Section 3: Vaccines in development and new vaccine strategies

Alternative vaccine delivery methods

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The earliest known route of vaccination was intranasal, by insufflation of scab material containing variola virus from smallpox patients, described in China around the first millennium AD (see Chapters 1 [history] and 30 [smallpox]). The cutaneous route for such variolation involved breaking the skin with a sharp instrument and was used in India perhaps as early as in China, but not documented until the 16th century. Variolation was supplanted by safer cutaneous vaccination using material from cowpox lesions, a method known in the 18th century and first published by Edward Jenner. After 15th century experiments with hypodermic injection, the introduction of the needle and syringe (N-S) in the mid 19th century by Pravaz, Rynd and Wood, began a new era in medicine. Pasteur used a Pravaz syringe to inoculate sheep in the famed controlled challenge experiment demonstrating anthrax ‘vaccination,’ a term henceforth broadened to the administration of immunizing agents for various diseases, not just smallpox.

Upon acceptance of the germ theory and resulting sterilization by the early 20th century, 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 vaccine antigen for cutaneous vaccination using N-S, and among non-medical intravenous drug users everywhere. Other drawbacks of N-S include needlestick injuries to health care workers, needle-phobia and discomfort for patients facing increasingly crowded immunization schedules, and the costs and complexity of safe disposal of sharps in the medical waste stream. In the early 21st century, preparedness efforts for threatened pandemics and bioterrorism, as well as new targets for disease control or eradication have rekindled an interest in mass vaccination campaigns, and stimulated research on vaccine delivery not requiring N-S.

Existing and potential alternatives to conventional intramuscular (IM) and subcutaneous (SC) vaccination by N-S are classified here into three major categories: cutaneous, jet injection and respiratory. The cutaneous route may be subdivided into 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. 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 earlier, the skin was one of the first tissues into which variola (smallpox) virus—and later cross-protecting cowpox virus—were introduced to prevent smallpox. Cutaneous immunization remains today the standard route for smallpox vaccine (now containing related vaccinia virus) (see Chapter 30 [smallpox]), as well as for administering Bacille Calmette–Guérin (BCG) to prevent tuberculosis (see Chapter 33 [tuberculosis]). Various adjectives have been used to describe vaccination into or onto the skin (e.g., cutaneous, dermal, epicutaneous, epidermal, intradermal, patch, percutaneous, skin, topical, and transcutaneous). In this chapter these are encompassed within the general term cutaneous vaccination.

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 (Fig. 61-1). The major constituent of this stratum Malpighii, as it is known, is the keratinocyte, which serves both a structural function in limiting the passage of water and other molecules, and an immunologic role. This cell germinates just above a basement membrane and then grows, flattens, matures and senesces in increasingly superficial strata until it reaches the surface and is sloughed. The main product of this cell is keratinohyalin, a dense lipid which 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.

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, 0.01 to 0.02 mm thick, represents 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 are found, including small blood vessels, lymphatic vessels, nerves, hair follicles, sweat and sebaceous glands. The subcutaneous tissue below, sometimes referred to as the hypodermis, consists primarily of fat, and varies widely in thickness among different body surfaces and, of course, individuals. Faster passive diffusion of therapeutic substances transcellularly through the dead and living keratinocytes, and via intercellular channels.
between them, correlates with smaller molecules (<500 Da), lower melting points, increased lipophilicy (and correspondingly lower water solubility), higher (saturated) concentrations, and the paucity of pendant groups that form hydrogen bonds that slow diffusion.

The specific mechanisms which produce the resulting immune response when vaccine antigen is introduced into the skin are not entirely clear. Upon stimulation, keratinocytes can produce pro-inflammatory cytokines (interleukin 1) and can themselves function as antigen-presenting cells by displaying major histocompatibility complex (MHC) class II antigens (HLA-DR), as well as intercellular adhesion molecules (ICAM-1). Epidermal Langerhans cells are believed to play a key role in cutaneous immunization, although other well-known immune system players also circulate or reside in the epidermis or dermis, such as CD8+ and CD4+ T lymphocytes, mast cells, macrophages, and dermal dendritic cells. 29–32

The immature Langerhans cells reside like sentinels among the keratinocytes in the epidermis, comprising about a quarter of the skin surface area,22,23 where they efficiently capture foreign antigen by phagocytosis or endocytosis. As with similar dendritic cells in other tissues (see Chapter 5 [immunologic adjuvants]), upon activation (Fig. 61–1) 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.

Classical intradermal injection with sharp instruments or needles

Traditional vaccination for smallpox

During the more than 200 years of cutaneous vaccination against smallpox (see Chapter 30 [smallpox]), 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 (see Fig. 61–2). 5 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 multiple smaller 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 for revaccination. 34 Multi-tines devices have also been used. 36,37

Tuberculosis vaccination

The Bacille Calmette-Guérin (BCG) vaccine for the prevention of disease from Mycobacterium tuberculosis was originally administered orally in the 1920s (see Chapter 33 [tuberculosis]). Safety concerns prompted a shift to cutaneous administration by ID needle injection (1927), 27 and later multiple puncture (1939), 38–41 scarification (1947), and multi-tine devices, 36 as described above for smallpox vaccine. BCG has also been delivered cutaneously by bifurcated needles 42 and jet injectors. 43

Mantoux method

The ID needle technique used for BCG was originally developed by Felix Mendel 44 and Charles Mantoux 45 in the early 20th century for the administration of tuberculin (now replaced by purified protein derivative) for the diagnosis of tuberculosis infection. It is now called the Mantoux method. This procedure has become the common route for ID injection of various antigens (Fig. 61–2E). A short-bevel, fine-gauge needle, usually 27 gauge (0.016 inch, 0.406 mm diameter), is inserted, bevel up, almost parallel at a 5–15 degree angle into slightly-stretched skin, often the volar surface of the forearm. 46 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 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 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 dose-sparing effect of ID vaccination, reducing needed antigen by up to 80 percent in reducing dose volume to 0.1 mL from the common 0.5 mL, has prompted renewed attention to this route because of concern for emerging threats like pandemic influenza, SARS, and bioterrorism that may leave populations vulnerable due to insufficient vaccine supply. Both old and new techniques can more easily achieve the effect of the Mantoux method in depositing the injectate into the skin to
produce a visible wheal of temporary induration. Since the 1960s, multi-use-nozzle jet injectors (discussed in more detail below) delivered smallpox, BCG, and other vaccines ID by use of specialized nozzles (Fig. 61–2G).47–49 Modern disposable-cartridge injectors are being adapted with spacers to achieve that same route (Fig. 61–2H).50–52 Requiring less skill than the Mantoux method, a new investigational ID syringe with a 30-gauge needle (outer diameter \[\text{OD} \approx 0.305 \text{ mm}\]) that projects only 1.5 mm beyond its depth-limiting hub is inserted perpendicularly to deposit the dose into the skin (Fig. 61–2F).53,53a A 34-gauge equivalent (OD \[\approx 0.178 \text{ mm}\]) for animal models produced good immune responses to recombinant protective antigen (rPA) for anthrax,54,54a conventional hemagglutinin (HA) and plasmid DNA antigens for influenza,55 and live recombinant yellow fever vector for Japanese encephalitis vaccines.56 ID-immunized rabbits challenged with \(\approx 100 \text{ LD}_{50}\) of \textit{Bacillus anthracis} spores had identical survival rates (no adjuvant: 100%, aluminum salt adjuvant [alum]: 100%, CpG: 83%) as IM-immunized controls.54

**Other intradermal vaccines**

In addition to smallpox and BCG, mentioned above, as well as combined BCG-smallpox vaccine,58,59 over a dozen other vaccine types have been administered ID.

**Influenza**

There is a substantial literature, since the 1930s, starting with Thomas Francis (of Salk polio vaccine trial fame),60 documenting the equivalence and occasionally improved immunogenicity of ID influenza vaccination by needle-syringe compared to larger doses by the SC and IM routes.57,61–79 On the other hand, a few studies found ID influenza responses less than IM or SC on some or all of the antigens that were studied.80–85 When identical amounts of reduced antigen were compared between the ID and IM or SC routes, there were conflicting results from mid-century trials using the whole-cell products of that era. Bruyn et al found GMTs in children receiving 0.2 mL intradermally of influenza vaccine to be higher than those receiving the same dose SC,84 as did Davies et al86 and Tauraso et al87 administering 0.1 mL, by both routes. When administering...
by ID one-tenth (0.1 mL) the SC dose (1.0 mL) in varying dilutions below the labelled dosage of 800 chick cell agglutinating (CCA) units per mL, Stille et al also found greater ID responses, but only when the SC dose was low, at 8 or 0.08 CCA (ID dose: 0.8 and 0.008, respectively).\textsuperscript{77} Conversely, SC responses exceeded ID ones when the standard SC dose was used or reduced by only one log (80 CCA, ID: 80 and 8 CCA, respectively). This suggested a linear ID dose-response curve, but a sigmoid SC one, which favored the ID route at the lower-dose end. On the other hand, when identical reduced doses for a new shifted ‘Asian’ strain were given by the two routes (80, 40, or 20 CCA, compared to 200 per full 1.0 mL), both McCarroll et al\textsuperscript{77} studying hospital employees 18 to 65 years of age, and Klein et al\textsuperscript{86} studying infants 2 months to 5 years of age, found little difference in responses between the ID and SC routes. McCarroll speculated the ID superiority in earlier studies was due to 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 Klein et al study, febrile reactions were reported among 34.7% (17/49) in the intradermal group and only 19.2% (10/52) in the subcutaneous group getting the same reduced dose.\textsuperscript{86} Similarly, local reactions of small areas of erythema and induration with 2 to 3 days of slight tenderness and itching were described for ‘all’ intradermal participants (ages 2 month to 5 years, \( n = 96 \)), while only 2 of 94 children vaccinated subcutaneously had local pain and induration. Considering the entire reduced-dose, ID influenza literature, one might conclude that this route may be considered when antigen shortages and distributive equity demand the use of the lower end of the dose-response curve, where ID may outperform the SC/IM routes. The increased reactions described in these whole-virus studies may be mitigated by the modern use of less reactogenic split-virus products.

Other conventional vaccines by intradermal route

The ID route was used extensively for the live, attenuated yellow fever French neurotropic vaccine (FNV), which was given by ID scarification in the 1940s and 1950s in Francophone Africa (see Chapter 36 [yellow fever]).\textsuperscript{86} The 17D strain showed both good\textsuperscript{90} and poor\textsuperscript{91} immune responses when jet-injected ID. The ID route also yielded mixed results for live measles vaccines.\textsuperscript{92-104}

Inactivated vaccines with good immune responses after ID injection include typhoid\textsuperscript{105} and rabies,\textsuperscript{106-110} the latter of which has been used widely for dose-sparing purposes in the developing world.\textsuperscript{111} Salk’s first clinical trials of inactivated poliovaccine administered it ID,\textsuperscript{112,113} a route widely used for millions of Danes in the mid-1950s,\textsuperscript{114,115} but studied little since despite good responses.\textsuperscript{116-121} Generally good results have been reported for ID hepatitis B,\textsuperscript{122-130} with some exceptions in infants\textsuperscript{131-135} and with recombinant vaccine.\textsuperscript{136-138} Mixed results have been reported for cholera\textsuperscript{139} and hepatitis A.\textsuperscript{140,141}

Other non-living vaccines studied rarely by this route include meningococcal A,\textsuperscript{142} diphtheria-tetanus-pertussis,\textsuperscript{143} tetanus\textsuperscript{144} and rabies,\textsuperscript{145} tickborne encephalitis\textsuperscript{146} and Rift Valley fever.\textsuperscript{147}

Investigational intradermal vaccines

ID injection—as well as IM—led to the serendipitous discovery in an influenza model\textsuperscript{148} that viral genes encoded into bacterial DNA would somehow get expressed in vivo into their protein antigens, a seminal event in the modern era of recombinant nucleic acid vaccinology.\textsuperscript{149} Gene proto-antigens were used to prevent influenza,\textsuperscript{150} HIV/AIDS,\textsuperscript{151,152} smallpox,\textsuperscript{153} and many other diseases are being inserted into both ‘naked’ DNA/RNA\textsuperscript{155} and various vectors such as modified vaccinia Ankara (MVA) virus, for delivery by the ID route. ID jet injection has been used for immunomodulators like interferon.\textsuperscript{156}

Novel methods to deliver antigen past the stratum corneum

Various commercial patch delivery systems developed since 1981 have demonstrated the ability of certain therapeutic agents (e.g., scopolamine, nitroglycerin, clonidine, estradiol, fentanyl, nicotine and testosterone) to diffuse passively into bare, untreated skin without the use of the active technologies or enhancers described below.\textsuperscript{157} But such passive diffusion usually works only for small molecules of certain physical characteristics. Thus, there are but a few animal models of immunization onto bare, untreated skin.\textsuperscript{156-158} 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.\textsuperscript{159-161} Some methods combine several processes.

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 upon subsequent application of various antigens and adjuvants to the skin in mice.\textsuperscript{162,163} Similarly, rubbing gauze, emery paper, EKG pads, or pumice on the skin removes cells by their abrasive effects, and have been found to enhance immune responses in humans.\textsuperscript{164}

Shaving and brushing

The razor and the brush work as well. In a clinical trial of adenovirus vectors encoded to express influenza HA 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\textsuperscript{TM} patch.\textsuperscript{165} Two doses 28 days apart at the highest dose level produced 4-fold rises in HI titer in 67% of the cutaneous vaccinees. Occasional mild erythema at the abdominal site was reported in 61% and rash/itching in 39% of patients. This same research team, studying mice, substituted an electric trimmer for shaving but otherwise used similar brushing to demonstrate that topical application of non-replicating Escherichia coli vectors overproducing antigens for Clostridium tetani and B. anthracis were immunogenic.\textsuperscript{166,167} Control animals demonstrated that depilation alone had little effect; what made the difference was the mild brushing that produced minimal irritation (Draize scores \( \leq 1 \)).\textsuperscript{168}

Uncoated microtines

Other methods to abrade the stratum corneum take advantage of low-cost fabrication techniques adapted from the microelectronics industry to produce arrays of large numbers of submicron- to millimeter-sized tines (sometimes referred to as solid microneedles) of silicon, metal, or other material.\textsuperscript{169,170} One technology that abrades the skin before or after topical application of the antigen or therapeutic agent is named a microenhancer array (MEA) and consists of a square or round chip of about 1 cm\textsuperscript{2} area of silicon or plastic microprojections that are mounted on a hand-held applicator (OnVax\textsuperscript{TM}, Fig. 61-3A).\textsuperscript{171}

Clinical studies of the MEA device in mice inoculated with DNA plasmids encoding firefly luciferase and HBsAg found similar or greater light emission and immune responses,
respectively, compared with control IM and experimental ID injections. Anthrax rPA with alum or CpG adjuvants applied by MEA device to mouse skin produced equivalent or better immune responses than IM controls (although not as good as an ID microneedle), while immune responses and challenge survival were significantly less among MEA-immunized rabbits compared to IM controls. 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 (JE) vaccine. Those vaccinated by swiping the MEA through a drop of vaccine already on the skin showed neutralizing antibody responses in the same range as for SC controls, while applying vaccine after the abrasion appeared less effective.
A clinical trial of the MEA measured transepidermal water loss (TEWL) as a surrogate indicator for removal of the stratum corneum following 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 showed little or no TEWL.259

Coated microtines

Another method to carry antigen across the stratum corneum is by coating it onto solid microscopic projections or microtines, from which it dissolves and diffuses while held for variable periods of time in the epidermal layer.170 But their suitability for human vaccination has not yet been fully demonstrated.21,177

One example of microtines is the investigational Macroflux® microprojection array,178 whose projections vary from 225 to 600 µm height and are packed into an area of 1 to 2 cm² at densities from 140 to 650 tines per cm². They are inserted by a spring-mounted applicator and held in place by an adhesive patch (Fig. 61–3B). In a hairless guinea pig model, ovalbumin as a representative large antigenic protein was applied to the tines and administered in two doses 4 weeks apart.179,180 Post-booster titers for the device were comparable to control IM, SC and ID Mantoux method injections at higher doses, and surpassed IM and SC routes at lower doses. Other preclinical studies of the Macroflux have demonstrated delivery of oligonucleotides181 and the peptide hormone desmopressin.192

Another array of microtines is termed a Microstructured Transdermal System (MTS),193 and consists of drug-coated pyramidal projections of 250 µm height, in a density of 1,300 projections per cm², again mounted on an adhesive patch and applied with a spring-powered applicator (Fig. 61–3C).184-187 In a rabbit model, several formulations in various ratios of tetanus toxoid and alum adjuvant coated onto the microtines induced antibody levels an order of magnitude higher than the presumed protective threshold (>0.2 IU), using just a fraction of the standard IM dose.21 Experimental placement of the device on human volunteers found it to be ‘well-tolerated,’ ‘non-intimidating and not painful.’218

Among others working with microtines, Coulman et al 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.219 After applying the microtines to the skin for 10 seconds, they were able to verify epidermal penetration and gene expression by a variety of histologic and photometric means. Kwon et al developed biodegradable microtines made by dissolving drug in carboxymethylcellulose and casting into a solid by centrifugation in a mold and air drying (DrugMAT™, VaxMAT™).190,191 Others conducting work with microtines (solid microneedles) include Corium214,195 and Valeritas (Micro-Trans™).196

Injecting microneedles

Hollow projections termed microneedles, produced by similar techniques as for the solid microtines described above, are designed to inject therapeutic agents through their tiny cannulae (Fig. 61–3D).20,176,178 Although harder to manufacture and more easily broken and clogged,174,217 flow rates of microneedles have been measured up to a remarkable 1 mL per minute per cannula.196 Common lengths are 0.2 to 0.5 mm, short enough to be painless since their depth does not reach nerve endings in the dermis198,199. Among those working on such microneedles are the Georgia Institute of Technology,198,200 Norwood Abbey,201 NanoPass (MicroPyramid™, MicronJet™),202 SpectRx (SimpleChoice™),190 and Valeritas.107

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 two ways to breach the stratum corneum. In one, a brief pulse of laser light ‘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.20,27,203-205 One device, the LAD (laser assisted drug delivery, Norwood Abbey)206 generates an erbium-doped yttrium-aluminum-garnet (YAG) laser beam whose energy is highly absorbed by skin (Fig. 61–3E).207 It was shown in adult volunteers to facilitate the anesthetic effect of the topical application of lidocaine,208 and is licensed in the U.S. and Australia for that purpose. In another method, a high-power pulsed laser creates a photomechanical wave that drives particles representing drug carriers through the stratum corneum,207,209 Preclinical or clinical studies for intended vaccination using such laser methods have not yet been reported.

Electrophoresics

Iontophoresis—first demonstrated a century ago in rabbits210—uses an electric current to drive charged molecules from an electrode of the same charge towards another of opposite charge located elsewhere on the body.22,27,211-215 Among licensed devices applying this technique for skin anesthesia are the LidoSite™ (Actyve™ technology)216 and the IONSYS™ (E-TRANS® technology).217 A related method is electro-osmosis, which induces a flow of solvent to carry non-charged molecules.190,218 Voltages above 1 volt in themselves increase skin permeability, perhaps by opening up pathways along hair follicles. But these techniques do not work well at higher molecular sizes, which characterize many vaccine antigen proteins.

Thermoporation and electroporation

Thermoporation, also termed microperoration, uses the heat of electrical resistance to vaporize tiny openings in the stratum corneum.22,27,219 In the PassPort™ system,220 a disposable array of metallic filaments is held momentarily against the skin by a device the size of a computer mouse which, upon activation, induces electric pulses in the filaments (Fig. 61–3F). An adhesive patch containing vaccine or therapeutic agent is then applied over the micropores just created. In a hairless mouse model, this technique elicited 10–100-fold greater cellular and humoral responses to an adenovirus vaccine compared to intact skin, as well as 100 percent protection to surrogate tumor challenge (27 percent for intact skin).221 In the same model, adenovirus-vectorized melanoma antigen applied to the micropores roughly doubled the average onset time of tumors by challenge, and protected 1 of 6 mice compared to 0 of 8 vaccinated controls with intact skin. Microporated recombinant influenza H5 hemagglutinin protected BALB/c mice from challenge with a lethal H5N1 strain.222 Skin micropores also permitted the passage of insulin in pharmacokinetic human trials with historical controls,223,224 and in the other direction allowed interstitial fluid to be extracted for potential glucose monitoring.225 Another method generates micropores with heat induced by radiofrequency waves (ViaDerm™).224

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.226-230 In vitro and in vivo preclinical studies of this technique demonstrated entry into or through the cells of larger molecules, such as heparin (12 kDa), peptides and proteins (such as luteinizing-hormone-releasing hormone), and oligonucleotides (up to 24-mer), which hold promise for
polysaccharides, proteins, nucleic acids, and even adenovirus vectors as vaccine antigens.212,213,214,215 IM electroporation is also being pursued to enhance vaccination with DNA antigens.216,217,218 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 ,236 Easy Vax Electrokinetics Device (EKD),237 ECF,238 MedPulse ,239,240,241 and TriGrid242,243,244 among others.

Sound energy

The convection between keratinocytes can be solubilized to facilitate drug or antigen delivery by ultrasonic waves and short-duration shock waves.245,246,247 These are theorized to induce cavitation—the formation and collapse of microbubbles—which disrupts the intercellular bilayers within 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 to the subcutaneous route when subjected to 20 kHz ultrasound.248 High-molecular weight molecules delivered include insulin, erythropoietin, interferon, and low molecular weight heparin.249,250,251,252 Various groups are pursuing ultrasound for enhanced drug delivery.253,245,246

Kinetic deposition

The transfection of cells by use of kinetic methods to deposit DNA-coated gold particles into them was pioneered in the 1980s.253 The Helios or PDS 100/1 HE ‘gene guns’254 and the Accell injector255 have become standard bench tools for ‘biolistic’ delivery of nucleic acid plasmids into a wide variety of plants and animals to tranfect them to express the coded genes.256,257 Delivery of DNA into the skin overcomes the usual polarized Th1 response when DNA is delivered into muscle.252,253,254 These devices are unavailable for human vaccination (patent rights are held by PowderMed255). Documenting the safety of DNA as antigen by any route remains a major regulatory obstacle for such a paradigm shift in human vaccination.255

Powder/particle technology

The proprietary terms epidermal powder immunization (EPI) and particle-mediated epidermal delivery (PMED) refer to the use of helium gas to blow into the epidermis at supersonic speeds powdered proteins, polysaccharides, or inactivated pathogens, or DNA-coated particles, respectively.258 This unique method of vaccination was developed in the early 1990s by Oxford BioSciences, which over the years was renamed PowderJet, acquired by Chiron,259 spun off as PowderMed,257 and acquired by Pfizer260 in 2006. Delivery is by either reusable (XR series) or single-use disposable (ND series) devices (Fig. 61–3G), with the latter targeted for commercialization.

Conventional protein antigens for delivery by EPI are spray-dried into powders of suitable density and size (20–70 μm),261,262 but the economics of manufacturing such formulations may be an obstacle.263 For DNA vaccines delivered by PMED, plasmids coding for desired antigens are coated onto gold beads (1–5 μm in diameter) and upon their deposition into epidermal antigen-presenting cells are eluted and transcribed.264 Human trials of DNA vaccines containing up to one order of magnitude less antigen than used for IM routes have induced humoral and cellular immune responses for hepatitis B in subjects both naïve and previously vaccinated with conventional vaccine.265,266 PMED vaccination has also been studied for DNA priming in trials of malaria vaccine candidates and produced the first seroprotective immune responses by a DNA vaccine for seasonal influenza.267,268 Clinical trials still ongoing or unpublished studied antigens for H5 avian influenza (DNA),271 herpes simplex virus 2,272 HIV and non-small cell lung cancer.273,274

In the hepatitis B and influenza trials cited above, 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 subjects267 through day 180 in 21 (25%) of 84 injection sites210 and beyond 12 months in 5 (25%) of 20 patients with long-term followup.267 No anti-double-stranded DNA antibodies were detected. The disposition of the gold particles was studied in pigs, in whom most particles were deposited in the stratum corneum and epidermis, and eventually sloughed by exfoliation by 28 days.255 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. 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 encephalitis viruses,266 hantaviruses,273 HIV,274 malaria,275 SARS coronavirus276 and smallpox.277

Other kinetic methods

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 where channels are created in the stratum corneum, to which drug is then applied.278 Another method employs a fast and powerful contractile fiber-activated pump to fire drug at the skin with sufficient velocity to penetrate the epidermis.279 A miniaturized form of traditional jet injection uses piezoelectric transducers to propel liquid microjects into the skin.280

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.158,281 Rubbing the skin with acetone also enhances antigen passage by extracting epidermal lipids.159

Bacterial exotoxins

Discovery of the remarkable adjuvant effect of bacterial ADP-ribosylating exotoxins, such as the B (binding) subunits of cholera toxin (CT) and the structurally-similar, heat-labile toxin (LT) of enterotoxigenic E. coli (ETEC), has prompted much interest and work (see Chapter 9 [Cholera]).156,282–285 For safety reasons, these toxins have been engineered, or mutants selected, to reduce toxicity while retaining adjuvanticity.286–289 Nevertheless, one such use as adjuvant in a licensed intranasal influenza vaccine was hypothesized as the cause of temporary paralysis of the 7th cranial nerve, prompting market withdrawal.290

Iontal technology

Skin vaccination using CT or LT as adjuvants and antigens has been advanced principally by Iomai,290 which calls the process transcutaneous immunization,293,294 although others have also studied this technique.295 Such toxins may be administered by themselves as antigen to induce immunity against ETEC causing traveler’s diarrhea or against Vibrio cholerae, either with296 or without297,298 ETEC colonization factor (Fig. 61–1). A randomized, blinded field trial among travelers to Central America found 75% efficacy for the LT patch in protecting from moderate/severe diarrhea.299 Their adjuvant effect has been exploited for influenza vaccines, which have generally the lowest rates of immune response and efficacy among licensed vaccines, particularly in the very young and old. Applying an
LT patch near the site of injection of conventional parenteral influenza vaccine was found to improve HI titers in the serum and mucosa of both young and aged mice,301,302 and to increase or show an improving trend for adult volunteers over 60 years.303 The use of CT or LT as cutaneous adjuvant has resulted in improved immune responses or challenge protection in animal models for tetanus,314 anthrax,305,306 malaria307 and Helicobacter pylori.308 Clinical trials found no serious reactions, but pruritis and maculopapular rash at the patch site, were found in 13%,303 74%298 and 100%300 of patients exposed to LT-containing patches for 6 hours; 17% progressed to vesicle formation.309 Delayed type hypersensitivity contact dermatitis was observed when using recombinant colonization factor.302

Chemical, protein and colloidal enhancers

Chemical penetration enhancers under consideration as skin adjuvants, alone or in conjunction with iontophoresis, ultrasound, and electroporation methods, include oleic and retinoic acids,241 dimethylsulfoxide (DMSO), ethanol, limonene and polysorbate, among others.25 Flagellin, a bacterial surface component protein, was engineered to express influenza nucleoprotein and applied to the bare skin of mice, inducing virus-specific interferon-γ T cells.156 Certain colloids may serve as antigen carriers.22 Deformable lipid vesicles (‘transferrosomes’) containing tetanus toxoid applied to animal skin yielded comparable immune responses with alum-adjuvanted tetanus toxoid given IM.29

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.301,302,314,315 Another method is termed a needle-free solid dose injector (GlideTM,312) It uses a spring-loaded device to push a sharp, pointed, biodegradable ‘pioneer tip’ and the solid or semisolid medication behind it in the chamber—both with the width of a grain of rice—into subcutaneous tissues.

Jet injection

Jet injectors (JIs) squirt liquid under high pressure to deliver medication needle-free into targeted tissues.5 In France, the 1950s6 (Fig. 61–4A),7,316,317,318 and the United States in the late 1960s,319 the technology was filed for patent in 1936,321 and reintroduced in the 1940s as the Hypospray8,322,323 for patient self-injection with insulin (Fig. 61–4B, Table 61–1). In the 1950s, the U.S. military developed high-speed models (once referred to as ‘jet guns’) for mass vaccination programs (Fig. 61–4C).324,325 Over the last half-century, JIs have administered hundreds of millions, if not billions, of vaccine doses for mass campaigns against smallpox,127,351 polio,347,348 meningitis,396,397 influenza,398,399 yellow fever,385,392,393,394 cholera395 and other diseases.396,397–399 During the swine influenza vaccine campaign of 1976–1977 in the U.S., a substantial proportion of the approximately 80 million doses distributed that season were administered by JIs (CDC, unpublished data).390 JIs have also been used for a wide variety of therapeutic drugs, including local391–393 and pre-general394–396 anesthetics, antibiotics,397–399 anticoagulants,400–402 antivirals,403,404 corticosteroids,405,406 cytotoxic drugs410 immunomodulators,155 insulin,325,348,412 and other hormones.413–415 and vitamins.416

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) 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, while some use compressed gas such as carbon dioxide (CO2) or nitrogen (N2) (Table 61–1). Two investigational ones are powered by the expanding pressure of chemical combustion.424,425 The source of energy to compress the spring is usually supplied manually or pedally through an integral or separate tool to apply mechanical advantage and/or hydraulic pressure. A few use electrical power from batteries or wall (main) electrical current.

Although devices vary, peak pressures within the dose chambers range from 14–35 MPa (~2,000–5,000 psi) and occur quite early in order 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 tailoff at the end of the piston’s stroke. The velocity of the jet stream exceeds 100 meters per second.417 Complete injection lasts about 1/5 to 1/2 second, depending on volume delivered, orifice cross-section, and other variables.

JIs may be classified in various ways: by their energy storage and sources described above, by intended market (human vs. veterinary), by intended usage (e.g., repeated self-administration of insulin by the same patient vs. use to vaccinate consecutive patients), by how the dose chamber is filled (medication vial attached ‘on tool’ vs. filled ‘off tool’), by reusability of the entire device (single-use disposable vs. reusable), and by reusability of the fluid pathway and patient-contact components (multi-use vs. disposable). This last criterion results in a key distinction between multi-use-nozzle jet injectors (MUNJIs) and disposable-cartridges jet injectors (DCJIs), with major implications for immunization safety (discussed below).

Deposition in target tissues

In vivo imaging indicated jet-injected medication tends to spread along paths of least resistance in a generally conical distribution.289,415–423 The depth achieved depends primarily on the power imparted to the liquid and variables such as orifice diameter, viscosity of the dose, tautness and thickness of the skin and fat layer, and angle of injection, among other factors.395,396,397,417,418,424,425 The SC compartment is the only one accessible by most marketed DCJIs, as well as by MUNJIs used in veterinary markets.347,351 Self-administration of insulin, hormones, and other drugs. Most MUNJIs developed for mass vaccination campaigns are powered to reach IM tissues, e.g., the Ped-O-Jet and Med-E-Jet, as is one DCJI, the Biojector® 2000, which varies the orifice of different cartridges to deliver either IM or SC.41 Given great patient variation, it is no surprise that imaging studies suggest JIs often miss the intended IM or SC compartment.426 But this may have little clinical relevance, and be no different than needle injections for which fat pad thickness is often underestimated in selecting needle length, or which is not fully inserted.327,428

As mentioned in the cutaneous immunization section above, jet injectors are capable of classical ID delivery by use of specialized nozzles (Fig. 61–2G). The most widely used Ped-O-Jet® administered tens of millions of smallpox vaccine doses for the first half of the WHO Smallpox Eradication Program in South America and West Africa in the late 1960s to early 1970s, until invention of the simpler and swifter bifurcated needle.149,381 Jet injectors also delivered ID the BCG vaccine125,124 and various tuberculosis skin testing antigens (TST).445–447 However, variations in consequent TST reaction sizes suggest WHO to discourage JI use for BCG and TST.448 In the absence of an ID nozzle, many have attached 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.39,377,276,441,447 This ID technique is still pursued.
Alternative vaccine delivery methods

Chapter

61

investigationally for local anesthesia\(^448\) and DNA vaccines (Fig. 61–2H). \(^51,52,330\) Intrapulmonary injections (between the ribs) of antibiotics, bronchodilators, and steroids were performed in Russia.\(^333\)

**Immune response**

A large clinical literature documents 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.\(^314,315,317\) Among inactivated and toxoid vaccines, this includes anthrax,\(^445,446,30,32\) cholera,\(^451\) whole cell diphtheria-tetanus-pertussis (DTPw),\(^138,139,381,452\) hepatitis A,\(^131,456,457\) hepatitis B,\(^452,453,455,456,457\) influenza,\(^7,37,38,39,40,41,46\) plague,\(^356,470\) polio,\(^462\) typhoid,\(^355,397,452,463\) and typhoid-diphtheria.\(^42\) With the exception of the variable delayed hypersensitivity responses to BCG discussed earlier, other live vaccines inducing suitable immune responses when administered byJI into their
<table>
<thead>
<tr>
<th>Current/Last Manufacturer</th>
<th>Trade name(s)</th>
<th>Year(s)</th>
<th>Market/Primary Use(s)</th>
<th>Energy Source/Storage</th>
<th>Type</th>
<th>Filling</th>
<th>Target Tissue</th>
<th>References</th>
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<tr>
<td>Antares Pharma</td>
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<td>1972</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IM, SC</td>
<td>375, 409</td>
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<td></td>
<td>Medi-Jectors II™, III™, IV™</td>
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<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-F</td>
<td>SC</td>
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<td>Hu/In</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valeo™ (MJ 8)™</td>
<td>2000s</td>
<td>Hu/In, Gh</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>Md, Sd</td>
<td>SC</td>
<td>316</td>
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<td></td>
<td>Medi-Jector MJ 10™</td>
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<td>Hu/</td>
<td>Ga/Ga</td>
<td>SUDJI</td>
<td>Mf</td>
<td>SC</td>
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<td>Vibex™</td>
<td>2001</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>Mini-needle DCJI, SUDJI</td>
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<td>ID, SC</td>
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<td></td>
<td>Vaccijet™ électrique, Avijet™</td>
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<td>Ve/Va</td>
<td>Ba/Sp</td>
<td>MUNJI</td>
<td>On-I, via tube</td>
<td>ID, IM, SC</td>
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<td></td>
<td>Vaccijet™ manuel</td>
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<td>Ve/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>ID, IM</td>
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<td>Avant Medical</td>
<td>Guardian™ 101™</td>
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<td>Hu/Un, Va</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>Off</td>
<td>SC</td>
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<td>Becton, Dickinson</td>
<td>Velodermic™ I™, II™</td>
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<td>Ga/Ga (N₂)</td>
<td>DCJI</td>
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<td>313, 328, 348, 385</td>
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<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-F</td>
<td>SC</td>
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<td>Vitajet® 3 (Cool, Click®, SeroJet™, mhi-500™™)</td>
<td>1996</td>
<td>Hu/In Gh</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
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<td>Iject™</td>
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<td>Ga/Ga (N₂)</td>
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<td>Mf</td>
<td>SC</td>
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<td>Vitavax™</td>
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<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
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<td>Vetjet™</td>
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<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
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<td>Mhi-500™™</td>
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<td>Hu/In</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
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<td>Ch/Ch</td>
<td>SUDJI</td>
<td>Mf</td>
<td>SC, IM, ID</td>
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<td>LectraJet™ M3™</td>
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<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>Off</td>
<td>ID, IM, SC</td>
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<td></td>
<td>LectraVet™</td>
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<td>Ve/Va, Mu</td>
<td>Ba/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IM, SC</td>
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<td>EMS Electro Medical Systems™</td>
<td>Swiss Injector™, EMS/RPM™</td>
<td>1990s</td>
<td>Un/Un</td>
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<td>MUNJI</td>
<td>On-F</td>
<td>IM</td>
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<td>EuroJet Medical™</td>
<td>E-Jet 500</td>
<td>2003</td>
<td>Hu, Ve/Ho, In, St, Va</td>
<td>Ma/Sp</td>
<td>DCJI</td>
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<td>E-Jet 50</td>
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<td>Ma/Sp</td>
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<td>Felton™</td>
<td>BI-100™, HSI-500™</td>
<td>1990s</td>
<td>Hu/Va</td>
<td>Pe/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IM, SC</td>
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<td></td>
<td>Pulse 200, 250</td>
<td>1990s</td>
<td>Ve/Mu</td>
<td>Ga/Ga</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IM, SC</td>
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<td>H. Galante et Compagnie™</td>
<td>Device for the Aquapuncture™</td>
<td>1865</td>
<td>Hu/Mu</td>
<td>Ma/Ma</td>
<td>MUNJI</td>
<td>ON-I</td>
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<td>Sensa-Jet™</td>
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<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>Off</td>
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<td>Heng Yang Weida Science Technology™</td>
<td>Pro-Jeey 2000</td>
<td></td>
<td>Hu/Un</td>
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<td>INJEX – Equidyne Systems™</td>
<td>INJEX™ 30 and 50™ models, ZipTip™</td>
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<td>Ma/Sp</td>
<td>DCJI</td>
<td>Off</td>
<td>SC</td>
<td>24, 415, 467</td>
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<td></td>
<td>Jet Syringe™, ROJEX™</td>
<td>2000s</td>
<td>Hu/In, Gh</td>
<td>Ma/Sp</td>
<td>SUDJI</td>
<td>Mf or Off</td>
<td>SC</td>
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<td>Syrjet™</td>
<td>1960s</td>
<td>De, Hu/An, St</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>Md, Sd</td>
<td>ID, SC</td>
<td>413, 422, 485, 510</td>
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<td>MADA Medical Products™</td>
<td>MadaJet™, MadaJet™ XL™, MadaJet™®</td>
<td>1980s</td>
<td>De, Hu/An, St</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>Md</td>
<td>ID, SC</td>
<td>155, 399, 513</td>
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<td>The Medical House PLC™</td>
<td>mhi-500™ &amp; InsulinJet™</td>
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<td>Hu/In</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
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<td>SQ-PEN™</td>
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<td>Hu/In</td>
<td>Ma/Sp</td>
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<td>On-F</td>
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<td>SQ-X™</td>
<td>2002</td>
<td>Hu/In</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
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**Table 61–1  Historical, Currently-marketed, and Investigational Jet Injectors Used, Studied, or Proposed for Vaccination—cont’d**

<table>
<thead>
<tr>
<th>Current/Last Manufacturer</th>
<th>Trade name(s)</th>
<th>Year(s)/</th>
<th>Market/Primary Use(s)</th>
<th>Energy Source/Storage</th>
<th>Type</th>
<th>Filling</th>
<th>Target Tissue</th>
<th>References</th>
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</thead>
<tbody>
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<td>Medical International Technologies</td>
<td>Med-Jet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1990s</td>
<td>Hu/An, Va</td>
<td>Ga/Ga (CO&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>MUNJI</td>
<td>ON-I</td>
<td>IM, SC</td>
<td></td>
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<tr>
<td></td>
<td>Agro-Jet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1990s</td>
<td>Ve/Mu, Va</td>
<td>Ga/Ga (CO&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>MUNJI</td>
<td>ON-I</td>
<td>IM, SC</td>
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<td>Microbiological Research Establishment&lt;sup&gt;361&lt;/sup&gt;</td>
<td>Porton Needleless Injector&lt;sup&gt;®&lt;/sup&gt;, Port-O-Jet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1962</td>
<td>Hu/Va</td>
<td>Pe/Sp</td>
<td>MUNJI</td>
<td>ON-I</td>
<td>ID, SC</td>
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<td>1990s</td>
<td>Hu/In</td>
<td>Ga/Ga (CO&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>SUDJI</td>
<td>On-F</td>
<td>SC</td>
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<td>Nidec Tosok Corporation&lt;sup&gt;363&lt;/sup&gt;</td>
<td>Hyjetor&lt;sup&gt;™&lt;/sup&gt;</td>
<td>1970s</td>
<td>Hu/Un</td>
<td>Pe/Hy</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IM, SC</td>
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<td>PATH&lt;sup&gt;353&lt;/sup&gt;</td>
<td>MEDI-VAX&lt;sup&gt;™&lt;/sup&gt;</td>
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<td>Hu/Va</td>
<td>Pe/Ga (air)</td>
<td>DCJI</td>
<td>On-I</td>
<td>SC, IM</td>
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<td>Ga/Ga (N&lt;sub&gt;2&lt;/sub&gt;)</td>
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<td>PharmaJet&lt;sup&gt;™&lt;/sup&gt;</td>
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<td>Ma/Sp</td>
<td>DCJI</td>
<td>Off</td>
<td>ID, IM, SC</td>
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<td>IsaJet&lt;sup&gt;™&lt;/sup&gt; *, Isa40 Isa10</td>
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<td>Hu, Ve/Un</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IDm</td>
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<tr>
<td></td>
<td>Mesoflash&lt;sup&gt;®&lt;/sup&gt; M10 *</td>
<td>1980s</td>
<td>Ve/Un</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IDm</td>
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<td>Mesoflash&lt;sup&gt;®&lt;/sup&gt; M30 * and M40 *</td>
<td>1980s</td>
<td>Hu/Un</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>IDm</td>
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<td>Im-O-Jet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1980s</td>
<td>Hu/Va</td>
<td>Pe/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>SC</td>
<td>131, 356, 474, 477</td>
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<td>Mini-Imojet&lt;sup&gt;®&lt;/sup&gt; *, PM SC&lt;sup&gt;®&lt;/sup&gt;</td>
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<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>Mf</td>
<td>SC</td>
<td>24, 78, 355, 356, 452, 454</td>
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<tr>
<td>Robert P. Scherer Co.&lt;sup&gt;360&lt;/sup&gt;</td>
<td>Hypospray&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1940s</td>
<td>Hu/In</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>Off</td>
<td>ID, SC</td>
<td>313, 322, 323, 403, 404, 408, 413, 416, 418, 424</td>
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<tr>
<td></td>
<td>Hypospray Professional&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1950s</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>ID, IM, SC</td>
<td>95, 435</td>
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<td>Hypospray Multidose Jet Injector&lt;sup&gt;™&lt;/sup&gt; K&lt;sup&gt;®&lt;/sup&gt;, K-2&lt;sup&gt;®&lt;/sup&gt;, K-3&lt;sup&gt;®&lt;/sup&gt; models</td>
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<td>Hu/Va</td>
<td>El/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>ID, IM, SC</td>
<td>73, 86, 385, 393, 394, 420, 435, 439, 488, 490, 497</td>
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<tr>
<td>Schuco International&lt;sup&gt;361&lt;/sup&gt;</td>
<td>Panjet&lt;sup&gt;™&lt;/sup&gt; multiple models, Intrajet, SchucoJet&lt;sup&gt;™&lt;/sup&gt;</td>
<td>1960s</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-F, Md</td>
<td>ID, SC</td>
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<td>Shimadzu Corporation&lt;sup&gt;362&lt;/sup&gt;</td>
<td>ShimaJET</td>
<td>Hu/In, Va</td>
<td>Ma/Sp</td>
<td>DCJI</td>
<td>On-F</td>
<td>SC</td>
<td>363, 486</td>
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<td>SICIM&lt;sup&gt;364&lt;/sup&gt;</td>
<td>JET2000</td>
<td>Hu/Va</td>
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<td>MUNJI</td>
<td>On-I</td>
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<td>DG-77</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
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<td>Société AKRA DermoJet&lt;sup&gt;385&lt;/sup&gt;</td>
<td>DermoJet Standard, Dermojet type HR, Dermojet model G</td>
<td>1960s</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I, Md</td>
<td>ID, IDm, SC</td>
<td>43, 92, 93, 101, 138, 140, 142, 143, 144, 396, 411, 413, 443, 463</td>
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<tr>
<td>Dermojet Automatic, Vacci-Jet</td>
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<td>Valeritas&lt;sup&gt;197&lt;/sup&gt;</td>
<td></td>
<td>2000s</td>
<td>Hu/Un</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-I</td>
<td>SC</td>
<td>331</td>
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<tr>
<td>Z &amp; W Manufacturing&lt;sup&gt;305&lt;/sup&gt;</td>
<td>Press-O-Jet&lt;sup&gt;TM&lt;/sup&gt;, IntraJet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1950s</td>
<td>Hu/Va</td>
<td>Ma/Sp</td>
<td>MUNJI</td>
<td>On-F</td>
<td>SC / IM</td>
<td>371, 385, 389, 413, 462, 468</td>
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<td>Zogenix&lt;sup&gt;207&lt;/sup&gt;</td>
<td>IntraJet&lt;sup&gt;®&lt;/sup&gt;</td>
<td>1990s</td>
<td>Hu/Ho</td>
<td>Ga/Ga (N&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>SUDJI</td>
<td>Mf</td>
<td>SC</td>
<td>368</td>
</tr>
</tbody>
</table>

**Market / Primary Uses:**
- **Hu** = human medicine
- **De** = dentistry
- **Un** = unspecified
- **Ve** = veterinary
- **An** = anesthetic
- **Av** = antiviral
- **Gh** = growth hormone
- **Ho** = hormone(s)
- **In** = insulin
- **Mu** = multiple
- **St** = steroids
- **Un** = unspecified
- **Va** = vaccine(s)

**Energy Source / Storage:**
- **Ba** = battery
- **Ch** = chemical
- **Ga** = compressed gas cylinder or electrical compressor
- **Hy** = hydraulic fluid pressurized in foot-pump accumulator
- **Sp** = metal spring

**Type:**
- **MUNJI** = multi-use-nozzle jet injector
- **DCJI** = disposable-cartridge jet injector
- **SUDJI** = single-use disposable jet injector (entire unit discarded after use)

**Filling:**
- **On-F** = on tool; primary container (vial) attaches to injector to fill dose chamber temporarily during filling, but removed before injection
- **On-I** = on tool; primary container (vial) remains attached to injector to fill dose chamber repeatedly, staying attached during injections
- **Off** = off tool; vial fills dose chamber (cartridge) before insertion into injector
- **Md** = multiple doses possible from dose chamber before refilling required
- **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

<sup>1</sup> Device investigational, or not yet sold commercially for routine use in humans or animals.
<sup>2</sup> The mini-500<sup>TM</sup> (“The Medical House<sup>®</sup>”) device contains Vitajet® 3 technology licensed by Bioject to The Medical House.
<sup>3</sup> The Vetjet<sup>®</sup> (“by Merial<sup>®</sup>”) is the Vitajet<sup>®</sup> 3 design licensed by Bioject to Merial for delivery to cats of PureVax<sup>®</sup> brand of feline leukemia virus vaccine.
<sup>4</sup> The cool.click® and SeroJet<sup>®</sup> devices are the Vitajet® 3 design licensed by Bioject to Serono<sup>®</sup> for delivery of the Seronorm<sup>®</sup> and Serostim<sup>®</sup> brands of somatropin (recombinant human growth hormone) for treatment of growth hormone deficiency and AIDS-wasting diseases, respectively.
<sup>5</sup> Versions of the Vision<sup>®</sup> injector are licensed to Ferring Pharmaceuticals BV (ZomaJet<sup>®</sup>), SciGen Ltd (SciTojet<sup>®</sup>), and JCR Pharmaceuticals (Twin-Jector<sup>®</sup> EZ II).
<sup>6</sup> Device withdrawn from market, no longer manufactured, or abandoned in development.
<sup>7</sup> Approximate year(s) first introduced to market, investigational development initiated, or patent filed.
<sup>8</sup> The ZipTip<sup>®</sup> (“by Pfizer”) is the INJEX design licensed to Pfizer for delivery of Genotropin<sup>®</sup> recombinant human growth hormone.
usual tissue compartment are measles,\textsuperscript{353} mumps-rubella,\textsuperscript{467} measles-smallpox,\textsuperscript{377,378,391} measles-smallpox-yellow fever,\textsuperscript{377,391} smallpox,\textsuperscript{146,402,377,381,447} diphtheria,\textsuperscript{406,416} and yellow fever.\textsuperscript{60} and yellow fever.\textsuperscript{200}

The immunogenicity or efficacy of traditional meningococcal polysaccharide vaccines administered by JIs have been demonstrated for serogroup A in the clinic\textsuperscript{127} and in outbreaks in the meningitis belt of western sub-Saharan Africa,\textsuperscript{396,475} as well as for serogroup C in South America,\textsuperscript{378,492} and Africa.\textsuperscript{366,477}

Jet injection of the newer Vi capsular polysaccharide typhoid vaccine resulted in 87% seroconversion vs. 69% by needle-measles-mumps-rubella,\textsuperscript{467} measles-smallpox,\textsuperscript{377,378,391} measles-polysaccharide vaccines administered by JIs have been demonstrated for serogroup A in the clinic\textsuperscript{137} and in outbreaks in the meningitis belt of western sub-Saharan Africa,\textsuperscript{396,475} as well as for serogroup C in South America,\textsuperscript{378,492} and Africa.\textsuperscript{366,477}

A wide variety of investigational recombinant nucleic acid vaccines are being delivered in preclinical and clinical trials using various JIs.\textsuperscript{351,353,354,360,483,496}

Reactogenicity

Comparisons of immediate pain between JIs and needles used to deliver IM and SC injections depend on the medication involved. Insulin, other non-irritating drugs, and non-adjuvanted vaccines are reported to result in either reduced or equivalent pain compared to needles,\textsuperscript{322,377,381,410,415,416,424,467} but not always.\textsuperscript{461} True double-blinded, needle-controlled studies for such subjective criteria are nearly impossible to design and thus lacking.

Vaccines with alum adjuvants or other irritating components tend to result in higher frequencies of delayed local reactions (e.g., soreness, edema, erythema) when jet-injected, probably because small amounts remain in the track through skin and superficial tissue. These include vaccines for diphtheria-tetanus-pertussis (whole-cell),\textsuperscript{391,394,467} hepatitis A,\textsuperscript{452,455,455} hepatitis B,\textsuperscript{353,490,493} tetanus,\textsuperscript{353,395,397,402,403,404} diphtheria,\textsuperscript{424} tetanus-diphtheria-polio\textsuperscript{400} and typhoid.\textsuperscript{452,464,500,522} 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 following II vaccination with tetanus toxoid absorbed to alum,\textsuperscript{60} and pigmented macules persisted in a few hepatitis B vaccinees.\textsuperscript{450}

Other adverse events

Bleeding, and less often ecchymosis, are reported to occur at the jet injection site more frequently than with needle injections.\textsuperscript{377,322,540,371,374,381,389,405,410,411,414,418,424,464,465,468,495} Rarely, the jet stream may cause a laceration if the health care worker has not properly immobilized the limb and injector in relation to the patient. Hematomas of the injection site may occur because blood or HBsAg remained in nozzle orifices despite recommended alcohol swabbing between injections.\textsuperscript{496} Others, however, reported negative results in bench or animal testing to try to detect contamination.\textsuperscript{396,405,499,500} or pointed to the lack of epidemiologic evidence of a problem.\textsuperscript{389}

Safety of multi-use-nozzle jet injectors (MUNJIs)

Beginning in the 1960s, concerns arose for potential iatrogenic transmission of bloodborne pathogens by multi-use-nozzle jet injectors (MUNJIs), which use the same nozzle to inject consecutive patients without intervening sterilization.\textsuperscript{396,405,499,500} 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.\textsuperscript{498} Others, however, reported negative results in bench or animal testing to try to detect contamination.\textsuperscript{396,405,499,500} or pointed to the lack of epidemiologic evidence of a problem.\textsuperscript{389} Then in 1985, Brink et al described a caseful animal model in which a Med-E-Jet transmitted lactic dehydrogenase (LDH) virus between mice in 16 (33%) of 49 animals.\textsuperscript{389}

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.\textsuperscript{304,305} Subsequent clinical,\textsuperscript{500} field,\textsuperscript{396} animal\textsuperscript{311,312} and epidemiologic,\textsuperscript{333,334} studies added more evidence that MUNJIs could transmit pathogens between patients. This led to warnings and discontinuation of their use by public health authorities.\textsuperscript{333,334} and market withdrawal of the Ped-O-Jet and discontinuation of its U.S. military use in 1997.\textsuperscript{500}

There have been efforts in the 2000s to reengineer MUNJIs with disposable caps or washers with a central hole for the jet stream to prevent blood or tissue fluid from reaching the nozzle.\textsuperscript{322} However, clinical studies revealed the caps were unable to prevent HBV contamination of subsequent in vitro injections assayed by PCR after injections of high-titer HBV-carrier volunteers.\textsuperscript{316,322} MUNJIs also face doubts raised by high-speed microcinematography revealing extensive splashback,\textsuperscript{317} and the challenge of proving that contamination does not occur and of convincing policymakers to set any level of acceptable risk. Despite the withdrawal of MUNJIs for vaccination, models such as the MadaJet\textsuperscript{464} and SyriJet\textsuperscript{62} continue to be used in dentistry and medicine for delivery of local anesthetics.

MUNJIs allowed a single health worker to vaccinate 600 or more patients per hour.\textsuperscript{351,375,377,399} Their withdrawal poses challenges for conducting mass immunization campaigns for disease control programs and in response to pandemic or bioterror threat. Indeed, while the Soviet biological warfare effort was underway in secret,\textsuperscript{519} numerous clinical trials were published of high-speed Russian MUNJIs capable of rapidly protecting soldiers or civilians against potential bio warfare agents such as anthrax, botulism, plague, smallpox and tularemia.\textsuperscript{514,449,490,493,520,523}

Disposable-cartridge jet injectors (DCJJs)

To overcome concerns over MUNJIs and their withdrawal, since the early 1990s, a new generation of safer, disposable-cartridge jet injectors (DCJJs) have appeared on the market (Table 61–1).\textsuperscript{306} Each cartridge has its own sterile orifice and nozzle and is discarded between patients. Most are used for self-administration of insulin and other hormones. An exception is the Biojector\textsuperscript{200} (Fig. 61–2H)\textsuperscript{86} which was designed for vaccination and delivers approximately one million doses per year at private, public, and U.S. Navy and Coast Guard immunization clinics. Another DCJI for SC delivery only, the Injex\textsuperscript{50} (Fig. 61–4I),\textsuperscript{50} produced satisfactory immune responses to measles-mumps-rubella vaccine boosters.\textsuperscript{50}

To meet developing world needs for needle-free vaccination systems that are economical, autodisinfectable to prevent reuse, and suitable for both mass campaigns and routine immunization, DCJJs such as the PharmJet\textsuperscript{50} and the investigational LectraJet\textsuperscript{24,335} and the Vitavax\textsuperscript{74} are in research and development (Fig. 61–4 K, L, M, N). Financial support for DCJI R&D has been provided by private sources, by the U.S. Government (CDC), and by the Program for Appropriate Technology in Health (PATH)\textsuperscript{50} under a grant from the Bill and Melinda Gates Foundation.

Respiratory vaccination

Since early in the history of immunization, the respiratory tract has been considered a highly promising route for vaccine delivery. However, only since the year 2000 have advances in respiratory vaccines and their delivery systems begun to play a role in routine immunization practices, as heralded by the licensure of an intranasal (IN), live attenuated influenza vaccine (FluMist\textsuperscript{88}) in the United States (see Chapter 16 [influenza, live]). Two

\[ \text{usual tissue compartment are measles, mumps-rubella, measles-smallpox, measles-smallpox-yellow fever, smallpox, diphtheria, and yellow fever.} \]
major advantages of respiratory immunization are that it avoids needles and generally provides stronger mucosal immunity than parenteral immunization.

The great majority of human pathogens gain access across mucosal surfaces in the gastrointestinal, respiratory, or genitourinary tracts. Mucosal immunity includes humoral and cellular components and prevents infection at these portals of entry. In contrast, systemic (humoral and cellular) immunity clears infection only after 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 reduces the risk of transmission to others, in addition to preventing clinical disease. Prevention of initial infection is important for diseases in which effective systemic immunity has been difficult to achieve, such as for tuberculosis and AIDS.

Every mucosal surface for administering vaccines has been studied with a variety of antigens in animal models, including the oral, conjunctival, rectal and vaginal routes. Several human vaccines are already licensed and in use for delivery by oral ingestion, including vaccines for polio and cellular rotavirus, typhoid and adenovirus, which are described in detail in other chapters. This chapter, however, will focus only on vaccines and technologies for respiratory tract immunization, including devices for depositing vaccines in the target area, delivery systems to optimize presentation of antigen to the respiratory immune tissues, and adjuvants to enhance the immune response.

Antigen presentation and processing in the respiratory tract

Pathogens and vaccine antigens enter the respiratory tract in airborne particles through oral or nasal inhalation and deposit on respiratory surfaces. Air inspired through the nose is effectively filtered by the nasal hairs, by the external nasal valves which restrict the airflow from the nares into the internal nasal passages and by the convolutions of the turbinates. For example, Djupesland et al showed only 25% of large, high speed droplets (average 43 μm) of a nasal spray and nasal inhalation of smaller aerosol particles (0.015 μm) 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 about 150 square centimeters (0.015 m²) in the nasal airways.520 The lower airways in humans do not typically have organized lymphoid tissues, but they do have abundant numbers of intraepithelial dendritic cells and alveolar macrophages which process antigens.530

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.531 T cells also play a major role in immunologic priming 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 mucosal immune cells and tissues is known as the common mucosal immune system.520,533 Because the respiratory tract is exposed to a myriad of non-pathogenic macromolecules, there are mechanisms for down-regulating the immune response to antigenic exposure. This is known as immunological tolerance and must be considered when developing respiratory immunization strategies.534

Challenges for respiratory delivery of vaccines

The first challenge in respiratory immunization is to identify the appropriate target tissue. Most respiratory drugs traditionally target two areas. The nasal passages are the desired site of action for decongestants, while the lower airways are targeted by asthma medications. The optimal target tissue is not yet determined for most potential respiratory vaccines and may be different for different vaccines. The pharyngeal tonsils are likely the primary target because of their key role in immunologic responses, however, some vaccines may require deposition in the lower airways. Scientific methods for evaluating and comparing different vaccine target tissues areas are not yet well developed. Interspecies differences in respiratory immunologic tissue organization makes it difficult to use animal models to determine optimal vaccine target tissues. Moreover, the relative size and anatomy of the respiratory tract of many animals (e.g., sheep) differ greatly from humans. For example, in small animals such as rodents, the use of nose drops may result in deposition to the entire respiratory tract which would not be the case in humans. Balmelli, et al estimated that 30% of 20 μL of vaccine given to mice as IN drops deposited into the lungs.535 A second challenge to research is the lack of susceptibility in many animal models to many human diseases of interest. This makes it difficult to use live vectors as vaccines or to do challenge studies to determine vaccine protection. Such limitations impede the translation of promising results from animal research into safe and effective vaccines for human use.

A third challenge for respiratory immunization is the difficulty in delivering a consistent dose. The mass or volume of the dose 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 anatomy and physiology in the vaccinees (animals) or vaccinees (humans).520 Fortunately, for many vaccines there is a wide margin between the dose necessary to induce protection and the dose 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 dose accuracy and consistency is critical, suggests that this challenge can be overcome for respiratory vaccines.537
A fourth major challenge is that accepted ‘correlates of protection’ for mucosal immune responses have yet to be determined. In contrast, for many diseases there are well-established laboratory assays of systemic immunity—such as antibody titers above certain cutoffs—that have served for many years as indicators of protection from disease. Several immunization safety issues represent further challenges for respiratory vaccines. One is the risk that vaccine viruses, antigen, or adjuvant might affect nearby cranial nerves, or travel along the olfactory nerve through the cribiform plate into the brain with resulting adverse central nervous system effects. Another risk that must be addressed is cross-contamination, in which respiratory pathogens from one patient may contaminate the respiratory immunization device, with the risk of their spread to subsequent patients using the device. Other safety issues for vaccines targeting lower airways include the possible induction or exacerbation of bronchospasm and/or pulmonary inflammation, which can be life-threatening. Also, respiratory vaccine aerosols may spread beyond the intended vaccinee to other persons in the vicinity. Finally, certain live virus or bacterial vaccines might have a pathogenic effect on persons immunocompromised by HIV or other conditions.

Remaining challenges relate to the delivery devices. Although many devices already exist for delivering drugs to the respiratory tract, very few of them are designed for vaccine delivery. 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 mentioned above. Although single-
use, disposable devices might solve this, they may be costly. Some aerosol drug delivery devices require patient education to obtain the needed cooperation for adequate dose delivery, which is difficult in the brief time typical for vaccination. Some respiratory delivery methods are not effective for young children, who receive many vaccines. Although current respiratory drug delivery devices typically target the anterior nasal passages or the lower airway, respiratory vaccination may work best by deposition in the quite different area of the pharyngeal tonsils. New delivery technologies to meet the requirements of respiratory immunization are required if this route is to become practical and accepted. As a young field, published research on devices used in respiratory vaccination of humans or animals is limited. In most reported animal studies, the IN delivery device is not mentioned at all, or a laboratory pipette unsuitable for humans is used for instillation.

Current progress in vaccination via the respiratory tract

Respiratory vaccination devices

The only device currently licensed and in use in the United States for respiratory delivery of a vaccine is the AccuSpray™ (Becton, Dickinson and Company (BD)), which is used to deliver FluMist™ influenza vaccine. The AccuSpray™ is a nasal spray syringe preloaded for single patient use (Fig. 61–5F). It produces particles with a mean aerosol diameter of 70 microns in a total dose of 0.5 mL, with 0.25 mL delivered consecutively through each nostril. Key advantages of this device are that it is simple to use, inexpensive, disposable and very difficult to refill and reuse. The large particle size minimizes deposition to the lower airways which reduces the risk of pulmonary adverse events.

Another respiratory immunization device that has been used in humans is the jet nebulizer system known as the Classic Mexican Device (CMD, Fig. 61–5E). With slight modifications, this nebulizer delivered live attenuated measles vaccines in multiple clinical trials in Mexico and South Africa, and in a mass campaign which vaccinated over 3 million Mexican children against measles. The system consists of a general-use compressor which delivers air to a jet nebulizer (non-medical) which holds the vaccine in crushed ice to maintain potency. The vaccine aerosol is delivered through a disposable cone (modified paper cup) which is held close to the patient’s face for 30 seconds. Typically, the aerosolized vaccine dose is roughly 0.15 mL, and the mass median aerosol diameter of the emitted particles is 4.3 μm.421

The OptiMist™ is a breath-activated nasal spray device for liquid or powders which delivers only during oral exhalation.424 Because oral exhalation closes the connection between nose and throat, pulmonary deposition is avoided and delivery to the posterior nasal segments is increased (Fig. 61–5A,B). In a human study, inactivated influenza vaccine self-administered using the OptiMist™ resulted in significant increases in virus-specific IgA in nasal secretions and protective levels of virus-specific serum antibodies after two doses in >80% of subjects from each group.424

A Combitips-plus syringe (Eppendorf) was used to deliver a dry powder Neisseria meningitidis vaccine IN to human subjects. IN-vaccinated subjects had serum bactericidal antibody titers comparable to 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 to two-thirds of injection vaccinees reporting mild injection pain.425 BD has demonstrated the utility of a novel device for delivery of vaccine powder (Fig. 61–5C). Air from a syringe barrel ruptures the membranes of a capsule containing the vaccine and delivers the powder to the nasal tract. The device was effective in nasal delivery of influenza vaccine to rats and of anthrax vaccine to rabbits.426

The Centers for Disease Control and Prevention (CDC) developed a nebulizer for vaccine delivery which utilizes a disposable aerosol-generating element and disposable patient interface to prevent cross contamination (Fig. 61–5D). The aerosol it generates can provide either 10-25 μm droplets for upper airway delivery or <5 μm droplets for lower airway delivery, and can be used with a disposable nasal prong, oral prong or mask. Delivery of live attenuated measles vaccine with this device through a nasal prong was shown to be safe and immunogenic in macaques. Ongoing research focuses on maximizing delivery to the nasopharynx. The AccuInhale™ company has acquired the rights to manufacture and distribute this technology.

Adjuvants for respiratory delivery of vaccine

Non-replicating antigens delivered via the respiratory tract are typically poorly immunogenic and may require adjuvants to stimulate an appropriate immune response. Adjuvants which have been studied for respiratory delivery of vaccines include bacterial toxins and their derivatives, other bacterial components, bacterial DNA motifs, cytokines and chemokines, plant derivatives and other adjuvants (Table 61–2). Cholera toxin (CT) and E. coli heat labile toxin (LT) are potent respiratory immunization adjuvants but are considered too toxic for use in humans. Although the pathogenesis of Bell’s palsy has not been clearly defined, CT and LT have been shown to accumulate in the olfactory bulbs of Balb/c mice following nasal administration, sometimes with concurrent inflammation, which suggests a risk for adverse neurological effects. As a result, recent adjuvant research has focused on alternative subunits and variants of CT and LT. Several of these, such as CT1A-DD, do not accumulate in the olfactory bulb of BALB/c mice.

Other bacterial products which induce potent activation of the innate immune system include bacterial lipopolysaccharide (LPS) and its derivative, monophosphoryl lipid A (MPL), as well as bacterial outer membrane protein proteosomes, flagellins, lipopeptides and filamentous hemagglutinins which contain CpG motifs found in bacterial DNA. These motifs are recognized as pathogen associated molecular patterns (PAMPs) by the innate immune system and are potent adjuvants. Abe et al found that a non-typeable Haemophilus influenzae (NTHi) vaccine, delivered IN with CpG ODNs, produced similar mucosal IgA and serum IgG responses as vaccine delivered with CT. Enhanced clearance of NTHi from the nasopharynx following challenge was shown equally in both groups. However, in another study, daily injection of high dose (60 μg) CpG resulted in lymphoid follicle destruction and immunosuppression with liver necrosis after 20 days. Therefore, potential adverse effects of CpG ODNs should be carefully monitored.

Because many adjuvants induce enhanced immune responses through the activation of chemokines and cytokines, investigators have studied these molecules themselves as adjuvants that...
### Table 61–2  Examples of Adjuvants for Respiratory Vaccination Successfully Tested in Animals

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Vaccines</th>
<th>Studied In</th>
<th>Serum IgG</th>
<th>Mucosal IgA</th>
<th>Challenge Protection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial Toxins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cholera Toxin (CT)</td>
<td>Trichomonas, Malaria, <em>Chlamydia trachomatis</em>, <em>Streptococcus pyogenes</em></td>
<td>Mice</td>
<td>+++∧</td>
<td>+/-</td>
<td>+</td>
<td>554, 555, 556, 557</td>
</tr>
<tr>
<td>CT-B subunit</td>
<td>Pneumococcus, Group A <em>Streptococcus</em>, Human Papilloma Virus (HPV), Tetanus, Gonorrhea, Group B <em>Streptococcus</em>, <em>Porphyromonas gingivalis</em>, Diphtheria, Simian Immunodeficiency Virus (SIV)</td>
<td>Mice</td>
<td>+++++∧</td>
<td>+++∧</td>
<td>+</td>
<td>562, 563, 564, 565, 566, 567, 568, 569, 570</td>
</tr>
<tr>
<td>CT mutants, CTA1-DD</td>
<td><em>C. trachomatis</em>, Human Immunodeficiency Virus (HIV), Influenza, <em>Helicobacter pylori</em>, HPV</td>
<td>Mice, Macaques</td>
<td>+∧</td>
<td>∧∧</td>
<td>+∧∧∧∧</td>
<td>564, 571, 572, 573, 574, 575, 576, 581</td>
</tr>
<tr>
<td><em>Escherichia coli</em> heat labile toxin (LT)</td>
<td>Meningococcus, <em>P. gingivalis</em>, Measles</td>
<td>Mice</td>
<td>+++∧</td>
<td>+∧</td>
<td>+</td>
<td>558, 559, 568</td>
</tr>
<tr>
<td>LT-B subunit</td>
<td>Meningococcus</td>
<td>Mice</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>784</td>
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<tr>
<td>LT mutants</td>
<td>Influenza, Meningococcus, Ricin, <em>P. gingivalis</em>, Measles</td>
<td>Mice, Humans</td>
<td>+++∧∧</td>
<td>+∧∧</td>
<td>+∧∧∧∧</td>
<td>559, 568, 577, 578, 579, 580</td>
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<tr>
<td><strong>Other Bacterial Products</strong></td>
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<td></td>
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<tr>
<td>Proteosomes, Outer membrane vesicles</td>
<td>Respiratory Syncytial Virus (RSV), Leishmania, Influenza, Hepatitis B, Measles, Plague,</td>
<td>Mice, Humans</td>
<td>+++++∧∧∧</td>
<td>+++∧∧∧</td>
<td>+++++∧∧∧∧</td>
<td>582, 583, 584, 585, 586, 587, 587, 581</td>
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<tr>
<td>Lipopolysaccharide</td>
<td>Measles, Leishmania, Meningococcus, Influenza, Plague</td>
<td>Mice</td>
<td>+∧</td>
<td>+∧</td>
<td>+</td>
<td>585, 586, 587</td>
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<tr>
<td>Monophosphoryl Lipid A (MPL)</td>
<td>Anthrax, SIV, Meningococcus</td>
<td>Mice, Rabbits, Macaques</td>
<td>+++∧</td>
<td>+++∧</td>
<td>+∧</td>
<td>588, 589</td>
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<tr>
<td>Lipopeptides</td>
<td>HIV, Measles</td>
<td>Mice, Cotton rats</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>590, 591</td>
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<tr>
<td>Flagellins</td>
<td>Plague, Tetanus</td>
<td>Mice, Monkeys</td>
<td>+∧</td>
<td>+∧</td>
<td>+∧</td>
<td>592, 593</td>
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<td><strong>Bacterial DNA Motifs</strong></td>
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<tr>
<td><strong>Cytokines/Chemokines</strong></td>
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<tr>
<td>Interleukins (IL-1, IL-5, IL-6, IL-12, IL-15, IL-23) GM-CSF Type 1 Interferon</td>
<td>Tuberculosis, Human Papilloma Virus (HPV), Herpes Simplex Virus (HSV), HIV, Simian/Human Immunodeficiency Virus (SHIV), Pneumococcus, Influenza</td>
<td>Mice, Macaques</td>
<td>+++∧∧∧∧++</td>
<td>+++∧∧</td>
<td>+++∧∧∧∧</td>
<td>573, 600, 601, 602, 603, 604, 605, 607</td>
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<td><strong>Plant Derivatives</strong></td>
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<tr>
<td>Quillaja Saponins</td>
<td><em>P. gingivalis</em>, HIV</td>
<td>Mice</td>
<td>∧∧</td>
<td>∧∧</td>
<td>∧</td>
<td>568, 611</td>
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<tr>
<td><strong>Other Adjuvants</strong></td>
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<td></td>
</tr>
<tr>
<td>Chitin, Chitosan</td>
<td>Anthrax, Influenza</td>
<td>Mice, Rabbits</td>
<td>∧∧</td>
<td>∧∧</td>
<td>+</td>
<td>588, 804</td>
</tr>
</tbody>
</table>

+ Denotes a respiratory vaccination study in which an immune response was demonstrated using the adjuvant, but unadjuvanted vaccine was not studied as a control.
∧ Denotes a respiratory vaccination study in which the immune response was increased with the adjuvant compared to vaccination without the adjuvant.
might minimize any adjuvant toxicity (Table 61–2).\textsuperscript{603–605} Chemokines and cytokines have been added directly to the vaccine, or encoded for expression by a live vector or DNA vaccine.\textsuperscript{606} Bracci and colleagues found a single IN dose of an inactivated influenza vaccine provided full protection against virus challenge in mice when type 1 IFN was included as an adjuvant. The same vaccine dose was only partially effective (40%) without it.\textsuperscript{607}

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.\textsuperscript{608} Chitin and chitosan have mucoadhesive properties and stimulate the innate immune system.\textsuperscript{609} In humans, the addition of chitosan to an IN vaccine based on CRM-197 diphtheria antigen significantly increased toxin-neutralizing antibody levels.\textsuperscript{610} The saponins of the Quillaja sapo•aria tree are potent adjuvants with high toxicity. Quil A, QS-21 and ISCOPREP 703 are subcomponents with less toxicity.\textsuperscript{611} As adjuvant to an IN DNA HIV-1 vaccine studied in QS-21 and ISCOPREP 703 are subcomponents with less toxicity.\textsuperscript{612} The same vaccine dose was only partially effective (40%) without it.\textsuperscript{613} Quil A and ISCOPREP 703 are commonly used as components of immuno•stimulating complexes (ISCOMs), antigen delivery vehicles described in more detail in the next section. Combining adjuvants may synergistically enhance immune protection with respiratory immunization. For example, IN immunization of mice with a recombinant influenza HA (rHA) antigen, with a combination of proteosomes and LPS adjuvants, enhanced serum IgG and mucosal IgA antibodies up to 250-fold compared to vaccine alone.\textsuperscript{614}

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) must penetrate mucosal barriers to gain access to appropriate cells to activate the immune system. The vehicles or vectors which may 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, ISCOMs, microparticles and nanoparticles (Table 61–3).\textsuperscript{615–619}

Live viruses

Viruses are prototypical antigen delivery vehicles because they enter and commande•er cells to replicate themselves, thus multiplying the available antigen which 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.\textsuperscript{620–626} Their major risks are possible rever•sion to virulence, potential neurotoxicity via the olfactory route, and the risk of pathogenic effects in immunocompromised persons.

Live, attenuated cold adapted influenza vaccine (CAIV, FluMist\textsuperscript{[®]}) is the only vaccine currently licensed for delivery by the respiratory tract. Its development, testing and licensure are reviewed in detail in Chapter 16 [influenza, live]. As a model respiratory immunization, IN CAIV demonstrates several potential benefits of live virus respiratory immunization. It produces both mucosal and systemic immunity and provides higher protective efficacy than injected inactivated vaccine.\textsuperscript{[®],626–628} It also provides heterotypic immunity against influenza strains that had antigenically drifted from the vaccine strains.\textsuperscript{629} Finally, it may reduce the risk of influenza transmission because it reduces respiratory shedding among children challenged with a vaccine virus.\textsuperscript{630} Also, modest coverage with CAIV among school children reduced influenza-related illness rates in unvaccinated adults in a community.\textsuperscript{631}

Apart from influenza, measles has been the disease for which vaccine delivery via the respiratory tract has been most thoroughly studied. In a review by Cutts et al through 1997,\textsuperscript{632} and in more recent studies, three basic immune response patterns were revealed upon measles vaccine delivery. First, drops or sprays delivered to the conjunctiva, oral or nasal mucosa produced inconsistent immune responses.\textsuperscript{633–635} Second, among older children (>12 months), delivery of small-particle aerosols via inhalation typically produced immune responses in very high proportions of subjects. Immune responses to aerosol vaccines were usually equivalent to or greater than to injected vaccines.\textsuperscript{636–638} For example, Dilraj et al found that 96.4%, 94% and 86% of schoolchildren who received aerosol measles vaccine had antibody titers >300 IU/L at 1, 2 and 6 years after vaccination, respectively, compared to 91.4%, 87% and 73% among injected vaccinees.\textsuperscript{639–641} In addition to the clinical trials, de Castro reported >3.7 million children in Mexico were vaccinated by aerosol with no serious adverse events noted.\textsuperscript{642} A subsequent outbreak investigating showed that 0.8% of aerosol-vaccinated children compared to 14.6% among injection vaccinees and 26.2% among the unvaccinated. The third pattern noted is that the aerosol route among children ≤12 months of age usually produced an immune response lower than that by injection when the two routes are compared directly.\textsuperscript{643–646} For example, Wong-Chew et al found vaccination by injection provided immunity in 100% of 12-month-old and 9-month-old infants, while the rates among aerosol recipients were only 86% and 23%, respectively.\textsuperscript{647–650}

No severe adverse events following aerosol measles vaccination have been reported in any of the studies. Rates of minor adverse events, when reported, have typically been less than or the same as vaccination by injection.\textsuperscript{651–655}

Based on the encouraging results of prior trials, the World Health Organization (WHO), in partnership with CDC and the American Red Cross, leads the Measles Aerosol Project. Its goal is licensure in the developing world of at least one live, attenuated aerosol measles vaccine consisting of the delivery device and the associated vaccine. The project has already documented immunogenicity, and safety (the lack of local or systemic toxicity) in animal studies.\textsuperscript{656} Three devices were selected for Phase I clinical trials based on the criteria of 1) critical performance data, 2) usability under field conditions, 3) vaccine potency during nebulization and 4) existing licensure for other uses. As of December, 2006, phase I clinical trials are in progress in India.

IN delivery of live attenuated rubella vaccine was investigated during the 1970s in multiple clinical trials.\textsuperscript{657} Ganguly et al demonstrated that drops or spray produced mucosal IgA antibody, equivalent serum IgG antibody, and better protection against reinfection by IN challenge of vaccine virus compared to subcutaneous vaccination.\textsuperscript{658} The IN subjects, however, had higher rates of mild adverse events, usually rhinitis and sore throat. More recently, Sepulveda et al found aerosolized measles-rubella combination vaccine in school-age children not previously vaccinated against rubella produced high levels of rubella immunity, equivalent to subcutaneous administration. Fewer adverse events were reported in the aerosol group.\textsuperscript{659}

Recombinant viruses acting as vectors by incorporation of a gene expressing a heterologous antigen have similar advantages as conventional attenuated live virus vaccines. They deliver the antigen code into cells and get it replicated to activate the immune system. Viruses used as vaccine vectors ideally should have very low pathogenic potential, even in the immunocompromised, and the capacity to hold the necessary
### Table 61–3 Delivery Systems and Vehicles for Vaccination via the Respiratory Tract

<table>
<thead>
<tr>
<th>Vaccine Delivery Vehicle</th>
<th>Vaccines</th>
<th>Studied In</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Live Viruses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Viral Vectors</strong></td>
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<td></td>
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<tr>
<td>Adenovirus</td>
<td>HIV, Severe Acute Respiratory Syndrome (SARS), Rotavirus, SIV, HSV, Rabies, Plague, RSV, Tetanus</td>
<td>Mice, Hamsters, Cotton Rats, Ferrets, Monkeys</td>
<td>169, 171, 589, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706</td>
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<tr>
<td>Modified Vaccinia Virus of Ankara</td>
<td>HIV, Vaccinia, Parainfluenza, SARS, SHIV</td>
<td>Mice, Monkeys</td>
<td>603, 707, 708, 709, 710</td>
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<tr>
<td>Adeno-associated Virus</td>
<td>Influenza, HPV, Alzheimer’s (A beta peptide)</td>
<td>Mice</td>
<td>678, 679, 680</td>
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<tr>
<td>Vesicular Stomatitis Virus</td>
<td>Tuberculosis, Plague, HIV</td>
<td>Mice</td>
<td>681, 682, 683, 684, 685, 686, 687, 688, 689</td>
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<tr>
<td><strong>Live Bacteria</strong></td>
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<tr>
<td>Attenuated Homologous Vaccines</td>
<td>BCG (tuberculosis), Pertussis</td>
<td>Mice, Possum</td>
<td>708, 711a, 711b, 713a, 718, 720, 722, 723, 724, 725, 726, 727, 732, 733, 734, 735</td>
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<tr>
<td><strong>Bacterial Vectors</strong></td>
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<tr>
<td>Food Grade Bacteria</td>
<td>HPV, Tetanus</td>
<td>Mice</td>
<td>714, 715, 716, 717, 719</td>
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<td><strong>DNA Vaccine</strong></td>
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<tr>
<td>Naked DNA</td>
<td>HIV, Tuberculosis, <em>H. Pylori</em>, HPV, SHIV, HSV, Rotavirus, Coxsackie virus</td>
<td>Mice, Monkeys</td>
<td>753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 778, 780, 781</td>
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<tr>
<td>Bacteria Vectored DNA</td>
<td>Measles, Hepatitis B, HIV, HSV, Tetanus, <em>Chlamydia pneumoniae</em></td>
<td>Mice, Cotton Rats, Guinea Pigs</td>
<td>773, 774, 775, 776, 777</td>
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<tr>
<td><strong>Non-replicating Delivery Systems</strong></td>
<td></td>
<td></td>
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<tr>
<td>Liposomes</td>
<td>Meningococcus</td>
<td>Mice</td>
<td>782, 783, 784, 785</td>
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<tr>
<td>Virus Like Particles</td>
<td>SIV, HIV</td>
<td>Mice</td>
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<td>Virosomes</td>
<td>Influenza, Carcinoembryonic antigen</td>
<td>Mice, Humans</td>
<td>788, 789, 790</td>
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<tr>
<td>ISCOMS</td>
<td>Diphtheria, Influenza, Bovine Respiratory Syncytial Virus</td>
<td>Mice, Guinea pigs, Cows</td>
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<tr>
<td><strong>Microparticles and Nanoparticles</strong></td>
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<td></td>
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<tr>
<td>PGA/PLGA particles</td>
<td>Hepatitis B, <em>E. coli</em>, Malaria</td>
<td>Mice</td>
<td>796, 798, 800, 801, 803</td>
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<tr>
<td>Chitin/ Chitosan particles</td>
<td><em>Bordetella bronchiseptica</em>, Meningococcus, Influenza</td>
<td>Mice</td>
<td>578, 580, 799, 802, 804</td>
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<td>Dry Powder Formulations</td>
<td>Anthrax, Influenza, Measles</td>
<td>Mice, Rabbits, Monkeys</td>
<td>546, 588, 805, 806, 807, 807a</td>
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</tbody>
</table>
foreign genes expressing the desired antigens, promoters and adjuvants. Viruses which naturally infect or grow in respiratory tissues are especially well suited as vectors for respiratory immunization. Some viruses studied as vaccine vectors in animal models include adenoviruses, poxviruses, vesicular stomatitis virus and adeno-associated virus.\textsuperscript{689–691} IN adenovirus vectors produced immune responses against many diseases in several animal models (Table 61–2).\textsuperscript{169,171,690–706} For example, a replication defective adenovirus expressing \textit{M. tuberculosis} antigen delivered IN to mice provided better protection against respiratory challenge than BCG vaccine.\textsuperscript{697} Vaccinia strains, such as modified vaccinia Ankara (MVA), have also been used as effective vectors for respiratory immunization.\textsuperscript{695,707–709} For example, an IN MVA vector expressing an HIV-1 antigen induced antigen-specific mucosal CD8\textsuperscript{+} T-cells in genital tissue and who developed models of influenza virus infection with airway colonization and vaginal antibodies.\textsuperscript{710} One caveat to vectored vaccines is that they may reduce its effectiveness.

**Live bacteria**

Bacteria have a major advantage over viruses as vaccine vectors because of their higher capacity for insertion of the heterologous genes expressing antigens, adjuvants, or plasmids for DNA vaccination (described in the next section).\textsuperscript{711} Animal models of respiratory immunization have been used to study attenuated respiratory pathogens such as \textit{Mycoabacterium bovis} bacille Calmette–Guérin (BCG) and attenuated \textit{Bordetella pertussis}, as well as non-respiratory pathogens such as salmonella and shigella (Table 61–2).\textsuperscript{711–713} Commensal bacteria such as food grade strains of lactococcus, lactobacillus and \textit{Streptococcus gordonii} have also been explored as vaccine vectors.\textsuperscript{714–716} Bacterial expression of adjuvants such as CTB, IL-6 and IL-12 has been shown to increase the respiratory vaccine immune response.\textsuperscript{717,718} A potential risk of administering live microbes was revealed in mice who developed strong systemic immune reactions of the lungs after IN but not subcutaneous vaccination.\textsuperscript{720} As with viruses, pre-existing immunity to the bacterial vector may diminish the immune response.\textsuperscript{721}

Several studies in mice have demonstrated an improved immune response to conventional BCG vaccine delivered IN or by aerosol inhalation, compared to injection.\textsuperscript{722} The study that also included a challenge found superior protection of the respiratory route over injection. Attenuated \textit{M. tuberculosis} has also been immunogenic by the respiratory route.\textsuperscript{723} Recombinant BCG has been used to express various heterologous antigens, including simian immunodeficiency virus, \textit{Borrelia burgdorferi} and \textit{Streptococcus pneumoniae}.\textsuperscript{724–726} IN, live attenuated pertussis vaccine protected against pertussis in mice.\textsuperscript{727,728} IN recombinant \textit{B. pertussis} expressing antigens of \textit{Clostridium tetani}, \textit{Haemophilus influenzae}, \textit{Neisseria meningitidis}, or \textit{Schistosoma mansoni} demonstrated strong immune responses in mice.\textsuperscript{726–729} Attenuated recombinant salmonella vaccines produced strong immune responses against a wide variety of pathogens when delivered IN in rodents.\textsuperscript{730–733} Similar results were reported for IN shigella vectors against enterotoxigenic \textit{E. coli} and tetanus.\textsuperscript{730,734}

**DNA vaccines**

DNA vaccination involves the delivery of eponymous plasmids directly into host cells to express the desired antigens.\textsuperscript{735} Delivery of ‘naked’ DNA to the respiratory tract as a vaccine has been studied in animal models for many diseases.\textsuperscript{736–738} For example, Kuklin found 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.\textsuperscript{739} Live attenuated bacteria, especially salmonella and shigella, have been vectored to produce DNA for IN vaccination.\textsuperscript{739,740} For example, cotton rats vaccinated with attenuated salmonella vaccine expressing DNA encoding for measles antigens resulted in significant reduction in measles virus titters in lung tissues following challenge.\textsuperscript{740} Virosomes, liposomes and microparticles—discussed next—have also delivered respiratory DNA vaccines.\textsuperscript{741–743}

**Non-replicating vaccine delivery systems**

Non-replicating vaccine delivery systems, including ISCOMs, liposomes, microparticles, nanoparticles, virosomes and viruses-like particles (VLP), mimic live viruses and deliver antigens and adjuvant. They are particles about the same size as viruses, allowing similar uptake by antigen presenting cells. Many include a lipid component to increase cell membrane permeability, as well as 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.\textsuperscript{744–747} A liposomal HIV-1 delivered IN to mice resulted in strong IgG and IgA responses in serum and vaginal washes.\textsuperscript{748} VLPs are aggregates of viral proteins that may include a lipid component.\textsuperscript{749} IN immunization of mice with a VLP influenza vaccine demonstrated a higher antibody response than injection of the same vaccine, and provided 100% protection to challenge by 5 LD\textsubscript{50}.\textsuperscript{750,751} Virosomes have the lipid bilayer membranes with embedded viral proteins and resemble viruses except they lack the genetic material needed to replicate.\textsuperscript{752–754} An IN virosomal anti-cancer vaccine enhanced the immunologic and protective activity of the vaccine in mice.\textsuperscript{755}

ISCOMs are cage-like structures roughly 40 nm size composed of 12 subunits of saponin (such as Quil A) and cholesterol. Several antigens administered IN in ISCOM-based vaccines produced strong systemic immune responses.\textsuperscript{575,755–757} For example, an IN respiratory syncytial virus ISCOM vaccine induced high levels of serum IgG and IgA in the respiratory tract which persisted for 22 weeks.\textsuperscript{758} Respiratory delivery can also be enhanced by packaging antigens and adjuvants into microparticles or nanoparticles composed of polymers of biodegradable materials such as polylactic acid (PLA) and polylactide co-glycolide (PLGA), or into biopolymers such as chitin or chitosan.\textsuperscript{764–766} Microparticles can be designed to slowly release antigens to increase the duration of antigen presentation. Carcaboso et al reported that mice immunized IN with a synthetic malaria vaccine encapsulated into 1.5 micron microparticles of PLGA had significantly higher antigen-specific serum IgG titters than control mice vaccinated subcutaneously.\textsuperscript{767} IN immunization of mice with an influenza vaccine in chitin microparticles yielded protection against virus challenge, even against a non-vaccine strain.\textsuperscript{803}

**Dry powder aerosol formulations**

Vaccines based on any of the above delivery systems could potentially be produced as dry powders with particle sizes suitable for delivery to the respiratory tract.\textsuperscript{805–807} With appropriate formulation, powders can be highly thermostable which reduces the need for the cold chain. Powders can be prepackaged in inexpensive, single use respiratory delivery devices and delivered dry without aqueous reconstitution. Dry powder delivery to the lung typically requires active inhalation and thus may be difficult with small children. However, two potential delivery solutions for this age group are direct nasal delivery and dis-
Respiratory vaccination in veterinary practice

The respiratory route is common in veterinary medicine. Aerosol vaccines for the IN route or pulmonary inhalation are commercially available for cows (bovine herpes virus-1, parainfluenza virus-3), pigs (Sus scrofa), horses (influenza, Streptococcus equi), cats (feline calicivirus, feline herpesvirus-1) and chickens (infectious bronchitis virus, infectious laryngotracheitis virus, Newcastle disease virus). Most almost of the respiratory veterinary vaccines use live attenuated pathogens.

Respiratory vaccines against potential biological weapons and pandemic threats

Many bioterror or biowarfare agents cause life-threatening respiratory infections, and could be dispersed as aerosols. Thus, vaccine-induced mucosal immunity may be very useful. Compared to the parenteral route, respiratory vaccination increased survival following aerosol exposure of deadly agents in animal studies. For example, a microsphere-based liquid anthrax vaccine delivered IN to mice completely protected against aerosol challenge with anthrax spores. Two doses of human parainfluenza virus vectored Ebola vaccine were highly immunogenic in macaques and protected all animals against lethal Ebola virus challenge. A powdered formulation anthrax vaccine with CPG ODNs administered IN to rabbits also provided full protection. Other bioterror agents for which respiratory vaccines have shown increased protection against aerosol challenge include Francisella tularensis, staphylococcal enterotoxin B (SEB), Burkholderia mallei (glanders) and Yersinia pestis (plague). The threat of a global pandemic of respiratory disease such as influenza or severe acute respiratory syndrome (SARS) is a major public health concern. Respiratory vaccination may be useful in a pandemic setting because of the ease of administration for mass vaccination and the potential for enhanced mucosal immunity resulting in decreased disease transmission. Simple respiratory vaccination devices, such as single use dry powder inhalers, could be widely distributed to avoid the need to congregate for mass vaccination. 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 provided high levels of specific cytotoxic lymphocytes in Balb/c mice. Two doses of IN, live attenuated, H5N1 influenza A vaccine fully protected mice and ferrets against pulmonary replication of homologous and heterologous wild type H5N1 strains. Protection against antigenically diverse strains is highly desirable for a pandemic vaccine because of rapid changes in the influenza surface antigens.

Conclusion

Cutaneous, jet-injected, respiratory and other novel delivery methods may overcome the drawbacks of the traditional needle and syringe. However, demonstrating non-inferiority to the traditional route, respiratory vaccination increased with key factors including the Serum Institute of India (SII), CDC and the University of Colorado on a five-year project funded at over $19 million under the Grand Challenges in Global Public Health program to refine the formulation, complete animal and clinical testing, license the vaccine and establish dry powder measles vaccine production capacity at SII.

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627. Not used.


Alternative vaccine delivery methods


