and significant cross-immunity to five other H3N2 influenza virus strains not present in the vaccine.

Respiratory vaccination in veterinary practice

The respiratory route of vaccination is common in veterinary medicine.¹¹¹⁶ Aerosol vaccines for the IN route or by pulmonary inhalation are commercially available for cows (bovine herpes virus 1, parainfluenza virus 3), pigs (*Salmonella*), horses (influenza, *Streptococcus equi*), dogs (*Bordetella bronchiseptica*), cats (feline calicivirus, feline herpesvirus 1), and chickens (infectious bronchitis virus, infectious laryngotracheitis virus, Newcastle disease virus). Almost all of the respiratory veterinary vaccines have live attenuated pathogens. In the United States, more than 8 billion chickens are vaccinated yearly using live attenuated vaccines delivered as aerosols or spray.¹¹¹⁷

Respiratory vaccines for bioterror agents and pandemic threats

Many biological agents for potential bioterrorism or biowarfare cause life-threatening respiratory infections and would probably be disseminated as aerosols. Thus, vaccine-induced mucosal immunity may be advantageous. Compared with the parenteral injection, respiratory vaccination increased survival after aerosol exposures of deadly agents in animal studies.^{1048,1049} For example, a microsphere-based liquid anthrax vaccine delivered by the IN route to mice completely protected against aerosol challenge with anthrax spores.¹¹¹⁸ Two doses of human parainfluenza-virus-vectored Ebola vaccine were highly immunogenic in macaques and protected all animals against lethal Ebola virus challenge.¹¹¹⁹ A powdered formulation of anthrax vaccine with CpG ODNs administered intranasally to rabbits also provided full protection.¹⁰¹ Other bioterror agents for which respiratory vaccines have shown increased protection against aerosol challenge include *Francisella tularensis* (tularemia), staphylococcal enterotoxin B, *Burkholderia mallei* (glanders) and *Y. pestis* (plague).^{1050, 1120-1125}

The threatened pandemic of severe acute respiratory syndrome (SARS) in 2002-03, and the actual one of H1N1 influenza in 2009-10, illustrate the critical need for prompt development of new vaccines and their rapid delivery in all countries potentially affected. In responding to future threats when new vaccines may be required, respiratory delivery may be useful for the various reasons already described. Simple devices, such as single-use dry-powder inhalers, could be distributed by mail and self-administered for mass vaccination if congregating crowds for conventional campaigns were deemed unwise.

IN delivery of *Salmonella*-vectored vaccine against the SARS coronavirus resulted in higher production of specific IgG and IgA than orogastric, intraperitoneal, or intravenous administration, and it provided high levels of specific cytotoxic T lymphocytes in BALB/c mice.¹¹²⁶ Two IN doses of live attenuated H5N1 influenza A vaccine fully protected mice and ferrets against pulmonary replication of homologous and heterologous strains of wild-type H5N1.¹¹²⁷ Such cross-protection against diverse strains would be desirable for pandemic vaccine because of potential rapid changes in influenza surface antigens. For example, IN administration of inactivated, whole-virus H5N1 vaccine with adjuvant elicited immune responses with both sIgA in nasal, lung, and vaginal lavage, and IgG in serum, showing protective immunity against lethal H5N1 challenge and cross-clade protection.¹¹²⁸ Also, aerosolized LAIV provided heterologous protection against pandemic H1N1 virus challenge in ferrets.⁸⁰⁷

Conclusion

Cutaneous, jet-injected, and respiratory methods for vaccine delivery overcome the dangers and often the hidden costs of traditional needle and syringe. Some long-standing, many novel, these techniques may offer other advantages in terms of dosage sparing, immune response, economics, thermostability, patient and user preference, and expanded venues for use.

Many promising techniques described in this chapter, however, face daunting obstacles to bridge the gap between successful proofs of principle in animal models by academic laboratories, and the expensive and complicated series of clinical trials (particularly for the many target diseases lacking convenient laboratory assays that predict protection) and related studies and regulatory steps to achieve licensure.³⁰ Indeed, the financing of all these stages requires investors to envision methods for their commercial-scale manufacturing and to predict demand in a rather monopsonistic market.

Finally, at the downstream outlet of the vaccine R&D pipeline, public health and immunization program policymakers, end-user purchasers, and (nowadays) independent philanthropic entities must be convinced by their own economic analyses and other considerations to pay for these fruits of immunization science. Perhaps some of the new technologies described and illustrated herein will help fulfill the widely admired goal that "all people deserve the chance to live healthy and productive lives".¹¹²⁹

Disclosure

Coauthor M. J. P. is a coinventor with corresponding financial interests in the AeroVax (AerovectRx, Inc.,⁸⁰⁸ Creare, Inc.⁸⁰⁵) and dry-powder inhaler (CDC, Creare⁸⁰⁵) devices illustrated in Figures 61-7E,F and 61-8F, respectively.

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