

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]).¹ 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.² 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,³ the introduction of the needle and syringe (N-S) in the mid 19th century by Pravaz,^{4,5} Rynd⁶ and Wood,⁷ 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.⁸

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

Other drawbacks of N-S include needlestick injuries to health care workers,^{13,14} needle-phobia and discomfort for patients facing increasingly crowded immunization schedules,^{15,16} and the costs and complexity of safe disposal of sharps in the medical waste stream.¹⁷ 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 earlier interest in mass vaccination campaigns,¹⁸ and stimulated research on vaccine delivery not requiring N-S.¹⁹⁻²⁴

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.^{25,26}

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

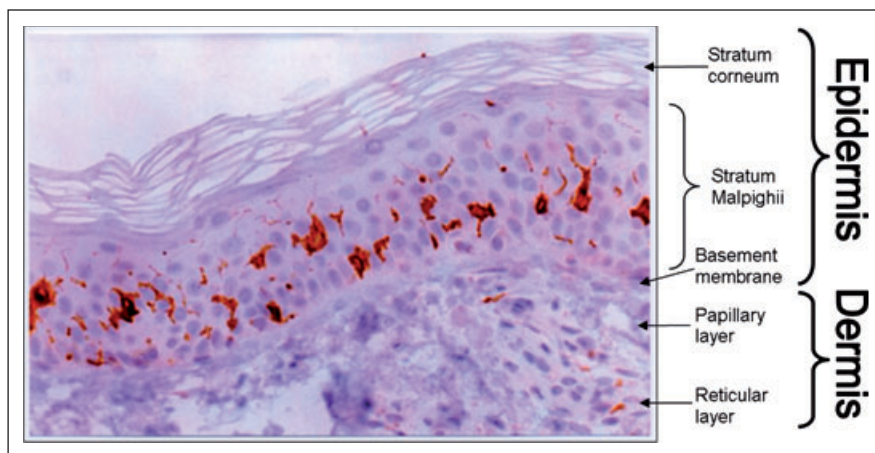


Figure 61-1 Activated Langerhans cells (dark stain) within epidermal Malpighian layer 48 hours after immunization by application of cutaneous patch containing heat-labile enterotoxin (LT) of *E. coli*. Full depth of dermis not shown. (Photograph from Glenn GM, Taylor DN, Xiuri Li, et al. Transcutaneous immunization: a human vaccine delivery strategy using a patch. *Nature Medicine* 6(12):1403–1406, 2000 (Fig. 3b, page 1405), with permission; and from Glenn GM, Kenney RT, Hammond SA, Ellingsworth LR. Transcutaneous immunization and immunostimulant strategies. *Immunol Allergy Clin N Am* 23:787–813, 2003²⁹⁵ (Fig. 1, p. 788), with permission.)

between them, correlates with smaller molecules (<500 Da), lower melting points, increased lipophilicity (and correspondingly lower water solubility), higher (saturated) concentrations, and the paucity of pendant groups that form hydrogen bonds that slow diffusion.^{22,27}

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).²⁸ 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,³³ 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).¹ In the 18th and 19th centuries, the scarification method involved scratching one or more lines into the skin with a needle, scalpel (lancet), or knife and rubbing vaccine into the resulting lesion. A rotary lancet first described in the 1870s consisted of a shaft attached to the center of a small disk, the opposite ‘patient’s side’ of which contained a central tine surrounded by 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 bac-

terial 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.^{35,36}

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),³⁷ 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 needles⁴² and jet injectors.⁴³

Mantoux method

The ID needle technique used for BCG was originally developed by Felix Mendel⁴⁴ and Charles Mantoux⁴⁵ 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

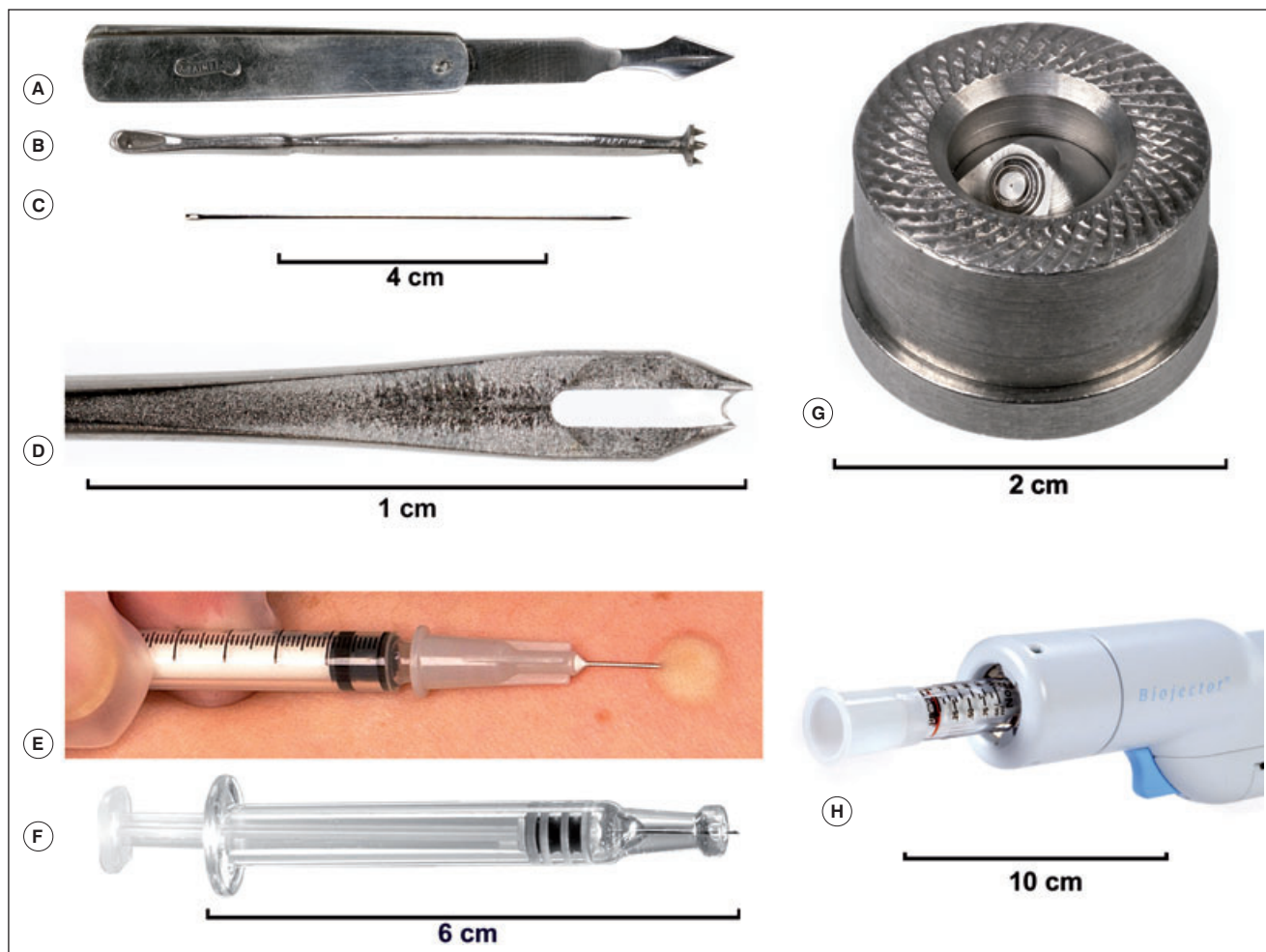


Figure 61-2 Devices for Classical Intradermal Vaccination. **(A)** Vaccinostyle, which scratches the skin before or after applying liquid vaccine. **(B)** Rotary lancet, twirled between thumb and fingers to abrade skin. **(C)** Surgical needle, pressed parallel to skin in multiple-pressure method. **(D)** Bifurcated needle, sharp end shown holding fluid by capillary action between tines. **(E)** 26-gauge hypodermic needle inserted by Mantoux method, creating wheal. **(F)** Investigational intradermal syringe (BD Micro-Delivery System; Becton, Dickinson and Co.⁵³) is inserted perpendicular to skin with 30-gauge needle projecting 1.5 mm beyond its hub. **(G)** Intradermal nozzle of Ped-O-Jet[®] multi-use-nozzle jet injector (Keystone Industries³⁴⁵) (see Fig. 61-4, C), showing 0.127 mm diameter orifice bored into inset sapphire. Recessed cone within nozzle directs jet stream at ~45° angle through short air gap into skin. **(H)** Investigational intradermal spacer on Biojector[®] 2000 disposable-cartridge jet injector cartridge #2 (Bioject, Inc.⁵⁰) used for subcutaneous injections; spacer creates a 2 cm air gap to weaken stream, leaving injectate in the skin. Items A, B, C, D, and G were used for smallpox vaccination; D is currently recommended.

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 [OD] ~0.305 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 ~0.178 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 ~100 LD₅₀ of *Bacillus anthracis* spores had identical survival rates (no adjuvant: 100%, aluminum salt adjuvant [alum]: 100%, CpG: 83%) as IM-immunized controls.⁵⁴ In clinical trials of conventional influenza HA antigen, the 30-gauge ID syringe proved feasible and immunogenic.⁵⁷

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,⁶⁴ as did Davies et al⁸⁶ and Tauraso et al⁷⁴ 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).⁷⁰ 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 CC, 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,⁸⁷ studying hospital employees 18 to 65 years of age, and Klein et al,⁸⁸ 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.⁸⁸ 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]).⁸⁹ The 17D strain showed both good⁹⁰ and poor⁹¹ immune responses when jet-injected ID. The ID route also yielded mixed results for live measles vaccines.⁹²⁻¹⁰⁴

Inactivated vaccines with good immune responses after ID injection include typhoid¹⁰⁵ and rabies,¹⁰⁶⁻¹¹³ the latter of which has been used widely for dose-sparing purposes in the developing world.¹¹⁴ Salk's first clinical trials of inactivated polio vaccine administered it ID,^{115,116} a route widely used for millions of Danes in the mid-1950s,^{117,118} but studied little since despite good responses.¹¹⁹⁻¹²³ Generally good results have been reported for ID hepatitis B,¹²⁴⁻¹³⁰ with some exceptions in infants¹³¹⁻¹³³ and with recombinant vaccine.^{133a-133c} Mixed results have been reported for cholera¹³⁴ and hepatitis A.^{135,136} Other non-living vaccines studied rarely by this route include meningococcal A,¹³⁷ diphtheria-tetanus-pertussis,^{138,139} tetanus,^{140,141} tetanus-diphtheria,¹⁴² tetanus-typhoid,^{143,144} tick-borne encephalitis^{145,146} and Rift Valley fever.¹⁴⁷

Investigational intradermal vaccines

ID injection—as well as IM—led to the serendipitous discovery in an influenza model¹⁴⁸ 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.¹⁴⁹ Gene proto-antigens to prevent influenza,¹⁵⁰ HIV/AIDS,^{151,152} smallpox¹⁵³ and many other diseases are being inserted into both 'naked' DNA/RNA¹⁵⁴ 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.¹⁵⁵

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.²⁷ 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.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.^{20,22,23,27,159,160} 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.¹⁶¹⁻¹⁶⁷ 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.¹⁶⁸

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 TegadermTM patch.¹⁶⁹ 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,¹⁷⁰ 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.^{171,172} Control animals demonstrated that depilation alone had little effect; what made the difference was the mild brushing that produced minimal irritation (Draize scores = 1).¹⁷³

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 sub-micron- to millimeter-sized tines (sometimes referred to as solid microneedles) of silicon, metal, or other material.^{22,174} 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 cm² area of silicon or plastic microprojections that are mounted on a hand-held applicator (OnVax^{TM53}, Fig. 61-3A).¹⁷⁵

Preclinical 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,

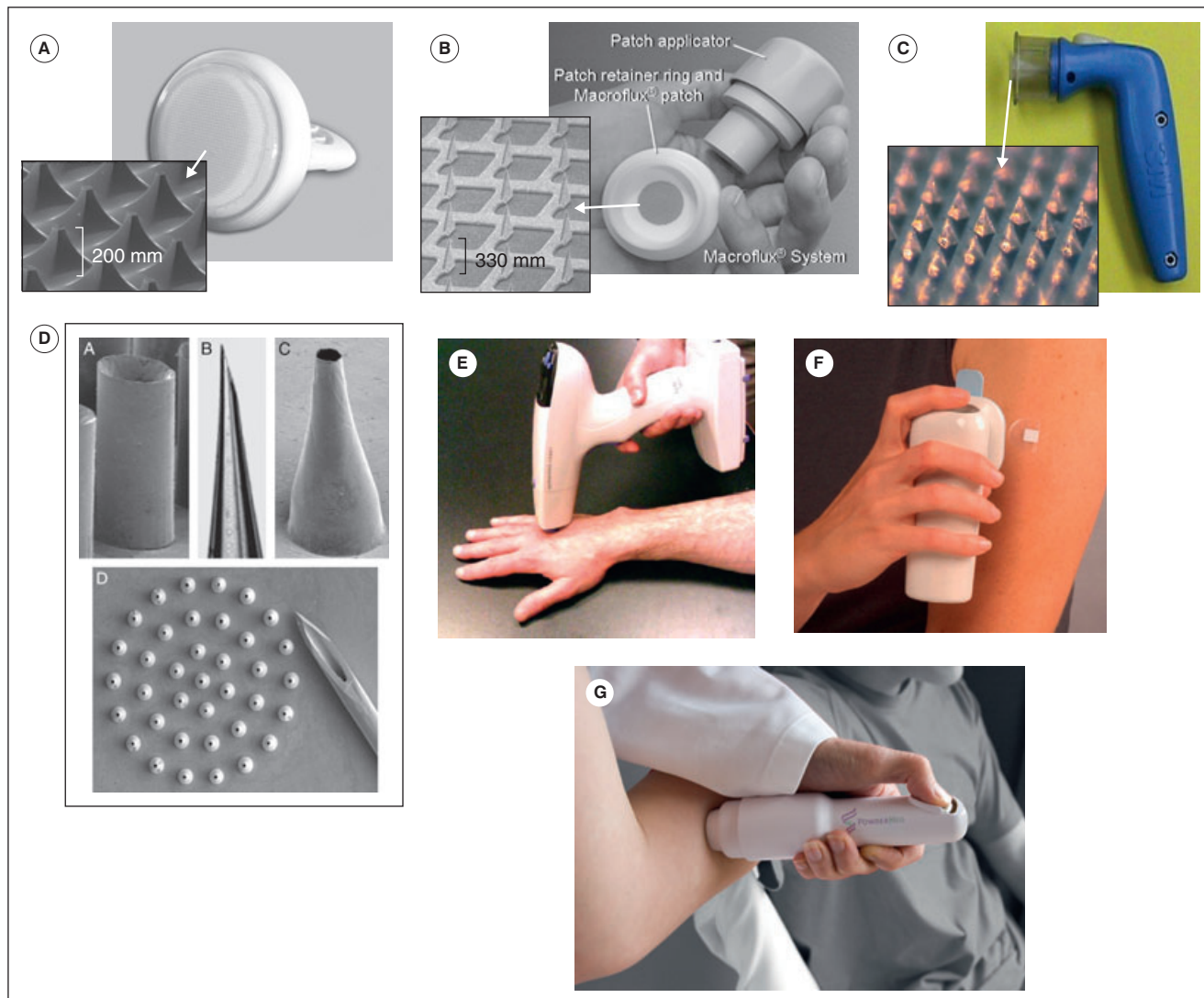


Figure 61-3 Investigational Devices for Cutaneous Vaccination by Mechanical, Electromagnetic, or Kinetic Methods of Disrupting or Penetrating Stratum Corneum. **(A)** OnVax™ hand-held applicator device for abrading skin before or after separate vaccine application (Becton, Dickinson and Co.⁵³). Inset: Scanning electron micrograph (SEM) of plastic microprojections (typical heights 150–200 μm each) of a microenhancer array (MEA) mounted on a hand-held applicator tool. (From Mikszta JA, Sullivan VJ, Dean C, et al. Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. *J Infect Dis* 191:278–288, 2005⁵⁴ (Fig. 1B, p. 281), with permission; and from Prausnitz MR, Mikszta JA, Raeder-Devens J. Microneedles. In: Smith EW, Maibach HI, (eds). *Percutaneous Penetration Enhancers*, 2nd ed. Boca Raton, FL 33487: CRC Press; 2006, 239–255¹⁷⁶ (Fig. 16.1(d), p. 241), with permission). **(B)** Macroflux® microneedle array (microprojections) patch and applicator (Macroflux Corporation¹⁷⁸). Inset: SEM of tines of 330 μm height embedded on the patch, to be coated with drug or antigen and applied into the skin. (From Cormier M, Johnson B, Ameri M, et al. Transdermal delivery of desmopressin using a coated microneedle array patch system. *J Control Release* 97(3):503–11, 2004 (Fig. 2b, p. 506),¹⁸² with permission; and from Matriano JA, Cormier M, Johnson J, et al. Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm Res* 19:63–70, 2002¹⁷⁹ (Fig. 1B, p. 64), with permission.) **(C)** Application device and microphotograph (inset) of microtines of Microstructured Transdermal System (3M Corporation¹⁸³).¹⁸⁸ (From Gordon RD, Peterson TA. Myths about transdermal drug delivery. *Drug Delivery Technology* 2003;3(4):2003 (Fig. 4),¹⁸⁵ with permission.) **(D)** Microneedles, conical and cylindrical (Georgia Institute of Technology²⁰⁰). View of circular array on end (DD) compared to 26-gauge hypodermic needle. (From McAllister DV, Wang PM, Davis SP, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: Fabrication methods and transport studies. *Proc Natl Acad Sci USA* 100:13755–13760, 2003¹⁹⁸ (Fig. 2, p. 13758), with permission; and from Prausnitz MR, Mikszta JA, Raeder-Devens J. Microneedles. In: Smith EW, Maibach HI, (eds). *Percutaneous Penetration Enhancers*, 2nd ed. Boca Raton, FL 33487: CRC Press; 2006, 239–255 (Fig. 16.4, p. 245),¹⁷⁶ with permission.) **(E)** Laser-assisted drug delivery (LAD) device (Norwood Abbey²⁰¹) ablates stratum corneum with laser beam before application of drug. **(F)** PassPort™ patch (Altea Therapeutics²²⁰) applied to patient chest; microporation induction device is held against the patch filaments and then activated to induce painless heat to generate micropores in stratum corneum. **(G)** Particle-Mediated Epidermal Delivery (PMED) Device (PowderMed²⁵⁷) propels microparticles coated with antigen or other drug into skin with supersonic helium gas.

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.⁵⁴ 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.⁵⁶ 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 EKG pads showed little or no TEWL.¹⁷⁵

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.¹⁷⁶ 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 cm^2 at densities from 140 to 650 tines per cm^2 . 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.¹⁸²

Another array of microtines is termed a Microstructured Transdermal System (MTS),¹⁸³ and consists of drug-coated pyramidal projections of 250 μm height, in a density of 1,300 projections per cm^2 , 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.¹⁸⁸ Experimental placement of the device on human volunteers found it to be 'well-tolerated,' 'non-intimidating and not painful.'¹⁸⁶

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.¹⁸⁹ 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[™]).¹⁹⁰⁻¹⁹² Others conducting work with microtines (solid microneedles) include Corium^{194,195} and Valeritas (Micro-Trans[™]).¹⁹⁷

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).^{20,176,198} Although harder to manufacture and more easily broken and clogged,^{174,176} flow rates of microneedles have been measured up to a remarkable 1 mL per minute per cannula.^{176a} Common lengths are 0.2 to 0.5 mm, short enough to be painless since their depth does not reach nerve endings in the dermis.^{22,198,199} Among those working on such microneedles are the Georgia Institute of Technology,^{198,200} Norwood Abbey,²⁰¹ NanoPass (MicroPyramid[™], MicronJet[™]),²⁰² SpectRx (SimpleChoice[™]),¹⁹⁶ and Valeritas.¹⁹⁷

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.^{20,27,203-206} One device, the LAD (laser assisted drug delivery, Norwood Abbey)²⁰¹ generates an erbium-doped yttrium-aluminum-garnet (YAG) laser beam whose energy is highly absorbed by skin (Fig. 61-3E).²⁰⁵ It was shown in adult volunteers to facilitate the anesthetic effect of the topical application of lidocaine,²⁰⁵ 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.

Electrophoretics

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.^{159,218} 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 *microporation*, 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).²¹⁹ In the same model, adenovirus-vectored 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.^{220a} Skin micropores also permitted the passage of insulin in pharmacokinetic human trials with historical controls,^{221,222} and in the other direction allowed interstitial fluid to be extracted for potential glucose monitoring.²²³ 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.^{22,225-230} *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.^{212,219,231–233}

IM electroporation is also being pursued to enhance vaccination with DNA antigens.^{230,234,235} 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 CythorLabTM,²³⁶ Easy VaxTM,¹⁹³ ElectrokineticTM Device (EKD),²³⁷ ECM,²³⁸ MedPulser[®],^{234,235,239} and TriGridTM,^{240,241} among others.

Sound energy

The connection between keratinocytes can be solubilized to facilitate drug or antigen delivery by ultrasonic waves and short-duration shock waves.^{20,22,159,242–244} 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.²⁴⁵ High-molecular weight molecules delivered include insulin, erythropoietin, interferon and low molecular weight heparin.^{22,243,246,247} Various groups are pursuing ultrasound for enhanced drug delivery.^{201,248,249}

Kinetic deposition

The transfection of cells by use of kinetic methods to deposit DNA-coated gold particles into them was pioneered in the 1980s.²⁵⁰ The Helios[®] or PDS 1000/HE ‘gene guns’²⁵¹ and the Accell injector²⁵² have become standard bench tools for ‘biolistic’ delivery of nucleic acid plasmids into a wide variety of plants and animals to transfect them to express the coded genes.^{253,254} Delivery of DNA into the skin overcomes the usual polarized Th1 response when DNA is delivered into muscle.^{21,255,256} These devices are unavailable for human vaccination (patent rights are held by PowderMed²⁵⁷). Documenting the safety of DNA as antigen by any route remains a major regulatory obstacle for such a paradigm shift in human vaccination.²¹

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 Powderject, 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.²¹ For DNA vaccines delivered by PMED, plasmids coding for desired antigens are coated onto gold beads (1–3 μm in diameter) and upon their deposition into epidermal antigen-presenting cells are eluted and transcribed.²⁶³

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 naive and previously vaccinated with conventional vaccine.^{264–267} PMED vaccination has also been studied for DNA priming in trials of malaria vaccine,^{268,269} and produced the first seroprotective immune responses by a DNA vaccine for seasonal influenza.^{150,270} 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 subjects,²⁶⁷ through day 180 in 21 (25%) of 84 injection sites¹⁵⁰ 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.²⁷⁵ 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 encephalitic viruses,²⁷⁶ hantaviruses,²⁷⁷ HIV,²⁷⁸ malaria,²⁷⁹ SARS coronavirus²⁸⁰ and smallpox.²⁸¹

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.²⁸² Another method employs a fast and powerful contractile fiber-activated pump to fire drug at the skin with sufficient velocity to penetrate the epidermis.²⁰¹ A miniaturized form of traditional jet injection uses piezoelectric transducers to propel liquid microjets into the skin.^{282a}

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.¹⁶⁸ Rubbing the skin with acetone also enhances antigen passage by extracting epidermal lipids.¹⁶³

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]).^{158,283–288} For safety reasons, these toxins have been engineered, or mutants selected, to reduce toxicity while retaining adjuvanticity.^{288–291} 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.²⁹²

Iomai technology

Skin vaccination using CT or LT as adjuvants and antigens has been advanced principally by Iomai,²⁹³ which calls the process *transcutaneous immunization*,^{294–296} although others have also studied this technique.²⁹⁷ Such toxins may be administered by themselves as antigen to induce immunity against ETEC causing traveler’s diarrhea or against *Vibrio cholera*, either with²⁹⁸ or without^{299,300} 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.^{300a} Their adjuvant effect has been explored 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,³⁰⁴ anthrax,^{305,306} malaria³⁰⁷ and *Helicobacter pylori*.³⁰⁸ Clinical trials found no serious reactions,²⁹⁹ 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.³⁰⁰ Delayed type hypersensitivity contact dermatitis was observed when using recombinant colonization factor.²⁹⁸

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,¹⁶⁷ dimethylsulfoxide (DMSO), ethanol, limonene and polysorbate, among others.²² Flagellin, a bacterial surface component protein, was engineered to express influenza nucleoprotein epitope and applied to the bare skin of mice, inducing virus-specific interferon- γ T cells.¹⁵⁸ Certain colloids may serve as antigen carriers.²³ Deformable lipid vesicles ('transfersomes') containing tetanus toxoid applied to animal skin yielded comparable immune responses with alum-adjuvanted tetanus toxoid given IM.³⁰⁹

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.^{310,311} Another method is termed a *needle-free solid dose injector* (Glide™).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 about 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.^{313–318} Invented in France in the 1860s (Fig. 61–4A),^{313,319,320} the technology was filed for patent in 1936,³²¹ and reintroduced in the 1940s as the Hypospray[®] 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).^{371–375} Over the last half-century, JIs have administered hundreds of millions, if not billions, of vaccine doses for mass campaigns against smallpox,^{1,376–381} measles,^{376,378,381–384} polio,^{374,385} meningitis,^{386–388} influenza,^{389,390} yellow fever,^{376,381,391,392} cholera³⁹³ and other diseases.^{18,394–397} During the swine influenza mass 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).³⁹⁸ JIs have also been used for a wide variety of therapeutic drugs, including local^{399,400} and pre-general^{401,402} anesthetics, antibiotics,^{403,404} anticoagulants,^{405,406} antivirals,⁴⁰⁷ corticosteroids,^{408,409} cytotoxics,⁴¹⁰ immunomodulators,^{155,411} insulin^{323,348,412} 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)^{316,368} 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.^{197,334} 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 1/3 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.^{328,418–423} 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.^{316,317,322,417,418,424,425} The SC compartment is the only one accessible by most marketed DCJIs, as well as by MUNJIs used in dental anesthesia^{345,346} and 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.⁵⁰ 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.^{427,428}

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 Programme in South America and West Africa in the late 1960s to early 1970s, until invention of the simpler and swifter bifurcated needle.^{1,49,381} Jet injectors also delivered ID the BCG vaccine^{429–434} and various tuberculosis skin testing antigens (TST).^{435–443} However, variations in consequent TST reaction sizes^{43,444} led WHO to discourage JI use for BCG and TST.^{445,446} 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.^{97,377,378,440,447} This ID technique is still pursued

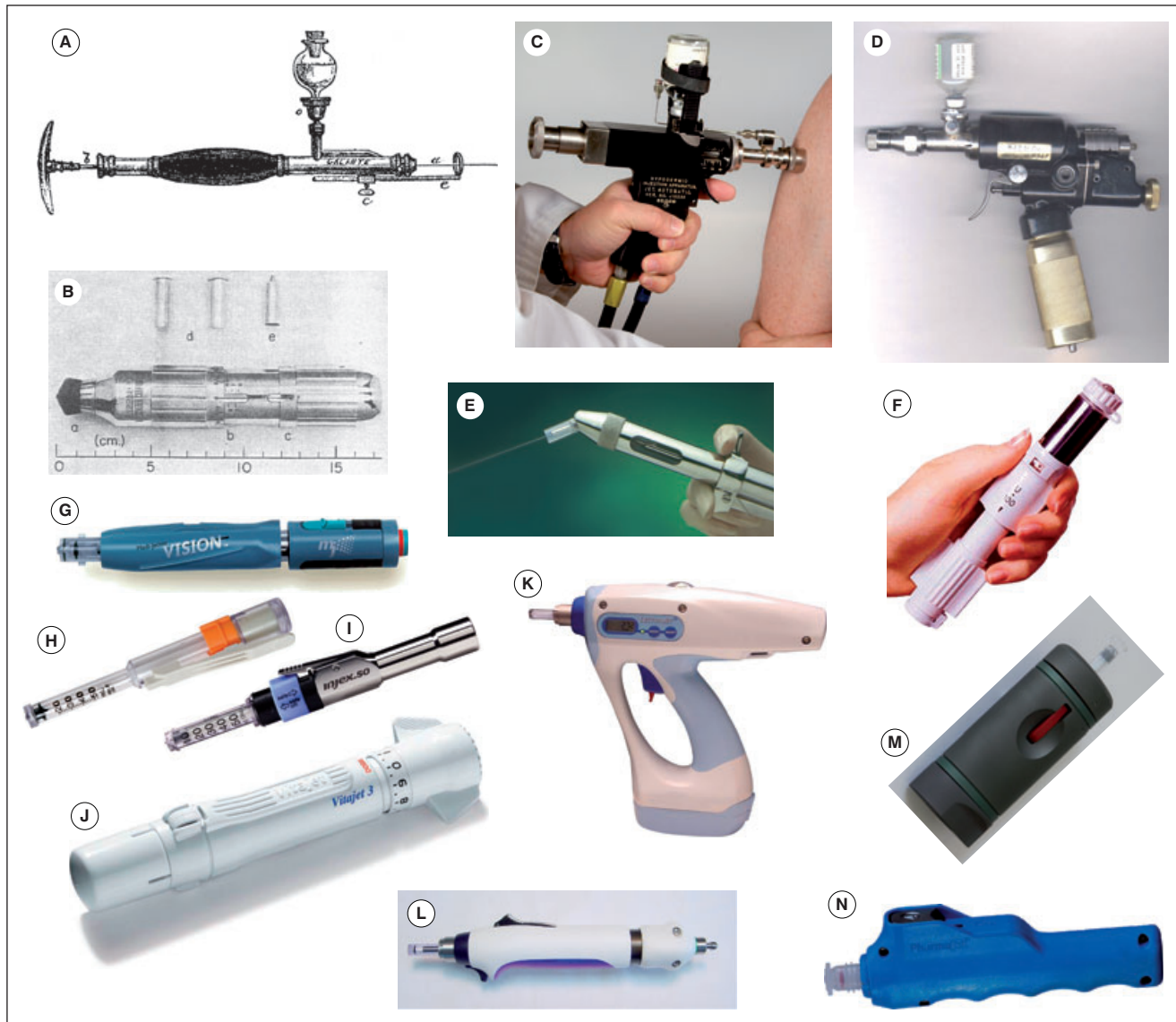


Figure 61-4 Selected Multi-use-Nozzle Jet Injectors (MUNJIS) and Disposable-cartridge Jet Injectors (DCJIs). MUNJIs: **(A)** Aqua-puncture device of Galante et Compagnie,³²⁰ circa 1866, of historical interest as first known jet injector. (From Béclard F. Présentation de l'injecteur de Galante, Séance du 18 décembre 1866, Présidence de M. Bouchardat. Bulletin de l'Académie Impériale de Médecine (France), 32:321–327, 1866³²⁰ with permission.) **(B)** Hypospray[®],³⁶⁰ the first commercial jet injector introduced in the 1940s, with reusable, resterilizable MetaPule[™] cartridges. (From Perkin FS, Todd, GM, Brown TM, Abbott HL. Jet injection of insulin in treatment of diabetes mellitus. Proceedings of the American Diabetes Association 10:185–199, 1950³²³ with permission.) **(C)** Ped-O-Jet[®],³⁴⁵ most widely used jet injector worldwide; metal springs compressed by hydraulic fluid from foot pump or electric pump; SC/IM and ID nozzles available. **(D)** Med-E-Jet[®],³⁴¹ springs compressed either by CO₂ gas cylinder within the handle, capable of about a dozen injections, or by connecting pneumatic hose to bottom of handle from separate tank or electric compressor pump; includes nozzle spacer for intradermal injections. **(E)** MadaJet[®],³⁴⁶ used primarily for local anesthesia in dentistry and medicine; plastic tube over nozzle intended to reduce splashback onto reusable nozzle. **(F)** GentleJet[®],³²⁴ used primarily for self-administration of insulin. DCJIs (also see Biojector[®] 2000 in Fig. 61–2H): **(G)** Medi-Jector[®] VISION[®],³²⁶ used primarily for self-administration of insulin. **(H)** J-Tip[®],³⁵² fully disposable upon single use; powered by compressed nitrogen gas. **(I)** Injex[®],³³⁹ metal spring compressed by separate cocking device. **(J)** Vitajet[™] 3,⁵⁰ used for self-administration of insulin and licensed under other tradenames (Table 61–1) for growth hormone. **(K)** and **(L)** Investigational LectraJet[®] HS (high-speed motorized) and LectraJet[®] M3 (manual) models,³³⁵ which utilize common cartridge capable of rapid, fingers-free loading and unloading from magazine. **(M)** Investigational Vitavax[™],⁵⁰ designed primarily for routine immunization with manual cocking of springs; different autolisabling cartridges for SC, IM, and ID injections. **(N)** PharmaJet[®],³⁵⁸ powered by metal spring compressed with off-tool device; blue model for adults, green and violet (not shown) for children-elderly and infants, respectively; spring power varied for SC, IM, and ID injections via common cartridge.

investigationally for local anesthesia⁴⁴⁸ 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,^{449,450,520,521} cholera,⁴⁵¹ whole cell diphtheria-tetanus-pertussis (DTPw),^{138,139,381,452} hepatitis A,^{452–455} hepatitis B,^{131,456,457} influenza,^{73,78,86,389,452,458–461} plague,^{450,450} polio,⁴⁶² tetanus,^{355,397,452,463} typhoid^{1452,464} and typhoid-diphtheria.¹⁴² With the exception of the variable delayed hypersensitivity responses to BCG discussed earlier, other live vaccines inducing suitable immune responses when administered by JI into their

Table 61-1 Historical, Currently-marketed, and Investigational Jet Injectors Used, Studied, or Proposed for Vaccination

Current/Last Manufacturer	Trade name(s)	Year(s)	Market/Primary Use(s)	Energy Source/Storage	Type	Filling	Target Tissue	References
Activa Brand Products ³²⁴	Predi-Jet™ w	1984	Hu/In	Ma/Sp	MUNJI	On-F	SC	214, 414
	Predi-Jet 50™ w							
	AdvantaJet™							
	GentleJet™							
	Freedom Jet™							
American Jet Injector ³²⁵	Am-O-Jet™	1995	Hu/Va	Pe/Sp	MUNJI	On-I	ID, IM	512
Antares Pharma ³²⁶	Medi-Jector w	1972	Hu/Va	Ma/Sp	MUNJI	On-I	IM, SC	375, 409
	Medi-Jectors II w, III w, IV w	1980s-90s	Hu/In	Ma/Sp	MUNJI	On-F	SC	423
	Medi-Jector Choice (MJ 6) w	1997	Hu/In	Ma/Sp	DCJl	On-F	SC	
	Medi-Jector Vision ^y (MJ 7, ZomaJet™, SciTojet™, Twin-Jector® EZ II)	1999	Hu/In, Gh	Ma/Sp	DCJl	On-F	SC	348
	Valeo™ (MJ 8) /	2000s	Hu/In, Gh	Ma/Sp	DCJl	Md, Sd	SC	316
	Medi-Jector MJ 10 /	1997	Hu/	Ga/Ga	SUDJl	Mf	SC	
	Vibex™/	2001	Hu/Va	Ma/Sp	Mini-needle DCJl, SUDJl	Mf, Off	ID, SC	316
	Vaccijet™ électrique, Avijet™		Ve/Va	Ba/Sp	MUNJI	On-I, via tube	ID, IM, SC	
	Vaccijet™ manuel		Ve/Va	Ma/Sp	MUNJI	On-I	ID, IM SC	
	Guardian™ 101 /	2002	Hu/Un, Va	Ma/Sp	DCJl	Off	SC	
Becton, Dickinson ⁵³	Velodermic™ / w	1940s	Hu/	Ga/Ga (N ₂)	DCJl			313, 328, 348, 385
	Biojector® 2000	1993	Hu/Va, Av	Ga/Ga (CO ₂)	DCJl	Off	ID, IM, SC	24, 51, 52, 232, 330, 331, 338, 401, 407, 426, 455, 457, 461, 471, 481, 482, 483, 484, 495
	Vitajet® w, Vitajet® II w	1984	Hu/In	Ma/Sp	MUNJI	On-F	SC	
	Vitajet® 3 (Cool, Click® s, SeroJet™ s, mhi-500™ m)	1996	Hu/In Gh	Ma/Sp	DCJl	On-F	SC	24, 417, 461
	lject™/	2000s	Hu/Un	Ga/Ga (N ₂)	SUDJl	Mf	SC	
Bioject ⁵⁰	Vitavax™ /	2004	Hu/Va	Ma/Sp	DCJl	On-F	SC	
	Vetjet™ p		Ve/Va	Ma/Sp	DCJl	On-F	SC	329
	Mhi-500™ m	2000s	Hu/In	Ma/Sp	DCJl	On-F	SC	347

Chemical Automatics Design Bureau (CADB) ³³²	BI-1, BI-1M TM , BI-2, BI-3, BI-3M, BIP-4, BI-8, BI-19, ISI-1, SSHA	1960s	Hu/Va	Ma/Sp	MUNJI	On-I	SC, IM	314, 333, 390, 449, 450, 470, 489, 511, 520, 521, 522, 523,
Crossject ³³⁴	Crossject TM /	2001	Hu/Un	Ch/Ch	SUDJI	Mf	SC, IM, ID	
D'Antonio Consultants, International (DCI) ³³⁵	LectraJet [®] HS ¹	1980s	Hu/Va	Ba/Sp	DCJI	Off	ID, IM, SC	24, 336
	LectraJet [®] M3 ¹	2000s	Hu/Va	Ma/Sp	DCJI	Off	ID, IM, SC	24
	LectraVet [®]	1980s	Ve/Va, Mu	Ba/Sp	MUNJI	On-I	IM, SC	
EMS Electro Medical Systems ³³⁷	Swiss Injector [®] /, EMS/RPM ¹	1990s	Un/Un		MUNJI	On-F	IM	338
	EMS/MPM ¹	1990s	Un/Un		MUNJI	Md	IM	338
EuroJet Medical ^{338a}	E-Jet 500	2003	Hu, Ve/Ho, In, St, Va	Ma/Sp	DCJI	Off	SC	
	E-Jet 50	2003	Hu/Va	Ma/Sp	DCJI	Off	SC	
Felton ³⁴²	BI-100 TM /, HSI-500 TM /	1990s	Hu/Va	Pe/Sp	MUNJI	On-I	IM, SC	24, 518
	Pulse 200, 250	1990s	Ve/Mu	Ga/Ga	MUNJI	On-I	IM, SC	
H. Galante et Compagnie ³⁴³	Device for l'Aqua-puncture ^w	1865	Hu/Mu	Ma/Ma	MUNJI	ON-I		320
Genesis Medical ³⁴⁰	Sensa-Jet TM /w	1990s	Hu/Va	Ma/Sp	DCJI	Off	SC	
Heng Yang Weida Science Technology ³⁴⁴	Pro-Jeey 2000		Hu/Un					
INJEX – Equidyne Systems ³³⁹	INJEX [®] 30 and 50 ¹ models, ZipTip TM z	2000	Hu/In, Gh	Ma/Sp	DCJI	Off	SC	24, 415, 467
	Jet Syringe TM /, ROJEX TM /	2000s	Hu/In, Gh	Ma/Sp	SUDJI	Mf or Off	SC	
Keystone Industries ³⁴⁵	Ped-O-Jet [®] w	1950s	Hu/Va	Pe, Ei/Sp	MUNJI	On-I	ID, IM, SC	1, 48, 49, 90, 336, 372, 375, 376, 377, 378, 381, 390, 391, 392, 397, 398, 410, 413, 431, 432, 447, 451, 453, 460, 463, 465, 469, 475, 487, 508, 509, 512, 517
	Syrjet TM	1960s	De, Hu/An, St	Ma/Sp	MUNJI	Md, Sd	ID, SC	413, 422, 485, 510
MADA Medical Products ³⁴⁶	MadaJet TM , MadaJet TM XL	1980s	De, Hu/An, St	Ma/Sp	MUNJI	Md	ID, SC	155, 399, 513
	Med-E-Jet [®]	Early 1970s	Hu/Va	Ga/Ga (CO ₂ , air)	MUNJI	On-I	ID, IM, SC	375, 402, 405, 448, 503, 504, 505, 506, 512
The Medical House PLC ³⁴⁷	mhi-500 ^{TM/m} (InsulinJet ^{TM/m})	2001	Hu/In	Ma/Sp	DCJI	On-F	SC	
	SQ-PEN TM	2002	Hu/In	Ma/Sp	DCJI	On-F	SC	
	SQ-X TM	2002	Hu/In	Ma/Sp	DCJI	On-F	SC	

Table 61-1 Historical, Currently-marketed, and Investigational Jet Injectors Used, Studied, or Proposed for Vaccination—cont'd

Current/Last Manufacturer	Trade name(s)	Year(s) ^y	Market/Primary Use(s)	Energy Source/Storage	Type	Filling	Target Tissue	References
Medical International Technologies ³⁴⁹	Med-Jet [®]	1990s	Hu/An, Va	Ga/Ga (CO ₂)	MUNJI	ON-I	IM, SC	
	Agro-Jet [®]	1990s	Ve/Mu, Va	Ga/Ga (CO ₂)	MUNJI	ON-I	IM, SC	
Microbiological Research Establishment ³⁵⁰	Porton Needleless Injector ^w , Port-O-Jet ^w	1962	Hu/Va	Pe/Sp	MUNJI	ON-I	ID, SC	421, 458, 499
National Medical Products ³⁵²	J-Tip [®]	1990s	Hu/In	Ga/Ga (CO ₂)	SUDJI	On-F	SC	400
Nidec Tosok Corporation ³⁵¹	Hjettor ^{TM/w}	1970s	Hu/Un	Pe/Hy	MUNJI	On-I	IM, SC	
PATH ³⁵³	MEDIVAX ^{TM/w}	1990s	Hu/Va	Pe/Ga (air)	DCJI	On-I	SC, IM	512
PenJet Corporation ³⁵⁷	PenJet ^{®/i}	1990s	Hu/Va	Ga/Ga (N ₂)	SUDJI	Mf	SC	
PharmaJet, Inc. ³⁵⁸	PharmaJet [/]	2000s	Hu, Ve/Va	Ma/Sp	DCJI	Off	ID, IM, SC	
Prolitec SA ³⁵⁹	IsaJet ^{TM/w} , Isa40 Isa10	1990s	Hu, Ve/Un	Ma/Sp	MUNJI	On-I	IDm	
Sanofi Pasteur ³⁵⁴	Mesoflash [®] M10 ^w	1980s	Ve/Un	Ma/Sp	MUNJI	On-I	IDm	
	Mesoflash [®] M30 ^w and M40 ^w	1980s	Hu/Un	Ma/Sp	MUNJI	On-I	IDm	
	Im-O-Jet ^w	1980s	Hu/Va	Pe/Sp	MUNJI	On-I	SC	131, 356, 474, 477
Robert P. Scherer Co. ³⁶⁰	Mini-Imojet ^{®/w} , PM 3C ^{®/w}	1980s	Hu/Va	Ma/Sp	DCJI	Mf	SC	24, 78, 355, 356, 452, 454
	Hypospray ^w	1940s	Hu/In	Ma/Sp	DCJI	Off	ID, SC	313, 322, 323, 403, 404, 408, 413, 416, 418, 424
	Hypospray Professional ^w	1950s	Hu/Va	Ma/Sp	MUNJI	On-I	ID, IM, SC	95, 435
Schuco International ³⁶¹	Hypospray Multidose Jet Injector ^{TM/w} , K ^w , K-2 ^w , K-3 ^w models	1952	Hu/Va	EI/Sp	MUNJI	On-I	ID, IM, SC	73, 86, 385, 393, 394, 420, 435, 439, 488, 490, 497
	Panjet TM multiple models, IntraJet, SchucoJet TM	1960s	Hu/Va	Ma/Sp	MUNJI	On-F, Md	ID, SC	131, 139, 433, 434
Shimadzu Corporation ³⁶²	ShimaJET		Hu/In, Va	Ma/Sp	DCJI	On-F	SC	363, 486
	JET2000		Hu/Va	Ma/Sp	MUNJI	On-I		512
	DG-77		Hu/Va	Ma/Sp	MUNJI	On-I		412

Société AKRA DermoJet ³⁶⁵	DermoJet Standard, Dermojet type HR, Dermojet model G	1960s	Hu/Va	Ma/Sp	MUNJI	On-I, Md	ID, IDm, SC	43, 92, 93, 101, 138, 140, 142, 143, 144, 396, 411, 413, 443, 463
	Dermojet Automatic, Vacci-Jet		Hu/Un	Ma/Sp	MUNJI	On-I	SC	
Valeritas ¹⁹⁷	Miri-Ject™/	2000s	Hu/Mu/Va	Ch/Ch	SUDJI	Mf	ID, IM, SC	331
Z & W Manufacturing ³⁶⁶	Press-O-Jet™	1950s	Hu/Va	Ma/Sp	MUNJI	On-F	SC / IM	371, 385, 389, 413, 462, 468
Zogenix ³⁶⁷	IntraJect®/	1990s	Hu/Ho	Ga/Ga (N ₂)	SUDJI	Mf	SC	368

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 via expanding gases of reaction or combustion, **Ga** = compressed gas, **El** = wall (mains) electricity, **Ma** = manual muscle, **Pe** = pedal muscle / **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: **Mf** = manufacturer prefilled only, **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 chambers 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

¹ Device investigational, or not yet sold commercially for routine use in humans or animals.

^m The mhi-500 (™ The Medical House³⁴⁷) device contains Vitajet[®] 3 technology licensed by Bioject to The Medical House.

ⁿ The Vitjet (™ by Merat³⁷⁰) device is the Vitajet[®] 3 design licensed by Bioject to Merat for delivery to cats of PureVax[®] brand of feline leukemia virus vaccine

^s The cool click[®] and Serolet[™] devices are the Vitajet[®] 3 design licensed by Bioject to Serono³⁶⁸ for delivery of the Saizen[®] and Serostim[®] brands of somatropin (recombinant human growth hormone) for treatment of growth hormone deficiency and AIDS-wasting diseases, respectively.

^v Versions of the Vision[®] injector are licensed to Ferring Pharmaceuticals BV (Zomaret[®]), SciGen Ltd (SciTojet[™]), and JCR Pharmaceuticals (Twin-Jector[®] EZ II).

^w Device withdrawn from market, no longer manufactured, or abandoned in development.

^y Approximate year(s) first introduced to market, investigational development initiated, or patent filed.

^z The ZipTip (™ by Pfizer) is the INJEX design licensed to Pfizer for delivery of Genotropin[®] recombinant human growth hormone.

usual tissue compartment are measles,^{93,95,101,377,381-383,391,447,465,466} measles-mumps-rubella,⁴⁶⁷ measles-smallpox,^{377,378,391} measles-smallpox-yellow fever,^{377,391} smallpox,^{1,48,49,377,381,447,465,468-470} BCG-yellow fever,⁹⁰ and yellow fever.^{89,90,91,377,381,392}

The immunogenicity or efficacy of traditional meningococcal polysaccharide vaccines administered by JIs have been demonstrated for serogroup A in the clinic^{137,472} and in outbreaks in the meningitis belt of western sub-Saharan Africa,^{386,473-477} as well as for serogroup C in South America,⁴⁷⁸⁻⁴⁸⁰ and Africa.^{386,477} Jet injection of the newer Vi capsular polysaccharide typhoid vaccine resulted in 87% seroconversion vs. 69% by needle-syringe ($p < 0.05$).⁴⁵² There have not yet been published clinical studies of JI for modern protein-conjugated polysaccharide vaccines for *Haemophilus influenzae* type b, pneumococcus, or meningococcus.

A wide variety of investigational recombinant nucleic acid vaccines are being delivered in preclinical and clinical trials using various JIs.^{51,330,331,336,363,481-486}

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,^{322,377,389,401,415,416,424,467} but not always.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),^{139,381,394,452} hepatitis A,^{452,453,455,487} hepatitis B,^{131,456,457} tetanus,^{355,395,397,452,463,488} tetanus-diphtheria,¹⁴² tetanus-diphtheria-polio³⁹⁴ and typhoid.^{452,464,489,522} 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 JI vaccination with tetanus toxoid adsorbed to alum,⁴⁹⁰ and pigmented macules persisted in a few hepatitis B vaccinees.⁴⁵⁶

Other adverse events

Bleeding, and less often ecchymosis, are reported to occur at the jet injection site more frequently than with needle injections.^{78,322,348,371,373,374,385,389,401,405,414,416,424,444,452,462,491-493} Rarely, the jet stream may cause a laceration if the health care worker has not properly immobilized the limb and injector in relation to each other during injection.^{322,373,389,416,452} Rare case reports of other adverse events include transient neuropathy^{494,495} and hematoma.^{409,496}

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.^{488,492,493,497} 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.⁴⁹⁸ Others, however, reported negative results in bench or animal testing to try to detect contamination,^{372,405,499,500} or pointed to the lack of epidemiologic evidence of a problem.^{394,499,501,502} Then in 1985, Brink et al described a careful animal model in which a Med-E-Jet transmitted lactic dehydrogenase (LDH) virus between mice in 16 (33%) of 49 animals.⁵⁰³

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.⁵⁰⁴⁻⁵⁰⁶ Subsequent clinical,⁵⁰⁷ field,^{508,509} bench,⁵¹⁰ animal^{511,512} and epidemiologic,^{513,514} studies added more evidence that MUNJIs could transmit pathogens between patients. This led to warnings and discontinuation of their use by public health authorities,^{515,516} and market withdrawal of the Ped-O-Jet and discontinuation of its U.S. military use in 1997.^{318,517}

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.³⁴² 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.^{518,518a} MUNJIs also face doubts raised by high-speed microcinematography revealing extensive splashback,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 MadaJet³⁴⁶ and SyriJet³⁴⁵ 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.^{315,373,375,389} 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,⁵¹⁹ numerous clinical trials were published of high-speed Russian MUNJIs capable of rapidly protecting soldiers or civilians against potential biowarfare agents such as anthrax, botulism, plague, smallpox and tularemia.^{314,449,450,470,489,520-523}

Disposable-cartridge jet injectors (DCJIs)

To overcome concerns over MUNJIs and their withdrawal, since the early 1990s, a new generation of safer, disposable-cartridge jet injectors (DCJIs) have appeared on the market (Table 61-1).³¹⁸ 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[®] 2000 (Fig. 61-2H)⁵⁰ 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[®] 50 (Fig. 61-4I),³³⁹ produced satisfactory immune responses to measles-mumps-rubella vaccine boosters.⁴⁶⁷

To meet developing world needs for needle-free vaccination systems that are economical, autolisabling to prevent reuse, and suitable for both mass campaigns and routine immunization, DCJIs such as the PharmaJet³⁵⁸ and the investigational LectraJet[®] 24,³³⁵ and the Vitavax[™] 50 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)³⁵³ 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[®]) in the United States (see Chapter 16 [influenza, live]). Two

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 infection at the mucosal surface may be especially 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, cholera, 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 traditional nasal spray traversed the external nasal valve.⁵²⁴

Particles that deposit on nasal mucus join the flow of mucus which is swept by ciliated epithelia toward the pharynx, where it is swallowed. Immune surveillance of antigens in the mucus flow occurs by uptake into epithelial cells, intraepithelial dendritic cells, surface macrophages and microfold (M) cells.^{525,526} M cells are specialized epithelial cells which take up macromolecules, viruses and bacteria by endocytosis, and then present them to lymphocytes and dendritic cells that congregate in special pockets in the M cells. The predominant organized lymphoid tissue of the human respiratory tract is located in the pharynx, where the adenoids and other tonsils (collectively known as Waldeyer's ring) surround the nasal and oral passages. The epithelium overlying these tissues is rich with M cells.⁵²⁷ Increased deposition of vaccine antigen in the posterior nasal passages and nasopharynx near Waldeyer's ring may be desirable to maximize the immune response. Breath actuation of a nasal spray and nasal inhalation of smaller aerosol particles (5–20 μm) are two methods to increase nasopharyngeal deposition (Fig. 61–5A,B).^{524,528}

The nasal filtration system is bypassed by mouth breathing (e.g., for vaccine delivery, through a mask or oral prong). In such case, particles impact in the oropharynx, larynx, or trachea. The bifurcation of the trachea into the right and left bronchi starts a series of bifurcations which trap airborne particles. Only very small, light, and slow-moving particles inhaled via either

nose or mouth succeed in navigating the tortuous pulmonary passages to deposit in the lower airways. The smallest particles (<3 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 m^2) in the nasal airways.⁵²⁹ 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 mucosal immunological memory responses. Some lymphocytes exposed to antigen in the respiratory tract migrate to provide protection at remote mucosal sites, such as the vagina. This integrated network of immune cells and tissues is known as the *common mucosal immune system*.^{532,533} 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 candidates because of their key role in immunologic priming, 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 common research animals 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).⁵³⁶ 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 (ExuberaTM), a drug for which dose accuracy and consistency is critical, suggests that this challenge can be overcome for respiratory vaccines.⁵³⁷

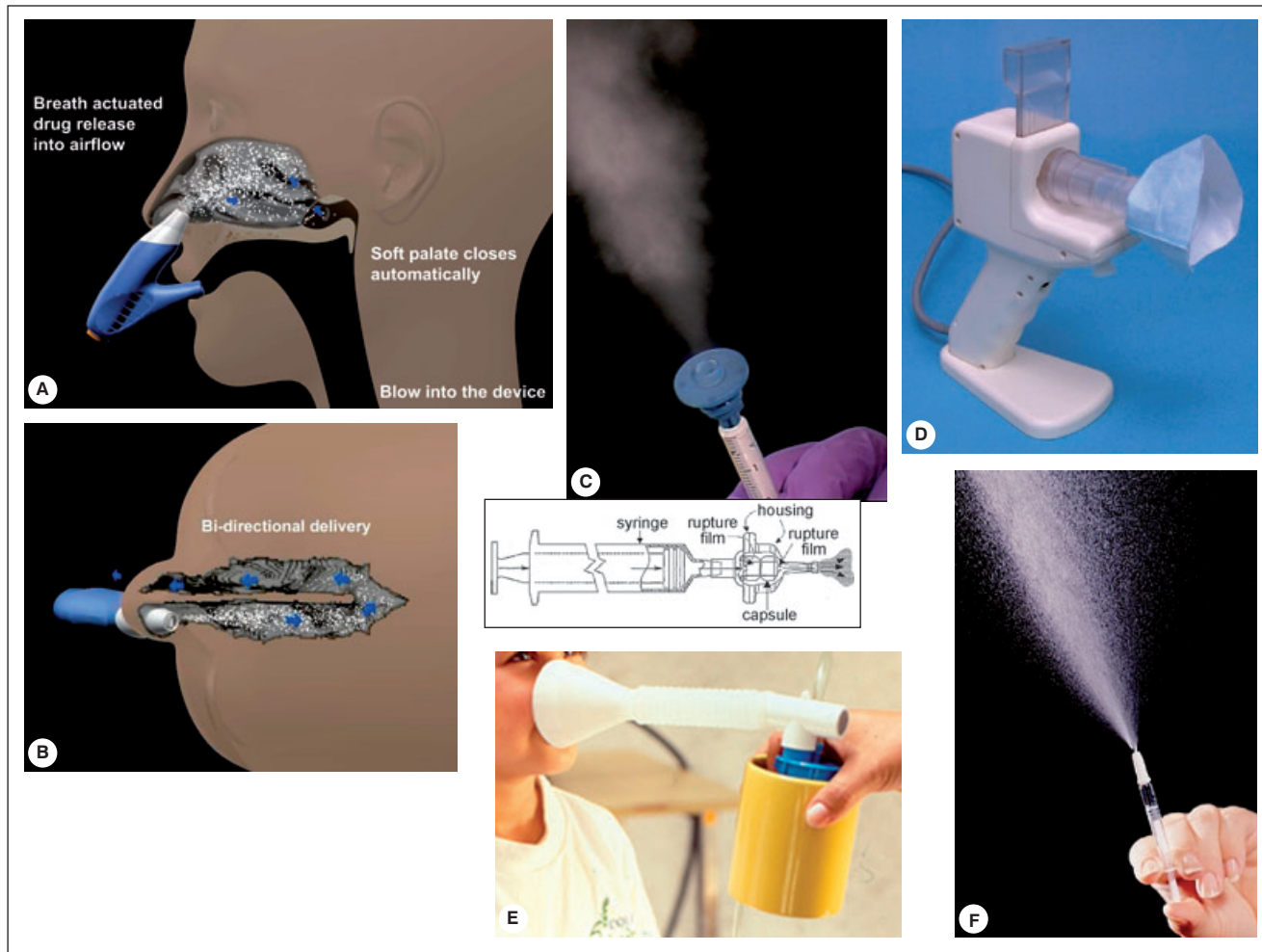


Figure 61-5 Selected Devices for Respiratory Vaccination. **(A–B)** Computer-assisted rendering of sagittal **(A)** and coronal **(B)** sections illustrating intranasal delivery by investigational Optimist™ (OptiNose AS⁴³) device. Exhaling into the device lifts the soft palate, closing off the nasal cavity. The breath actuates the release of liquid or powder particles and carries them beyond the nasal valve to target sites. The air flow passes through the communication posterior to the nasal septum and exits through the other nasal passage. **(C)** Investigational dry powder inhaler prototype (Becton, Dickinson and Co.⁵³). Air from the syringe barrel ruptures the membranes of a capsule containing the vaccine powder and delivers it to the nasal tract. Inset shows detail of vaccine capsule. (Inset from Huang J, Garmise JR, Crowder MT, et al. A novel dry powder influenza vaccine and intranasal delivery technology: introduction of systemic and mucosal immune responses in rats. *Vaccine* 23:794–801, 2004⁵⁴⁶ [Fig. 1a, p. 796], with permission.) **(D)** AeroLife™ prototype (investigational, AerovectRx, Inc.,⁵⁴⁸ originally known as the VaccinAire™ device, developed by Centers for Disease Control and Prevention and Creare, Inc.⁸¹⁹). The nebulizer utilizes battery-powered piezoelectric energy to drive an aerosol from a perforated mesh plate to a disposable patient interface (nasal prong, oral prong or mask). Droplet size can be tailored for upper or lower airway delivery. **(E)** Classic Mexican Device (investigational); a non-medical electric compressor (not shown) delivers roughly 9 liters of air per minute at a pressure of 30–40 pounds per square inch to a jet nebulizer which is kept in crushed ice to maintain vaccine potency. The vaccine aerosol (roughly 0.15 cc) is delivered through a disposable paper cone held close to the patient's face for 30 seconds.^{538–541} **(F)** AccuSpray™ nasal spray syringe (Becton, Dickinson and Co.⁵³); licensed to deliver FluMist™ influenza vaccine. Prefilled and stored frozen for single patient use after thawing. The total volume is 0.5 mL, a dose separator stops delivery at 0.25 mL, and the remaining 0.25 mL is delivered to the opposite nostril.

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,²⁹² 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)),⁵³ 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.^{538,539,540,541} The system consists of a general-use (non-medical) compressor which delivers air to a jet nebulizer (IPI™) 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.⁵⁴²

The OptiMist™ is a breath-actuated nasal spray device for liquid or powders which delivers only during oral exhalation.⁵⁴³ 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).⁵²⁴ 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.⁵⁴⁴

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.⁵⁴⁵

BD⁵³ 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.^{54,546}

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.⁵⁴⁷ Ongoing research focuses on maximizing delivery to the nasopharynx. The AerovectRx™ company⁵⁴⁸ 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).⁵⁴⁹⁻⁵⁵³ Cholera toxin (CT) and *E. coli* heat labile toxin (LT) are potent respiratory immunization adjuvants but are considered too toxic for use in humans.^{551,554-559} LT was an adjuvant in a commercially available IN influenza vaccine in Switzerland which was withdrawn from the market in 2001 due to an increased risk of Bell's palsy among vaccinees.^{292,560} 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.⁵⁶¹ As a result, recent adjuvant research has focused on alternative subunits and variants of CT and LT.⁵⁶²⁻⁵⁸⁰ Several of these, such as CTA1-DD, do not accumulate in the olfactory bulb of BALB/c mice.⁵⁸¹

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 hemagglutinins⁵⁸²⁻⁵⁹³ (Table 61-2). An IN, proteosome-based, inactivated influenza vaccine produced serum and mucosal antibodies in human subjects.⁵⁸³ CpG oligodeoxynucleotides (CpG ODNs) are short segments of synthetically constructed single stranded deoxynucleotides 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.⁵⁹⁴⁻⁵⁹⁷ 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.⁵⁹⁸ 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.⁵⁹⁹ 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

Adjuvant	Vaccines	Studied In	Serum IgG	Mucosal IgA	Challenge Protection	References
Bacterial Toxins						
Cholera Toxin (CT)	Trichomonas, Malaria, <i>Chlamydia trachomatis</i> , <i>Streptococcus pyogenes</i>	Mice	+ + ^	+ + ^	++	554, 555, 556, 557
CT-B subunit	Pneumococcus, Group A Streptococcus, Human Papilloma Virus (HPV), Tetanus, Gonorrhea, Group B Streptococcus, <i>Porphyromonas gingivalis</i> , Diphtheria, Simian Immunodeficiency Virus (SIV)	Mice	+++++ ^^	+++++ ^^^	+++	562, 563, 564, 565, 566, 567, 568, 569, 570
CT mutants, CTA1-DD	<i>C. trachomatis</i> , Human Immunodeficiency Virus (HIV), Influenza, <i>Helicobacter pylori</i> , HPV	Mice, Macaques	+ ^ ^ ^	+ ^ ^ ^	+ + ^ ^	564, 571, 572, 573, 574, 575, 576, 581
<i>Escherichia coli</i> heat labile toxin (LT)	Meningococcus, <i>P. gingivalis</i> , Measles	Mice	+ + ^	+ ^		558, 559, 568
LT-B subunit	Meningococcus	Mice	+	+		784
LT mutants	Influenza, Meningococcus, Ricin, <i>P. gingivalis</i> , Measles	Mice, Humans	+ + + + + ^^	+ + + + + ^^	^	559, 568, 577, 578, 579, 580
Other Bacterial Products						
Proteosomes, Outer membrane vesicles	Respiratory Syncytial Virus (RSV), Leishmania, Influenza, Hepatitis B, Measles, Plague,	Mice, Humans	+ + + ^ ^	+ + + ^ ^	+ + + + ^	582, 583, 584, 585, 586, 587, 817
Lipopolysaccharide	Measles, Leishmania, Meningococcus, Influenza, Plague	Mice	+ ^	+ ^	+	585, 586, 587
Monophosphoryl Lipid A (MPL)	Anthrax, SIV, Meningococcus	Mice, Rabbits, Macaques	+ + ^	+ + ^	+ ^	588, 589
Lipopeptides	HIV, Measles	Mice, Cotton rats	++	++	+	590, 591
Flagellins	Plague, Tetanus	Mice, Monkeys	+ ^	+ ^	+ ^	592, 593
Bacterial DNA Motifs						
CpG ODNs	Tetanus, Tuberculosis, <i>Haemophilus influenzae</i> , Trichomonas, <i>H. pylori</i> , <i>S. pyogenes</i>	Mice, Guinea pigs, Rabbits	+ + + + + ^	+ + + + +	+ + + +	554, 556, 594, 595, 596, 597, 598
Cytokines/Chemokines						
Interleukins (IL-1, IL-5, IL-6, IL-12, IL-15, IL-23) GM-CSF Type 1 Interferon	Tuberculosis, Human Papilloma Virus (HPV), Herpes Simplex Virus (HSV), HIV, Simian/Human Immunodeficiency Virus (SHIV), Pneumococcus, Influenza	Mice, Macaques	+ + + ^ ^^^	+ + + + ^ ^^	+ + ^ ^ ^ ^^	573, 600, 601, 602, 603, 604, 605, 607
Plant Derivatives						
Quillaja Saponins	<i>P. gingivalis</i> , HIV	Mice	^^	^^	^	568, 611
Other Adjuvants						
Chitin, Chitosan	Anthrax, Influenza	Mice, Rabbits	^^	^^	+ ^	588, 804

+ 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).⁶⁰⁰⁻⁶⁰⁵ Chemokines and cytokines have been added directly to the vaccine, or encoded for expression by a live vector or DNA vaccine.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.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.⁶⁰⁸ Chitin and chitosan have mucoadhesive properties and stimulate the innate immune system.⁶⁰⁹ In humans, the addition of chitosan to an IN vaccine based on CRM-197 diphtheria antigen significantly increased toxin-neutralizing antibody levels.⁶¹⁰ The saponins of the *Quillaja saponaria* tree are potent adjuvants with high toxicity. Quil A, QS-21 and ISCOPREP 703 are subcomponents with less toxicity.⁵⁵² As adjuvant to an IN DNA HIV-1 vaccine studied in mice, QS-21 consistently increased antigen-specific serum IgG and mucosal IgA compared to vaccine without adjuvant.⁶¹¹ Quil A and ISCOPREP 703 are commonly used as components of immunostimulating 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.⁵⁸⁷

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).⁶¹²⁻⁶¹⁶

Live viruses

Viruses are prototypical antigen delivery vehicles because they enter and commandeer 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.^{591,617-622,624-626,628-636} Their major risks are possible reversion 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[®])⁶³⁷ 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.^{638-641c} It also provides heterotypic immunity against influenza strains that had antigenically drifted from the vaccine strains.⁶⁴² Finally, it may reduce the risk of influenza transmission because it reduces respiratory shedding among children

challenged with a vaccine virus.⁶⁴² Also, modest coverage with CAIV among school children reduced influenza-related illness rates in unvaccinated adults in a community.⁶⁴³

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 1997,¹⁰⁴ 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.^{101,644-652} 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 vaccinees were usually equivalent to or greater than to injected vaccines.^{540,541,644,645,649,650,653-665} 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.^{541,664,665} 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.⁶⁶⁶ A subsequent outbreak investigation showed measles attack rates of 0.8% among 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.^{538,539,648,655-659,662,667,668} 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.^{538,539}

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.^{538,539,541,661,663,669} 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.⁵⁴⁷ 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.⁶⁷⁰⁻⁶⁷⁷ 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.⁶⁷² 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.⁶⁶¹

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

Vaccine Delivery Vehicle	Vaccines	Studied In	References
Live Viruses			
Homologous vaccines	Measles, Mumps, Rubella, Influenza, Varicella, SHIV, HIV, HSV, Yellow fever, Rotavirus, Parainfluenza, RSV, Smallpox	Mice, Cotton Rats, Monkey, Humans	101, 538, 539, 540, 541, 547, 591, 617, 618, 619, 620, 621, 622, 624, 625, 626, 628, 629, 630, 631, 632, 633, 634, 635, 636, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677
Viral Vectors			
Adenovirus	HIV, Severe Acute Respiratory Syndrome (SARS), Rotavirus, SIV, HSV, Rabies, Plague, RSV, Tetanus	Mice, Hamsters, Cotton Rats, Ferrets, Monkeys	169, 171, 589, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706
Modified Vaccinia Virus of Ankara	HIV, Vaccinia, Parainfluenza, SARS, SHIV	Mice, Monkeys	603, 707, 708, 709, 710
Adeno-associated Virus	Influenza, HPV, Alzheimer's (A beta peptide)	Mice	678, 679, 680
Vesicular Stomatitis Virus	Tuberculosis, Plague, HIV	Mice	681, 682, 683, 684, 685, 686, 687, 688, 689
Live Bacteria			
Attenuated Homologous Vaccines	BCG (tuberculosis), Pertussis	Mice, Possum	708, 711a, 711b, 713a, 718, 720, 722, 723, 724, 725, 726, 727, 732, 733, 734, 735
Bacterial Vectors			
Food Grade Bacteria	HPV, Tetanus	Mice	714, 715, 716, 717, 719
Attenuated Pathogens	Tuberculosis, Salmonella, HIV, <i>Borrelia burgdorferi</i> (Lyme disease), Pneumococcus, Tetanus, <i>H. influenzae</i> , Meningococcus, Plague, Rotavirus, Hepatitis B, <i>Clostridium difficile</i> , <i>E. coli</i> , SARS	Mice, Possum, Horse, Humans	711, 712, 713, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 817a
DNA Vaccine			
Naked DNA	HIV, Tuberculosis, <i>H. Pylori</i> , HPV, SHIV, HSV, Rotavirus, Coxsackie virus	Mice, Monkeys	753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 778, 779, 780, 781
Bacteria Vectors	Measles, Hepatitis B, HIV, HSV, Tetanus, <i>Chlamydia pneumoniae</i>	Mice, Cotton Rats, Guinea Pigs	773, 774, 775, 776, 777
Non-replicating Delivery Systems			
Liposomes	Meningococcus	Mice	782, 783, 784, 785
Virus Like Particles	SIV, HIV	Mice	569, 786, 787
Virosomes	Influenza, Carcinoembryonic antigen	Mice, Humans	788, 789, 790
ISCOMS	Diphtheria, Influenza, Bovine Respiratory Syncytial Virus	Mice, Guinea pigs, Cows	575, 791, 792, 793, 794, 795
Microparticles and Nanoparticles			
PGA/PLGA particles	Hepatitis B, <i>E. coli</i> , Malaria	Mice	796, 798, 800, 801, 803
Chitin/ Chitosan particles	<i>Bordetella bronchiseptica</i> , Meningococcus, Influenza	Mice	578, 580, 799, 802, 804
Dry Powder Formulations	Anthrax, Influenza, Measles	Mice, Rabbits, Monkeys	546, 588, 805, 806, 807, 807a

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.^{678–689} IN adenovirus vectors produced immune responses against many diseases in several animal models (Table 61–2).^{169,171,690–706} For example, a replication defective adenovirus expressing *M. tuberculosis* antigen delivered IN to mice provided better protection against respiratory challenge than BCG vaccine.⁶⁹⁷ Vaccinia strains, such as modified vaccinia Ankara (MVA), have also been used as effective vectors for respiratory immunization.^{603,707–709} For example, an IN MVA vector expressing an HIV-1 antigen induced antigen-specific mucosal CD8(+) T-cells in genital tissue and draining lymph nodes of mice, along with serum and vaginal antibodies.⁷¹⁰ One caveat to vectored vaccines is that pre-existing immunity in the population to the vector virus, either by natural exposure or by previous use in another vaccine, 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).⁶¹³ Animal models of respiratory immunization have been used to study attenuated respiratory pathogens such as *Mycobacterium bovis* bacille Calmette–Guérin (BCG) and attenuated *Bordetella pertussis*, as well as non-respiratory pathogens such as salmonella and shigella (Table 61–2).^{711–713} Commensal bacteria such as food grade strains of lactococcus, lactobacillus and *Streptococcus gordonii* have also been explored as vaccine vectors.^{714–717} Bacterial expression of adjuvants such as CTB, IL-6 and IL-12 has been shown to increase the respiratory vaccine immune response.^{718,719} A potential risk of administering live microbes was revealed in mice who developed dose-dependent granulomatous BCG infiltration of the lungs after IN but not subcutaneous vaccination.⁷²⁰ As with viruses, pre-existing immunity to the bacterial vector may diminish the immune response.⁷²¹

Several studies in mice have demonstrated an improved immune response to conventional BCG vaccine delivered IN or by aerosol inhalation, compared to injection.^{708,718,720,722–726} The studies that also included a challenge found superior protection of the respiratory route over injection. Attenuated *M. tuberculosis* has also been immunogenic by the respiratory route.⁷²⁷ Recombinant BCG has been used to express various heterologous antigens, including simian immunodeficiency virus, *Borrelia burgdorferi* and *Streptococcus pneumoniae*.^{728–731} IN, live attenuated pertussis vaccine protected against pertussis in mice.^{732–735} IN recombinant *B. pertussis* expressing antigens of *Clostridium tetani*, *Haemophilus influenzae*, *Neisseria meningitidis*, or *Schistosoma mansoni* demonstrated strong immune responses in mice.^{736–739}

Attenuated recombinant salmonella vaccines produced strong immune responses against a wide variety of pathogens when delivered IN in rodents.^{740–749} Similar results were reported for IN shigella vectors against enterotoxigenic *E. coli* and tetanus.^{750,751}

DNA vaccines

DNA vaccination involves the delivery of eponymous plasmids directly into host cells to express the desired antigens.⁷⁵² Delivery of 'naked' DNA to the respiratory tract as a vaccine has been studied in animal models for many diseases.^{753–771} 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.⁷⁷² Live attenuated bacteria, especially salmonella and shigella, have been vectored to produce DNA for IN vaccination.^{750,773–776} For example, cotton rats vaccinated with attenuated salmonella vaccine expressing DNA encoding for measles antigens resulted in significant reduction in measles virus titers in lung tissues following challenge.⁷⁷⁷ Virosomes, liposomes and microparticles—discussed next—have also delivered respiratory DNA vaccines.^{778–781}

Non-replicating vaccine delivery systems

Non-replicating vaccine delivery systems, including ISCOMs, liposomes, microparticles, nanoparticles, virosomes and virus-like particles (VLP), mimic live viruses in how they deliver antigen 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.^{782–784} A liposomal HIV-1 delivered IN to mice resulted in strong IgG and IgA responses in serum and vaginal washes.⁷⁸⁵ VLPs are aggregates of viral proteins that may include a lipid component.⁷⁸⁶ 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 LD₅₀.⁷⁸⁷ Virosomes have lipid bilayer membranes with embedded viral proteins and resemble viruses except they lack the genetic material needed to replicate.^{788,789} An IN virosomal anti-cancer vaccine enhanced the immunologic and protective activity of the vaccine in mice.⁷⁹⁰

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 and mucosal immune responses.^{575,791–795} 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.⁷⁹¹ Respiratory delivery can also be enhanced by packaging antigens and adjuvants into microparticles or nanoparticles composed of polymers of biodegradable materials such as polylactide (PLA) and polylactide co-glycolide (PLGA), or into biopolymers such as chitin or chitosan.^{796–802} 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 titers than control mice vaccinated subcutaneously with alum adjuvant.⁸⁰³ IN immunization of mice with an influenza vaccine in chitin microparticles yielded protection against virus challenge, even against a non-vaccine strain.⁸⁰⁴

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.^{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-

pensing the powder into a reservoir or 'spacer' from which the child can breathe normally. An IN influenza dry powder vaccine elicited high titers of nasal anti-influenza IgA as well as serum antibody titers equivalent to injected vaccine when administered to rats.⁵⁴⁶ The powder formulation showed no loss of potency when stored at 25°C and 25% relative humidity (RH) for up to 12 weeks. In one experiment it maintained full potency for 2 weeks at 40°C and 75% RH. Impermeable packaging which maintains powders dry at very low humidity may maintain potency to substantially increase their shelf life. IN dry powder formulations of an anthrax vaccine have provided complete protection against inhalational anthrax challenge (103 LD50) in rabbits while providing superior stability compared to liquid formulations.^{54,807a,807b}

Dry powder formulations have also been tested for measles vaccines. Early formulations milled to a fine powder retained adequate potency, but immune responses were poor when delivered to the respiratory tract of macaques.^{805,807} AKTIV-DRY⁸⁰⁸ used a novel spray-drying system to manufacture and test powder formulations of live attenuated measles vaccines. Measles virus plaque assays demonstrated potency losses in the drying process of 0 to 22%, which is comparable to losses seen with lyophilization.⁸⁰⁹ AKTIV-DRY is working with key partners 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.⁸¹⁰

Respiratory vaccination in veterinary practice

The respiratory route is common in veterinary medicine.⁸¹¹ Aerosol vaccines for the IN route or pulmonary inhalation are commercially available for cows (bovine herpes virus-1, parainfluenza virus-3), pigs (*Salmonella*), horses (influenza, *Streptococcus equi*), dogs (*Bordetella bronchiseptica*), cats (feline calicivirus, feline herpesvirus-1) and chickens (infectious bronchitis virus, infectious laryngotracheitis virus, Newcastle disease virus). Almost all of the respiratory veterinary vaccines 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 dispensed 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.⁸¹² Two doses of human parainfluenza virus vectored Ebola vaccine were highly immunogenic in macaques and protected all animals against lethal Ebola virus challenge.^{812a} A powdered formulation anthrax vaccine with CPG ODNs administered IN to rabbits also provided full protection.⁵⁴ 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).⁸¹³⁻⁸¹⁷

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 T lymphocytes in Balb/c mice.^{817a} 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.^{817b} 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 for existing vaccines will require expensive clinical data not yet generated for some of these methods.²¹ Economic analysis that recognizes the hidden costs of needles and syringes may justify the necessary R&D investment. For diseases not yet vaccine-preventable—such as gonorrhea, herpes simplex, HIV, *Chlamydia*, respiratory syncytial virus, parainfluenza and SARS—these alternate routes, taking advantage of the cutaneous or respiratory immune systems and their novel adjuvants and immunopotentiators, may finally provide vaccines to conquer them.

Acknowledgments

Credits for previously unpublished photography: Figure 61-2A,B,C,D,E,G,H, CDC Photographic Services. Figures 61-2F and 61-5C,F, Becton, Dickinson and Co.(BD).⁵³ Figure 61-3E, Norwood Abbey.²⁰¹ Figure 61-3F, Altea Therapeutics.²²⁰ Figure 61-3G, PowderMed.²⁵⁷ Figure 61-4E, Mada.³⁴⁶ Figure 61-4F, Activa Brand Products.³²⁴ Figure 61-4G, Antares Pharma.³²⁶ Figure 61-4H, National Medical Products.³⁵² Figure 61-4I, INJEX-Equidyne Systems.³³⁹ Figure 61-4J,M, Bioject.⁵⁰ Figure 61-4K,L, DCI.³³⁵ Figure 61-4N, PharmaJet.³⁵⁸ Figure 61-5A,B, OptiNose.⁵⁴³ Figure 61-5D, Creare.⁸¹⁸ Figure 61-5E, Jose Luis Valdespino (Instituto Nacional de Salud Pública, Mexico).

We are grateful to D.A. Henderson (University of Pittsburgh) for lending vaccinostyle and rotary lancet (Fig. 61-2A,B), Robert H. Thrun (Anchor Products Company) for surgical needle (Fig. 61-2C) and to the following organizations and individuals for photographs, pre-publication manuscripts, reference material, fact-checking and other assistance: 3M¹⁸³ (Cheryl A. Carlson), Activa Brand Products, Altea Therapeutics (Alan Smith, Frank Tagliaferri), Antares Pharma (Anne E. Olinger, Peter Sadowski), Avant Medical³²⁷ (Andrew C. Barnes), BD (Noel Harvey, Sherry Dean, Pat McCutchen, John Mikszta, Vince Sullivan), Bioject (Sergio Landau, Richard Stout), CDC Photographic Services (James Gathany, Greg Knobloch), Creare (James Barry), DCI³³⁵ (Rick Colvin; Linda, Nicholas Jr, Nicholas Sr. and Ronald D'Antonio), Georgia Institute of Technology (Mark Prausnitz), INJEX-Equidyne (Randy Willis), Iomai (Gregory Glenn, Wanda Hardy), Macroflux (Michel Cormier, Peter Daddona), Mada (Robert Sorbello), Mercer University (Ajay Banga), Merck (John Grabenstein), National Medical Products (Rekha Patel), Norwood Abbey (Peter Hansen), OptiNose⁵⁴³ (Per Gisle Djupesland), PATH (Darin Zehrung), PowderMed (Peter Loudon, Phil Price), TheraJect¹⁹⁰ (Sung-Yun Kwon), Weston Medical (Terry Weston), and others.

References

1. Fenner F, Henderson DA, Arita I, Je'ek Z, Ladnyi ID. Chapter 9. Development of the global smallpox eradication programme, 1958–1966 (pp. 365–419); Chapter 11. Smallpox vaccine and vaccination in the intensified smallpox eradication programme (pp. 539–592); Chapter 12. South America (pp. 593–625); Chapter 13. Indonesia (pp. 627–657); Chapter 17. Western and Central Africa (pp. 849–909). In: Smallpox and its Eradication, Geneva: World Health Organization, 1988 (ISBN 92 4 156110 6). Online. Available at: <http://www.who.int/smallpox/9241561106.pdf>
2. Hopkins DR. The Greatest Killer: Smallpox in History. Chicago, Ill: University of Chicago Press; 2002.
3. Feldmann H. Die Geschichte der Injektionen. Laryngo-Rhino-Otologie 79:239–246, 2000.
4. Blaessinger E. Un prestigieux centenaire polytechnicien Charles-Gabriel Pravaz (1791–1853). La Presse Médicale 61:1182–1183, 1953.
5. Martin M-E. Le centenaire de Pravaz. Maroc Med 32:736–737, 1953.
6. Rynd F. Description of an instrument for the subcutaneous introduction of fluids in affections of the nerves. Dublin Quart J Med Sci 32:13, 1861.
7. Wood A. New method of treating neuralgia by the direct application of opiates to the painful points. Edin Med Surg J 82:265–281, 1855.
8. Pasteur L. Compte rendu sommaire des expériences faites à Pouilly-le-Fort, près Melun, sur la vaccination charbonneuse (avec la collaboration de MM. Chamberland et Roux). Comptes Rendus de l'Académie des Sciences (Paris) 92:1378–1383, 1881.
9. Mendelsohn JA. 'Like all that lives': biology, medicine and bacteria in the age of Pasteur and Koch. Hist Philos Life Sci 24:3–36, 2002.
10. Simonsen L, Kane A, Lloyd J, et al. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. Bull WHO 77:789–800, 1999.
11. Hagan H, Des Jarlais DC. HIV and HCV infection among injecting drug users. Mt Sinai J Med 67:423–428, 2000.
12. Drucker E, Alcabes PG, Marx PA. The injection century: massive unsterile injections and the emergence of human pathogens. Lancet 358:1989–1992, 2001.
13. Prüss-Ustün A, Rapiti E, Hutin Y. Sharps Injuries: Global Burden of Disease from Sharps Injuries to Health-care Workers. Geneva: World Health Organization; 2003 (WHO Environmental Burden of Disease Series, No. 3). Online. Available at: www.who.int/quantifying_ehimpacts/publications/9241562463/en/index.html. Accessed July 15, 2006.
14. Panlilio AL, Orelan JC, Srivastava PU, et al. Estimate of the annual number of percutaneous injuries among hospital-based healthcare workers in the United States, 1997–1998. Infect Control Hosp Epidemiol 25:556–562, 2004.
15. Lieu TA, Black SB, Ray GT, et al. The hidden costs of infant vaccination. Vaccine 19:33–41, 2000.
16. Jacobson RM, Swan A, Adegbenro A, et al. Making vaccines more acceptable—methods to prevent and minimize pain and other common adverse events associated with vaccines. Vaccine 19:2418–2427, 2001.
17. Prüss A, Giroult E, Rushbrook P, (eds). Safe management of wastes from healthcare activities. Geneva, Switzerland: World Health Organization; 1999 (ISBN 92 4 154525 9). Online. Available at: www.who.int/water_sanitation_health/medicalwaste/wastemanag/en/. Accessed April 22, 2006.
18. Foege WH, Eddins DL. Mass vaccination programs in developing countries. Prog Med Virol 15:205–243, 1973.
19. Levine MM. Can needle-free administration of vaccines become the norm in global immunization. Nature Medicine 9:99–103, 2003.
20. Cross SE, Roberts MS. Physical enhancement of transdermal drug application: Is delivery technology keeping up with pharmaceutical development? Current Drug Delivery 1:81–92, 2004.
21. O'Hagan DT, Rappuoli R. Novel approaches to vaccine delivery. Pharmaceutical Research 21:1519–1530, 2004.
22. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nature Reviews 3:115–124, 2004.
23. Mitragotri S. Immunization without needles. Nature Reviews Immunology 5:905–916, 2005.
24. Giudice EL, Campbell JD. Needle-free vaccine delivery. Advanced Drug Delivery Reviews 58:68–89, 2006.
25. Cheng X, Koch PJ. In vivo function of desmosomes. J Dermatol 31:171–187, 2004.
26. Huber O. Structure and function of desmosomal proteins and their role in development and disease. Cell Mol Life Sci 60:1872–1890, 2003.
27. Hadgraft J. Skin, the final frontier. Int J Pharm 224:1–18, 2001.
28. Steinhoff M, Brzoska T, Luger TA. Keratinocytes in epidermal immune responses. Curr Opin Allergy Clin Immunol 1:469–476, 2001.
29. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 392:245–252, 1998.
30. Romani N, Holzmann S, Tripp CH, Kock F, Stoitzner P. Langerhans cells – dendritic cells of the epidermis. APMIS 111:725–740, 2003.
31. Sallusto F. Origin and migratory properties of dendritic cells in the skin. Curr Opin Allerg Clin Immunol 1:441–448, 2001.
32. Goldsby RA, Kindt TJ, Kuby J, Osborne BA. Immunology, 5th ed. New York: W.H. Freeman; 2003, 603.
33. Yu RC, Abrams DC, Alaibac M, Chu AC. Morphological and quantitative analyses of normal epidermal Langerhans cells using confocal scanning laser microscopy. Br J Dermatol 131:843–848, 1994.
34. Leake JP. Questions and answers on smallpox and vaccination. Public Health Rep 42:221–238, 1927.
35. Kravitz H. A simplified technique for vaccination against smallpox. Pediatrics 27:219–226, 1961.
36. Mérieux C, Mérieux A, Triau R. Tuberculination, vaccination B.C.G. ou antivariolique à l'aide d'une bague à pointes. Arch Mal Prof 27:444–449, 1966.
37. Lugosi L. Theoretical and methodological aspects of BCG vaccine from the discovery of Calmette and Guérin to molecular biology. A review. Tuber Lung Dis 73:252–261, 1992.
38. Birkhaug K. An experimental and clinical investigation of a percutaneous (Rosenthal) method of BCG vaccination. Nord Med 10:1224–1231, 1941.
39. Briggs IL, Smith C. BCG vaccination by the multiple puncture method in Northern Rhodesia. Tubercle 38:107–111, 1957.
40. Griffith AH. BCG vaccination by multiple puncture. Lancet 273:1170–1172, 1959.
41. Gheorgiu M. The present and future role of BCG vaccine in tuberculosis control. Biologicals 18:135–141, 1990.
42. Darmanger AM, Nekzad SM, Kuis M, et al. BCG vaccination by bifurcated needle in a pilot BCG vaccination programme. Bull WHO 55:49–61, 1977.
43. ten Dam HG, Fillastre C, Conge G, et al. The use of jet-injectors in BCG vaccination. Bull WHO 43:707–720, 1970.
44. Mendel F. Die von Pirquet'sche Hautreaktion und die intravenöse Tuberkulinbehandlung. Medizinische Klinik (München) 4:402–404, 1908.
45. Mantoux C. Intradermo-réaction de la tuberculine. Comptes Rendus de l'Académie des Sciences (Paris) 147:355–357, 1908.
46. Centers for Disease Control and Prevention. Mantoux tuberculosis skin test – facilitator guide. Atlanta: Department of Health and Human Services, CDC, Division of Tuberculosis Elimination. Online. Available at: <http://www.cdc.gov/tb/pubs/Mantoux/part1.htm>. Accessed August 19, 2007.
47. Ismach A. Intradermal nozzle for jet injection devices (U.S. Patent 3,140,713), assigned to Secretary of the Army on behalf of United States of America. Washington, DC: United States Patent Office; issued July 14, 1964.
48. Millar JD, Roberto RR, Wulff H, Wenner HA, Henderson DA. Smallpox vaccination by intradermal jet injection. Introduction, background and results of pilot studies. Bull WHO 41:749–760, 1969.
49. Neff JM, Millar JD, Roberto RR, Wulff H. Smallpox vaccination by intradermal jet injection. Evaluation in a well-vaccinated population. Bull WHO 41:771–778, 1969.
50. Bioject, Inc., Tualatin, OR 97062-7541, USA. Online. Available at: www.bioject.com.
51. Epstein JE, Gorak EJ, Charoenvit Y, et al. Safety, tolerability, and lack of antibody responses after administration of a PfCSP DNA malaria vaccine via needle or needle-free jet injection, and comparison of intramuscular and combination intramuscular/intradermal routes. Hum Gene Ther 13:1551–1560, 2002.
52. Bråve A, Ljungberg K, Boberg A, et al. Multigene/multisubtype HIV-1 vaccine induces potent cellular and humoral immune responses by needle-free intradermal delivery. Mol Ther 12:1197–1205, 2005.
53. Becton, Dickinson and Co., Franklin Lakes, NJ 07417-1815, USA. Online. Available at: www.bd.com/technologies/add.
- 53a. Laurent A, Mistretta F, Bottiglioli D, et al. Echographic measurement of skin thickness in adults by high-frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines. Vaccine 25(34):6423–6430, 2007.
54. Mikszta JA, Sullivan VJ, Dean C, et al. Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. J Infect Dis 191:278–288, 2005.
- 54a. Mikszta JA, Dekker JP, Harvey NG, et al. Microneedle-based intradermal delivery of the anthrax recombinant protective antigen vaccine. Infect Immun 74(12):6806–6810, 2006.
55. Alarcon JB, Waterston Hartley A, Harvey NG, Mikszta JA. Preclinical evaluation of microneedle technology for intradermal delivery of influenza vaccines. Clin Vaccine Immunol 14(4):375–381, 2007.
56. Dean CH, Alarcon JB, Waterston AM, et al. Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax™-JE) in non-human primates. Human Vaccines 1:106–111, 2005. [erratum: 1:179, 2005].
57. Belshe RB, Newman FK, Cannon J, et al. Serum antibody responses after intradermal vaccination against influenza. N Engl J Med 351:2286–2294, 2004.
58. Vaughan JP, Lindqvist K, Brooke D, Doyle RF. Combined BCG and smallpox immunization: a preliminary report on a method using the W.H.O. bifurcated needle. East Afr Med J 49:207–212, 1972.
59. Vaughan JP, Menu JP, Lindqvist KJ, Venneman A. A trial with a mixed BCG smallpox vaccine given intradermally. J Trop Med Hyg 76:262, 1973.
60. Francis T, McGill T. The antibody response of human subjects vaccinated with the virus of human influenza. J Exper Med 65:251–259, 1937.
61. Bruyn HB, Meiklejohn G, Brainerd HD. The use of influenza virus vaccine in children. Proceedings of the Western Society for Clinical Research, San Francisco. Am J Med 4:622, 1947.
62. Van Gelder D, Greenspan F, Dufresne N. Influenza vaccination: comparison of intracutaneous and subcutaneous methods. Naval Med Bull 47:197–206, 1947.
63. Weller TH, Cheever FS, Enders JF. Immunologic reactions following the intradermal inoculation of influenza A and B vaccine. Proc Soc Exp Biol Med 67:96–101, 1948.
64. Bruyn H, Meiklejohn G, Brainerd H. Influenza vaccination: a comparison of antibody response obtained by various methods of administration. J Immunol 62:1–11, 1949.
65. Bruyn H, Meiklejohn G, Brainerd H. Influenza vaccine: A study of serologic responses and incidence of reactions following subcutaneous and intradermal inoculation. Am J Dis Child 77:149–163, 1949.

66. Edwards HK, Wellings FM, Colwell FO, et al. Immunization against influenza in industry. *Int Med Surg* 27:638-640, 1958.
67. Hilleman MR, Flatley FJ, Anderson SA, et al. Antibody response in volunteers to Asian influenza vaccine. *JAMA* 166:1134-1140, 1958.
68. Kirkham LJ. Asiatic influenza in a midwestern town: with a comparison of intradermal and subcutaneous vaccination. *J Iowa State Med Soc* 48:593-598, 1958.
69. Sanger MD. Immunization after intradermal and subcutaneous injection of Asian influenza vaccine. *Ann Allergy* 17:173-178, 1959.
70. Stille WT, Woolridge RL, Gundelfinger BF. Antibody response to intracutaneous and subcutaneous influenza vaccination. *J Lab Clin Med* 53:751-754, 1959.
71. Beasley AR, Sigel MM, Schlaepfer GG, et al. Antibody responses of children to Asian influenza vaccine. *J Fla Med Assoc* 46:1367-1371, 1960.
72. Saslaw S, Carlisle HN. Effect of dosage on antibody response to polyvalent influenza virus vaccine in an aged population. *Am J Med Sci* 248:273-284, 1964.
73. Clark ML, Reinhardt H, Miller MC, Wilson R. Polyvalent influenza vaccine: comparison of jet injection with intradermal and subcutaneous syringe methods of administration. *J Lab Clin Med* 66:34-41, 1965.
74. Tauraso NM, Gleckman R, Pedreira FA, et al. Effect of dosage and route of inoculation upon antigenicity of inactivated influenza virus vaccine (Hong Kong strain) in man. *Bull WHO* 41:507-516, 1969.
75. Marks MI, Eller JJ. Intradermal influenza immunization. Experience with Hong Kong vaccine. *Am Rev Respir Dis* 103:579-581, 1971.
76. Brown H, Kasel JA, Freeman DM, et al. The immunizing effect of influenza A/New Jersey/76 (HswN1) vaccine administered intradermally and intramuscularly to adults. *J Infect Dis* 136(Suppl 2):S466-S471, 1977.
77. Halperin W, Weiss WI, Altman R, Diamond MA, et al. A comparison of the intradermal and subcutaneous routes of influenza vaccination with A/New Jersey/76 (swine flu) and A/Victoria/75: report of a study and review of the literature. *Am J Public Health* 69:1247-1251, 1979.
78. Spiegel A, Lemardeley P, Germanetto P, et al. Mini-Imojet® et vaccination anti-grippale dans les armées françaises. Tolérance immédiate et faisabilité. Actes: 4ème Séminaire International sur les Vaccinations en Afrique - 'Bâtir des Partenariats Durables pour les Vaccinations en Afrique', Yamoussoukro, Côte d'Ivoire, 13-16 March 1994. Association pour l'Aide à la Médecine Préventive. (eds): Parent du Château I, Schlumberger M, da Silva A, Stoeckel P. Lyon: Fondation Mérieux Collection, 1994, p. 344-345.
79. Kenney RT, Frech SA, Muenz LR, et al. Dose sparing with intradermal injection of influenza vaccine. *N Engl J Med* 351:2295-2301, 2004.
80. Boger WP, Liu OC. Subcutaneous and intradermal vaccination with Asian influenza vaccine. *JAMA* 165:1687-1689, 1957.
81. Saslaw S, Carlisle HN, Slutzker B. Antibody response to polyvalent influenza virus vaccine administered intradermally or subcutaneously in an aged population. *Am J Med Sci* 245:387-398, 1963.
82. Phillips CA, Forsyth BR, Christmas WA, et al. Purified influenza vaccine: clinical and serologic responses to varying doses and different routes of immunization. *J Infect Dis* 122:26-32, 1970.
83. Sigel MM, Edwards HK, Schlaepfer GA, et al. Preliminary findings on vaccination against Asian influenza [letter]. *JAMA* 165:1860-1861, 1957.
84. Hutchinson P, Izumi T, Davidson W. Influenza vaccines: intradermal administration. *Can Dis Wkly Rep* 3-28:110-111, 1977.
85. Herbert FA, Larke RP, Markstad EL. Comparison of responses to influenza A/New Jersey/76-A/Victoria/75 virus vaccine administered intradermally or subcutaneously to adults with chronic respiratory disease. *J Infect Dis* 140:234-238, 1979.
86. Davies JW, Simon WR. Antibody response to influenza immunization by jet injection. *Canadian J Public Health* 60:104-108, 1969.
87. McCarroll J, Kilbourne ED. Immunization with Asian-strain influenza vaccine: equivalence of the subcutaneous and intradermal routes. *N Engl J Med* 259:618-621, 1958.
88. Klein M, Huang M. The response of infants and children to Asian influenza vaccine administered by intradermal and subcutaneous routes. *J Pediatr* 58:312-314, 1961.
89. Durier C. Mass yellow fever vaccination in French Africa south of the Sahara. In: Smithburn KC, Durieux C, Koerber R., et al, (eds). *Yellow Fever Vaccination (Monograph Ser. No. 30)*. Geneva: World Health Organization; 1956:115-121.
90. Chambon L, Tommasi UB, Barne M, et al. Vaccination associée BCG-fièvre jaune avec un injecteur du type Ped-O-Jet. Rapport Final de la Xème Conférence Technique de l'OCCGE [Organisation de Coopération et de Coordination pour la Lutte Contre les Grandes Endémies], Bobo-Dioulasso, Upper Volta, 20-24 April 1970; 1:282-288.
91. Gateff C, Robin Y, Labusquière R, et al. Comparaison de deux vaccins anti-mariarils administrés par injecteur sous pression sans aiguille. *Médecine Tropicale (Marseille)* 32:193-197, 1972.
92. Rey M, Baylet R, Cantrelle P, et al. Vaccination contre la rougeole en milieu rural sénégalais par un vaccin vivant suratténué (Schwarz) au moyen d'un injecteur sans aiguille (Dermojet). Possibilités d'association avec le vaccin. *Bull Soc Méd Afrique Noire Lang Franç* 10:392-406, 1965.
93. Cooper C, Morley DC, Weeks MC, Beale AJ. Administration of measles vaccine by Dermojet. *Lancet* 1(7446):1076-1077, 1966.
94. Hong Kong Measles Vaccine Committee. Comparative trial of live attenuated measles vaccine in Hong Kong by intramuscular and intradermal injection. *Bull WHO* 36:375-384, 1967.
95. Calafiore DC, Nader PR, Lepow ML, et al. Attenuated measles virus vaccine dosage study - Cleveland Ohio, 1966. *Am J Epidemiol* 247-253, 1968.
96. Rey M, Cantrelle P, Lafaix C, et al. Enseignements d'une campagne expérimentale de vaccination contre la rougeole en milieu urbain. *Bulletin Société Médicale d'Afrique Noire de Langue Française* 13:291-310, 1968.
97. Weibel RE, Stokes J, Jr, Buynak EB, et al. Clinical-laboratory experiences with combined dried live measles-smallpox vaccine. *Pediatrics* 37:913-920, 1966.
98. Burland, W. Measles vaccination by the intradermal route. *Postgrad Med J* 45:323-326, 1969.
99. Stanfield JP, Bracken PM. Measles vaccination: studies in methods and cost reduction in developing countries. *Trans R Soc Trop Med Hyg* 65:620-628, 1971.
100. Wood PB, Shoeranda KS, Bracken PM, Houser NE. Measles vaccination in Zaire-when and how? *Trans R Soc Trop Med Hyg* 74:381-382, 1980.
101. Kok P, Kenya P, Ensoring H. Measles immunization with further attenuated heat-stable measles vaccine using five different methods of administration. *Trans R Soc Trop Med Hyg* 77:171-176, 1983.
102. Whittle H, Rowland M, Mann G, et al. Immunization of 4-6 month old Gambian infants with Edmonston-Zagreb measles vaccine. *Lancet* i:834-837, 1984.
103. de Moraes JC, Leon ME, Souza VA, et al. Intradermal administration of measles vaccines. *Bull Pan Am Health Organ* 28:250-255, 1994.
104. Cutts FT, Clements CJ, Bennett JW. Alternative routes of measles immunization: a review. *Biologicals* 25:323-338, 1997.
105. Tuft L. Active immunization against typhoid fever, with particular reference to an intradermal method. *J Lab Clin Med* 16:552-556, 1931.
106. Nicholson KG, Prestage H, Cole PJ, et al. Multisite intradermal antirabies vaccination. *Lancet* 2(8252):915-918, 1981.
107. Bernard KW, Roberts MA, Sumner J, et al. Human diploid cell rabies vaccine. Effectiveness of immunization with small intradermal or subcutaneous doses. *JAMA* 247:1138-1142, 1982.
108. Harverson G, Wasi C. Use of post-exposure intradermal rabies vaccination in a rural mission hospital. *Lancet* 2(8398):313-315, 1984.
109. Warrell MJ, Nicholson KG, Warrell DA, et al. Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post exposure rabies prophylaxis. *Lancet* 1(8437):1059-1062, 1985.
110. Phanuphak P, Khawplod P, Sirivichayakul S, et al. Humoral and cell-mediated immune responses to various economical regimens of purified Vero cell rabies vaccine. *Asian Pac J Allergy Immunol* 5:33-37, 1987.
111. Chutivongse S, Wilde H, Spuich C, et al. Post-exposure prophylaxis for rabies with antiserum and intradermal vaccination. *Lancet* 335:896-898, 1990.
112. Briggs DJ, Banzhoff A, Nicolay U, et al. Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. *Bull WHO* 78:693-698, 2000.
113. Madhusudana SN, Sanjay TV, Mehendra BJ, et al. Comparison of safety and immunogenicity of purified chick embryo cell rabies vaccine (PCECV) and purified Vero cell rabies vaccine (PVRV) using the Thai Red Cross intradermal regimen at a dose of 0.1 mL. *Human Vaccines* 2:200-204, 2006.
114. Wilde H, Khawplod P, Khamoltham T, et al. Rabies control in South and Southeast Asia. *Vaccine* 23:2284-2289, 2005.
115. Salk JE. Recent studies on immunization against poliomyelitis. *Pediatrics* 12:471-482, 1953.
116. Salk JE. Studies in human subjects on active immunization against poliomyelitis. I. A preliminary report of experiments in progress. *JAMA* 151:1081-1098, 1953.
117. von Magnus H. Polio vaccination 1955-1967 og fremtidige polio vaccinationer. *Ugeskr Laeg* 129:1759-1762, 1967.
118. von Magnus H. Salk: Control of polio with noninfectious vaccine. In: *Cellular Biology; Nucleic Acids and Viruses (Special Publications)*. New York: NY Academy of Sciences; 1957, 96-97.
119. Sigurdsson B, Gudnadóttir M, Pétursson G. Response to poliomyelitis vaccination. *Lancet* 1(7016):370-371, 1958.
120. Connolly JH, Dick GW, Corkin DL. Antibody response following intradermal or oral administration of formalinised poliomyelitis. *Lancet* 2(7042):333-336, 1958.
121. Samuel BU, Cherian T, Sridharan G, et al. Immune responses to intradermally injected inactivated poliovirus vaccine. *Lancet* 338(8763):343-344, 1991.
122. Samuel BU, Cherian MD, Rajasingh J, et al. Immune response of infants to inactivated poliovirus vaccine injected intradermally. *Vaccine* 10:135, 1992.
123. Nirmal S, Cherian T, Samuel BU, et al. Immune response of infants to fractional doses of intradermally administered inactivated poliovirus vaccine. *Vaccine* 16:928-931, 1998.
124. Halsey NA, Reppert EJ, Margolis HS, et al. Intradermal hepatitis B vaccination in an abbreviated schedule. *Vaccine* 4:228-232, 1986.
125. King JW, Taylor EM, Crow SD, et al. Comparison of the immunogenicity of hepatitis B vaccine administered intradermally and intramuscularly. *Rev Infect Dis* 12:1035-1043, 1990.
126. Bryan JP, Sjogren M, Iqbal M, et al. Comparative trial of low-dose, intradermal, recombinant- and plasma-derived hepatitis B vaccines. *J Infect Dis* 162:789-793, 1990.
127. Bryan JP, Sjogren MH, Perine PL, Legters LJ. Low-dose intradermal and intramuscular vaccination against hepatitis B. *Clin Infect Dis* 14:697-707, 1992.
128. Bryan JP, Sjogren MH, Macarthy P, et al. Persistence of antibody to hepatitis B surface antigen after low-dose, intradermal hepatitis B immunization and response to a booster dose. *Vaccine* 10(1):33-38, 1992.
129. Parish DC, Muecke HW, Joiner TA, et al. Immunogenicity of low-dose intradermal recombinant DNA hepatitis B vaccine. *Southern Med J* 84:426-430, 1991.
130. Egeman A, Aksit S, Kurugol Z, et al. Low-dose intradermal versus intramuscular administration

- of recombinant hepatitis B vaccine: a comparison of immunogenicity in infants and preschool children. *Vaccine* 16:1511-1515, 1998.
131. Whittle HC, Lamb WH, Ryder RW. Trial of intradermal hepatitis B vaccines in Gambian children. *Ann Trop Paediatr* 7:6-9, 1987.
 132. Woodruff BA, Moyer LA. Intradermal vaccination for hepatitis B [letter]. *Clin Infect Dis* 15:1063-1066, 1992.
 133. Coberly JS, Townsend T, Repke J, et al. Suboptimal response following intradermal hepatitis B vaccine in infants. *Vaccine* 12:984-987, 1994.
 - 133a. Centers for Disease Control and Prevention. Inadequate immune response among public safety worker receiving intradermal vaccination against hepatitis B—United States, 1990-1991. *MMWR* 40(33):569-572, 1991.
 - 133b. Payton CD, Scarisbrick DA, Sikotra S, Flower AJ. Vaccination against hepatitis B: comparison of intradermal and intramuscular administration of plasma derived and recombinant vaccines. *Epidemiol Infect* 110(1):177-180, 1993.
 - 133c. Turchi MD, Martelli CM, Ferraz ML, et al. Immunogenicity of low-dose intramuscular and intradermal vaccination with recombinant hepatitis B vaccine. *Rev Inst Med Trop Sao Paulo* 39(1):15-19, 1997.
 134. McBean AM, Agle AN, Compaore P, et al. Comparison of intradermal and subcutaneous routes of cholera vaccine administration. *Lancet* 1(7749):527-529, 1972.
 135. Brindle RJ, Morris CA, Berger R, Kurtz JB. Inadequate response to intradermal hepatitis A vaccine. *Vaccine* 12:483-484, 1994.
 136. Pancharoen C, Mekmullica J, Thisyakorn U, et al. Reduced-dose intradermal vaccination against hepatitis A with an aluminum-free vaccine is immunogenic and can lower costs. *Clin Infect Dis* 41:1537-1540, 2005.
 137. Gotschlich EC, Rey M, Triau R, Sparks KJ. Quantitative determination of the human immune response to immunization with meningococcal vaccines. *J Clin Investigation* 51:89-96, 1972.
 138. Rossier E, Heiz R. Essai clinique d'un vaccin mixte contre la diphtérie le tétanos et la coqueluche, administré par voie intradermique au moyen du 'Dermo-Jet'. *Schweiz Med Wochenschr* 98:1602-1608, 1968.
 139. Stanfield JP, Bracken PM, Waddell KM, Gall D. Diphtheria-tetanus-pertussis immunization by intradermal jet injection. *Br Med J* 2(807):197-199, 1972.
 140. Mérieux C. Single shot primovaccination against tetanus by needleless injectors. In: Echmann L. (ed.). *Principles on Tetanus. Proceedings, International Conference on Tetanus, 15-19 July 1966. Bern, Switzerland. Bern: Verlag Hans Huber; 1967, 423-436.*
 141. Dimache G, Stoean C, Durbaca S, et al. Study of specific immune response to unadsorbed concentrated tetanus vaccine administered by intradermal route to non-immunized persons in the last ten years. *Arch Roum Pathol Exp Microbiol* 49:51-62, 1990.
 142. Wegmann A, Heiz R, Baumann T. Auffrisch-Impfung mit einem Diphtherie-Tetanus-Impfstoff für Dermo-Jet mit niedrigem Diphtherietoxoidgehalt. *Schweiz Med Wochenschr* 106:112-114, 1976.
 143. Dimache G, Stoean C, Durbaca S, et al. Intradermal antitetanic-antiphoid booster by jet injection. *Roum Arch Microbiol Immunol* 50:117-125, 1991.
 144. Dimache G, Croitoru M, Velea V, et al. Intradermal antiphoid-antitetanus vaccination by jet injection. *Roum Arch Microbiol Immunol* 50:127-135, 1991.
 145. Zoulek G, Roggendorf M, Deinhardt F. Immune response to single dose, multisite, intradermal and to intramuscular administration of vaccine against tick-borne encephalitis virus. *Lancet* 2(8402):584, 1984.
 146. Zoulek G, Roggendorf M, Deinhardt F, Kunz C. Different immune responses after intradermal and intramuscular administration of vaccine against tick-borne encephalitis virus. *J Med Virol* 19:55-61, 1986.
 147. Kark JD, Aynor Y, Peters CJ. A Rift Valley fever vaccine trial: 2. Serological response to booster doses with a comparison of intradermal versus subcutaneous injection. *Vaccine* 3:117-122, 1985.
 148. Ulmer JB, Donnelly JJ, Parker SE, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 1745-1749, 1993.
 149. Plotkin SA. Vaccines: past, present and future. *Nature Med* 11(4s):S5-S11, 2005.
 150. Drape RJ, Macklin MD, Barr LJ, et al. Epidermal DNA vaccine for influenza is immunogenic in humans. *Vaccine* 24:4475-4481, 2006.
 151. Mwau M, Cebere I, Sutton J, et al. A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. *J Gen Virol* 85:911-919, 2004.
 152. Cebere I, Dorrell L, McShane H, et al. Phase I clinical trial safety of DNA- and modified virus Ankara-vectored human immunodeficiency virus type 1 (HIV-1) vaccines administered alone and in a prime-boost regime to healthy HIV-1-uninfected volunteers. *Vaccine* 24:417-425, 2006.
 153. Stittelaar KJ, van Amerongen G, Kondova I, et al. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. *J Virol* 79:7845-7851, 2005.
 154. Peachman KK, Rao M, Alving CR. Immunization with DNA through the skin. *Methods* 31:232-242, 2003.
 155. Nathan CF, Kaplan G, Levis WR, et al. Local and systemic effects of intradermal recombinant interferon-gamma in patients with lepromatous leprosy. *N Eng J Med* 315:6-15, 1986.
 156. Fan H, Lin Q, Morrissey GR, Khavari PA. Immunization via hair follicles by topical application of naked DNA to normal skin. *Nature Biotechnol* 17:870-872, 1999.
 157. Bolgiano B, Mawas F, Yost SE, et al. Effect of physico-chemical modification on the immunogenicity of *Haemophilus influenzae* type b oligosaccharide-CRM197 conjugate vaccines. *Vaccine* 19:3189-3200, 2001.
 158. Partidos CD, Beignon A-S, Mawab F, et al. Immunity under the skin: potential application for topical delivery of vaccines. *Vaccine* 21:776-780, 2003.
 159. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur J Pharm Sci* 14:101-114, 2001.
 160. Kendall M. Engineering of needle-free physical methods to target epidermal cells for DNA vaccination. *Vaccine* 24:4651-4656, 2006.
 161. Lu B, Federoff HJ, Wang Y, Goldsmith LA, Scott G. Topical application of viral vectors for epidermal gene transfer. *J Invest Dermatol* 108:803-808, 1997.
 162. Seo N, Tokura Y, Nishijima T, et al. Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. *Proc Natl Acad Sci U S A* 97:371-376, 2000.
 163. Takigawa M, Tokura Y, Hashizume H, et al. Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. *Ann NY Acad Sci* 941:139-146, 2001.
 164. Kahlon R, Hu Y, Orteu CH, et al. Optimization of epicutaneous immunization for the induction of CTL. *Vaccine* 21:2890-2899, 2003.
 165. Godefroy S, Peyre M, Garcia N, et al. Effect of skin barrier disruption on immune responses to topically applied cross-reacting material, CRM(197), of diphtheria toxin. *Infect Immun* 73:4803-4809, 2005.
 166. Choi MJ, Kim JH, Maibach HI. Topical DNA vaccination with DNA/lipid based complex. *Curr Drug Deliv* 3:37-45, 2006.
 167. Skountzou I, Quan F-S, Jacob J, et al. Transcutaneous immunization with inactivated influenza virus induces protective immune responses. *Vaccine* 24:6110-6119, 2006.
 168. Glenn GM, Kenney RT, Ellingsworth LR, et al. Transcutaneous immunization and immunostimulant strategies: capitalizing on the immunocompetence of the skin. *Expert Rev Vaccines* 2:253-267, 2003.
 169. Van Kampen KR, Shi Z, Gao P, et al. Safety and immunogenicity of adenovirus-vectored nasal and epicutaneous influenza vaccines in humans. *Vaccine* 23:1029-1036, 2005.
 170. Vaxin, Inc. Birmingham AL 35203 Online. Available at: www.vaxin.com.
 171. Shi Z, Zeng M, Yang G, et al. Protection against tetanus by needle-free inoculation of adenovirus-vectored nasal and epicutaneous vaccines. *J Virol* 75:11474-11482, 2001.
 172. Zhang J, Shi Z, Kong FK, et al. Topical application of *Escherichia coli*-vectored vaccine as a simple method for eliciting protective immunity. *Infect Immun* 74:3607-3617, 2006.
 173. Draize JH, Woodward G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther* 82:377-390, 1944.
 174. Tao SL, Desai TA. Microfabricated drug delivery systems: from particles to pores. *Adv Drug Deliv Rev* 55:315-328, 2003.
 175. Mikszta JA, Alarcon JB, Brittingham JM, et al. Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery. *Nature Med* 8:415-419, 2002.
 176. Prausnitz MR, Mikszta JA, Raeder-Devens J. Microneedles. In: Smith EW, Maibach HI, (eds). *Percutaneous Penetration Enhancers*, 2nd ed. Boca Raton, FL 33487: CRC Press; 2006, 239-255.
 - 176a. Personal communication, Mark R. Prausnitz, Georgia Institute of Technology, 2007.
 177. Prausnitz MR. Microneedles for transdermal drug delivery. *Adv Drug Deliv Rev* 56:581-587, 2004.
 178. Macroflux Corporation, Mountain View, CA 94039-7210 (formerly part of ALZA Corporation). Online. Available at: www.macroflux.com.
 179. Matriano JA, Cormier M, Johnson J, et al. Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm Res* 19:63-70, 2002.
 180. Widera G, Johnson J, Kim L, et al. Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system. *Vaccine* 24:1653-1664, 2006.
 181. Lin W, Cormier M, Samiee A, et al. Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux) technology. *Pharm Res* 18:1789-1793, 2001.
 182. Cormier M, Johnson B, Ameri M, et al. Transdermal delivery of desmopressin using a coated microneedle array patch system. *J Control Release* 97:503-11, 2004.
 183. 3M Corporation, Minneapolis, MN; 3M Drug Delivery Systems. Online. Available at: www.3m.com.
 184. Peterson TA, Wick SM, Ko C. Design, development, manufacturing, and testing of transdermal drug delivery systems. In: Ghosh T, Pfister W, Yum SI, (eds). *Transdermal and Topical Drug Delivery Systems*. Buffalo Grove, Illinois: Interpharm; 1997, 249-297.
 185. Gordon RD, Peterson TA. Myths about transdermal drug delivery. *Drug Delivery Technology*. 2003;3(4). Online. Available at: www.drugdeliverytech.com/cgi-bin/articles.cgi?idArticle=143. Accessed November 21, 2006.
 186. Raeder-Devens J. Microstructured Transdermal System (MTS), 2004. 3M Drug Delivery Systems Transdermal Publications page. Online. Available at: http://solutions.3m.com/wps/portal/3M/en_WW/DDS/DrugDeliverySystems/resources/publicationsposters. Accessed September 12, 2006.
 187. Johnson PR, Li J, Emery MR. Method development for quantification of tetanus toxoid and aluminum on microneedle arrays. WCBP 2005-9th Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products, 10-13 January 2005, Washington, DC (Abstract P-05-T).
 188. Wolter J, Brandwein D, Choi H, et al. Antigen-adjuvant dose response in a rabbit model using 3M's Microstructured Transdermal System. 33th Annual Meeting of the Controlled Release Society, 18-22 June 2005, Miami, Florida.
 189. Coulman SA, Barrow D, Anstey A, et al. Minimally invasive cutaneous delivery of

- macromolecules and plasmid DNA via microneedles. *Curr Drug Deliv* 3:65-75, 2006.
190. Theraject, Inc., Fremont, CA 94538. Online. Available at: www.theraject.com.
 191. Kwon S-Y, Oh S-J, Burkoth TL. Rapid intradermal drug delivery by a dissolvable micro-needle patch. 32th Annual Meeting of the Controlled Release Society, 18-22 June 2005, Miami, Florida (abstract no. 306). Online. Available at: http://theraject.com/news_events/news_events.html. Accessed September 13, 2006.
 192. Kwon S-Y. Acne treatment by a dissolvable micro-needle patch. 33rd Annual Meeting of the Controlled Release Society, 22-26 July 2006, Vienna, Austria. Online. Available at: http://theraject.com/news_events/news_events.html. Accessed September 13, 2006.
 193. Cyto Pulse Sciences, Inc. Glen Burnie, MD 21061. Online. Available at: www.cytospulse.com.
 194. Corium International, Inc., Menlo Park, CA 94025. Online. Available at <http://www.coriumgroup.com> (microtine technology acquired from Proctor & Gamble Company).
 195. Yuzhakov VV, Sherman FF, Owens GD, Garstein V (assignee: The Proctor & Gamble Company). Intracutaneous microneedle array apparatus (U.S. Patent 6,931,277 B1). Washington, DC: United States Patent and Trademark Office; issued August 16, 2005.
 196. SpectRx Company, Norcross, GA 30071. Online. Available at: www.spectrx.com, www.mysimplechoice.com.
 197. Valeritas, Inc., Westborough, MA 01581, USA. Online. Available at: www.valeritas.com (a wholly-owned subsidiary of Biovalve Technologies, Inc., www.biovalve.com).
 198. McAllister DV, Wang PM, Davis SP, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: Fabrication methods and transport studies. *Proc Natl Acad Sci USA* 100:13755-13760, 2003.
 199. Kaushik S, Hord AH, Denson DD, et al. Lack of pain associated with microfabricated microneedles. *Anesth Analg* 92:502-504, 2001.
 200. School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0100, USA. Online. Available at: www.chbe.gatech.edu.
 201. Norwood Abbey Ltd, Victoria, 3196, Australia. Online. Available at: www.norwoodabbey.com.
 202. NanoPass Technologies Ltd., Haifa, Israel 31043. Online. Available at: www.nanopass.com.
 203. Nelson JS, McCullough JL, Glenn TC, et al. Mid-infrared laser ablation of stratum corneum enhances in vitro percutaneous transport of drugs. *J Invest Dermatol* 97:874-879, 1991.
 204. Lee W-R, Shen S-C, Lai H-H, et al. Transdermal drug delivery enhanced and controlled by erbium:YAG laser: a comparative study of lipophilic and hydrophilic drugs. *J Control Release* 75:155-166, 2001.
 205. Baron ED, Harris L, Redpath WS, et al. Laser-assisted penetration of topical anesthetic in adults. *Arch Dermatol* 139:1288-1290, 2003.
 206. Singer AJ, Weeks R, Regev R. Laser-assisted anesthesia reduces the pain of venous cannulation in children and adults: a randomized controlled trial. *Acad Emerg Med* 13:623-628, 2006.
 207. Lee S, McAuliffe DJ, Flotte TJ, et al. Photomechanical transcutaneous delivery of macromolecules. *J Invest Dermatol* 111:925-929, 1998.
 208. Lee S, Kollias N, McAuliffe DJ, et al. Topical drug delivery in humans with a single photomechanical wave. *Pharm Res* 16:1717-1721, 1999.
 209. Lee S, McAuliffe DJ, Kollias N, et al. Photomechanical delivery of 100-nm microspheres through the stratum corneum: implications for transdermal drug delivery. *Lasers Surg Med* 31:207-210, 2002.
 210. Ludec S. Electric Ions and Their Use in Medicine. London: Robman; 1908.
 211. Panus PC, Campbell J, Kulkarni SB, et al. Transdermal iontophoretic delivery of ketoprofen through human cadaver skin and in humans. *J Control Release* 44:113-121, 1997.
 212. Banga AK. Electrically Assisted Transdermal and Topical Drug Delivery. London, UK: Taylor & Francis; 1998.
 213. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: overcoming the skin's barrier function. *Pharm Sci Technol Today* 3:318-326, 2000.
 214. Sugibayashi K, Kagino M, Numajiri S, et al. Synergistic effects of iontophoresis and jet injector pretreatment on the in-vitro skin permeation of diclofenac and angiotensin II. *J Pharm Pharmacol* 52:1179-1186, 2000.
 215. Kalia YN, Naik A, Garrison G, Guy RH. Iontophoretic drug delivery. *Adv Drug Deliv Rev* 56:619-658, 2004.
 216. Vyteris, Inc., Fair Lawn, New Jersey 07410. Online. Available at: www.vyteris.com.
 217. ALZA Corporation, Mountain View, CA 94039-7210 (wholly-owned subsidiary of Johnson & Johnson). Online. Available at: www.alza.com.
 218. Guy RH, Kalia YN, Delgado-Charro MB, et al. Iontophoresis: electrorepulsion and electroosmosis. *J Control Release* 64:129-32, 2000.
 219. Bramson J, Dayball K, Eveleigh C, et al. Enabling topical immunization via microporation: a novel method for pain-free and needlefree delivery of adenovirus-based vaccines. *Gene Ther* 10:251-260, 2003.
 220. Altea Therapeutics Corporation, Tucker, GA 30084. Online. Available at: www.alteatherapeutics.com.
 - 220a. Garg S, Hoelscher M, Belsler JA, et al. Needle-free skin patch delivery of a vaccine for a potentially pandemic influenza virus provides protection against lethal challenge in mice. *Clin Vaccine Immunol* 14(7):926-928, 2007.
 221. Smith AM, Eppstein JA, Delcher HK, et al. Transdermal insulin infusion through thermally created micropores in humans. *Diabetes* 50:A132, 2001.
 222. Smith AM, Woods TJ, Williams DJ, et al. Transdermal basal insulin delivery through micropores. *Diabetes* 51:A47 (abstract 191-OR), 2002.
 223. Smith A, Yang D, Delcher H, et al. Fluorescein kinetics in interstitial fluid harvested from diabetic skin during fluorescein angiography: Implications for glucose monitoring. *Diabetes Technology and Therapeutics* 1:21-27, 1999.
 224. TransPharma Medical Ltd., Lod 71291, Israel. Online. Available at: www.transpharma-medical.com.
 225. Prausnitz MR, Bose VG, Langer R, Weaver JC. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. *Proc Natl Acad Sci USA* 90:10504-10508, 1993.
 226. Prausnitz MR. A practical assessment of transdermal drug delivery by skin electroporation. *Advanced Drug Delivery Reviews* 35:61-76, 1999.
 227. Vanbever R, Pr at V. In vivo efficacy and safety of skin electroporation. *Advanced Drug Delivery Reviews* 35:77-88, 1999.
 228. Lombry C, Dujardin N, Pr at V. Transdermal delivery of macromolecules using skin electroporation. *Pharm Res* 17:32-7, 2000.
 229. Sintov AC, Krymberka I, Daniel D, et al. Radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. *J Control Release* 89:311-320, 2003.
 230. Prud'homme GJ, Glinka Y, Khan AS, Draghia-Akli R. Electroporation-enhanced nonviral gene transfer for the prevention or treatment of immunological, endocrine and neoplastic diseases. *Current Gene Therapy* 6:243-273, 2006.
 231. Zhang L, Nolan E, Kreitschitz S, Rabussay DP. Enhanced delivery of naked DNA to the skin by non-invasive in vivo electroporation. *Biochim Biophys Acta* 1572:1-9, 2002.
 232. Babiuk S, Baca-Estrada ME, Foldvari M, et al. Needle-free topical electroporation improves gene expression from plasmids administered in porcine skin. *Mol Ther* 8:992-998, 2003.
 233. Andre F, Mir LM. DNA electrotransfer: its principles and an updated review of its therapeutic applications. *Gene Ther* 11(Suppl 1): S33-S42, 2004.
 234. Otten GR, Schaefer M, Doe B, et al. Potent immunogenicity of an HIV-1 gag-pol fusion DNA vaccine delivered by in vivo electroporation. *Vaccine* 24:4503-4509, 2006.
 235. Li Z, Zhang H, Fan X, et al. DNA electroporation prime and protein boost strategy enhances humoral immunity of tuberculosis DNA vaccines in mice and non-human primates. *Vaccine* 24:4565-4568, 2006.
 236. Aditus Medical AB, SE-227 36 Lund, Sweden. Online. Available at: www.aditusmedical.com.
 237. ADViSYS, Inc., The Woodlands, Texas 77381. Online. Available at: www.advisys.net.
 238. BTX[®] Instrument Division, Harvard Apparatus, Inc., Holliston, MA 01746-1388. Online. Available at: www.btxonline.com.
 239. Inovio Biomedical Corporation, San Diego, CA 92121-1318. Online. Available at: www.inovio.com.
 240. Ichor Medical Systems, Inc., San Diego, CA 92121. Online. Available at: www.ichorms.com.
 241. Luxembourg A, Hannaman D, Ellefsen B, et al. Enhancement of immune responses to an HBV DNA vaccine by electroporation. *Vaccine* 24:4490-4493, 2006.
 242. Mitragotri S, Blankschtein D, Langer R. Ultrasound-mediated transdermal protein delivery. *Science* 269:850-853, 1995.
 243. Mitragotri S, Kost J. Low-frequency sonophoresis: a review. *Adv Drug Deliv Rev* 56:589-601, 2004.
 244. Lavon I, Kost J. Ultrasound and transdermal drug delivery. *Drug Discov Today* 9:670-676, 2004.
 245. Tezel A, Paliwal S, Shen Z, Mitragotri S. Low-frequency ultrasound as a transcutaneous immunization adjuvant. *Vaccine* 23:3800-3807, 2005.
 246. Tachibana K, Tachibana S. Transdermal delivery of insulin by ultrasonic vibration. *J Pharm Pharmacol* 43:270-271, 1991.
 247. Merino G, Kalia YN, Guy RH. Ultrasound-enhanced transdermal transport. *J Pharm Sci* 92:1125-1137, 2003.
 248. Sontra Medical Corporation, Franklin, MA 02038. Online. Available at: www.sontra.com.
 249. ImaRx Therapeutics, Inc., Tucson, AZ 85719. Online. Available at: www.imarx.com.
 250. Klein TM, Wolf ED, Wu R, Sanford JC. High velocity microprojectiles for delivering nucleic acids into living cells. *Nature* 327:70-73, 1987.
 251. Bio-Rad Laboratories, Inc., Hercules, CA 94547. Online. Available at: www.bio-rad.com.
 252. Agracetus campus, Monsanto Company, St. Louis, MO 63167. Online. Available at: www.monsanto.com.
 253. Wang S, Joshi S, Lu S. Delivery of DNA to skin by particle bombardment. In: Heiser WD, (ed.). *Gene Delivery to Mammalian Cells: Vol. 1: Nonviral Gene Transfer Techniques* [Methods in Molecular Biology series, vol. 245]. Totowa, New Jersey: Humana; 2003, 185-196.
 254. Williman J, Lockhart E, Slobbe L, et al. The use of Th1 cytokines, IL-12 and IL-23, to modulate the immune response raised to a DNA vaccine delivered by gene gun. *Vaccine* 24:4471-4474, 2006.
 255. McCluskie MJ, Brazolot Millan CL, et al. Route and method of delivery of DNA vaccine influence immune responses in mice and non-human primates. *Mol Med* 5:287-300, 1999.
 256. Weiss R, Scheibelhofer S, Freund J, et al. Gene gun bombardment with gold particles displays a particular Th2-promoting signal that overrules the Th1-inducing effect of immunostimulatory CpG motifs in DNA vaccines. *Vaccine* 20:3148-3154, 2002.
 257. PowderMed Limited, Oxford OX4 4ZZ, England, UK (subsidiary of Pfizer). Online. Available at: www.powdermed.com.
 258. Chen D, Maa YF, Haynes JR. Needle-free epidermal powder immunization. *Expert Rev Vaccines* 1:265-276, 2002.
 259. Chiron Corporation, Emeryville, CA 94608-2916. Online. Available at: www.chiron.com; a component of Novartis Vaccines and Diagnostics, www.novartis-vaccines.com.
 260. Pfizer, Inc., New York, NY 10017. Online. Available at: www.pfizer.com.
 261. Chen D, Weis KF, Chu Q, et al. Epidermal powder immunization induces both cytotoxic T-lymphocyte and antibody responses to protein antigens of influenza and hepatitis B viruses. *J Virol* 75:11630-11640, 2001.
 262. Chen D, Endres R, Maa YF, et al. Epidermal powder immunization of mice and monkeys with an influenza vaccine. *Vaccine* 21:2830-2836, 2003.
 263. Dean HJ, Fuller D, Osorio HE. Powder and particle-mediated approaches for delivery of

- DNA and protein vaccines into the epidermis. *Comp Immunol Microbiol Infect Dis* 26:373–388, 2003.
264. Tacket CO, Roy MJ, Widera G, et al. Phase I safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine* 17:2826–2829, 1999.
265. Roy MJ, Wu MS, Barr LJ, et al. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine* 19:764–778, 2001.
266. Rottinghaus ST, Poland GA, Jacobson RM, et al. Hepatitis B DNA vaccine induces protective antibody responses in human non-responders to conventional vaccination. *Vaccine* 21:4604–4608, 2003.
267. Roberts LK, Barr LJ, Fuller DH, et al. Clinical safety and efficacy of a powdered hepatitis B nucleic acid vaccine delivered to the epidermis by a commercial prototype device. *Vaccine* 23:4867–4878, 2005.
268. McConkey SJ, Reece WH, Moorthy VS, et al. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nature Med* 9:729–735, 2003.
269. Moorthy VS, McConkey S, Roberts M, et al. Safety of DNA and modified vaccinia virus Ankara vaccines against liver-stage *P. falciparum* malaria in non-immune volunteers. *Vaccine* 21:1995–2002, 2003.
270. Dean HJ, Chen D. Epidermal powder immunization against influenza. *Vaccine* 23:681–686, 2004.
271. Mant T. Safety Study of an Influenza Vaccine Against a Potential Pandemic Strain of Flu. Online. Available at: www.clinicaltrials.gov/ct/show/NCT00347529. Accessed November 22, 2006.
272. Komjathy S. A safety study to assess a DNA vaccine administered by particle mediated delivery to the skin in healthy subjects; 2006. Online. Available at: www.clinicaltrials.gov/ct/show/NCT00310271. Accessed November 22, 2006.
273. America's Health Insurance Plans. Emerging Vaccine Chart—July, 2006 Report. Online. Available at: www.ahip.org/content/default.aspx?docid=11691. Accessed November 26, 2006.
274. Oxford PharmaGenesis, Ltd. Pandemic influenza and biothreat preparedness: role of PMED(tm) DNA vaccines. Oxford, UK, 2005. Online. Available at: www.powdermed.com/pdf/Flu%20Brochure%20Dec%202005.pdf. Accessed November 26, 2006.
275. Pilling AM, Harman RM, Jones SA, et al. The assessment of local tolerance, acute toxicity, and DNA biodistribution following particle-mediated delivery of a DNA vaccine to minipigs. *Toxicol Pathol* 30:298–305, 2002.
276. Schmaljohn C, Vanderzanden L, Bray M, et al. Naked DNA vaccines expressing the prM and E genes of Russian spring summer encephalitis virus and Central European encephalitis virus protect mice from homologous and heterologous challenge. *J Virol* 71:9563–9569, 1997.
277. Hooper JW, Custer DM, Thompson E, Schmaljohn CS. DNA vaccination with the Hantaan virus M gene protects hamsters against three of four HFRS hantaviruses and elicits a high-titer neutralization antibody response in Rhesus monkeys. *J Virol* 75:8469–8477, 2001.
278. Chen D, Zuleger C, Chu Q, et al. Epidermal powder immunization with a recombinant HIV gp120 targets Langerhans cells and induces enhanced immune responses. *AIDS Res Hum Retroviruses* 18:715–722, 2002.
279. Sakai T, Hisaeda H, Nakano Y, et al. Gene gun-based co-immunization of merozoite surface protein-1 cDNA with IL-12 expression plasmid confers protection against lethal *Plasmodium yoelii* in A/J mice. *Vaccine* 21:1432–1444, 2003.
280. Kim TW, Lee JH, Hung Cf, et al. Generation and characterization of DNA vaccines targeting the nucleocapsid protein of severe acute respiratory syndrome coronavirus. *J Virol* 78:4638–4645, 2004.
281. Hooper JW, Thompson E, Wilhelmson C, et al. Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. *J Virol* 78:4433–4443, 2004.
282. Herndon TO, Gonzalez S, Gowrishankar TR, et al. Transdermal microconduits by microscission for drug delivery and sample acquisition. *BMC Med*. 2004;19(2):12. Online. Available at: <http://biomedcentral.com/1741-7015/2/12>. Accessed October 23, 2006.
- 282a. Arora A, Hakim I, Baxter J, et al. Needle-free delivery of macromolecules across the skin by nanoliter-volume pulsed microjects. *PNAS* 104(11):4255–4260, 2007.
283. Dickinson BL, Clements JD. Dissociation of *Escherichia coli* heat-labile enterotoxin adjuvant activity from ADP-ribosyltransferase activity. *Infect Immun* 63(5):1617–1623, 1995.
284. Williams NA, Hirst TR, Nashar TO. Immune modulation by the cholera-like enterotoxins: from adjuvant to therapeutic. *Immunol Today* 20:95–101, 1999.
285. Freytag LC, Clements JD. Bacterial toxins as mucosal adjuvants. *Curr Top Microbiol Immunol* 236:215–236, 1999.
286. Salmond RJ, Luross JA, Williams NA. Immune modulation by the cholera-like enterotoxins. *Expert Rev Mol Med* 2002:1–16, 2002.
287. Plant A, Williams NA. Modulation of the immune response by the cholera-like enterotoxins. *Curr Top Med Chem* 4:509–519, 2004.
288. Holmgren J, Adamsson J, Anjuere F, et al. Mucosal adjuvants and anti-infection and anti-immunopathology vaccines based on cholera toxin, cholera toxin B subunit and CpG DNA. *Immunol Lett* 97:181–188, 2005.
289. Rappuoli R, Pizza M, Douce G, Dougan G. Structure and mucosal adjuvant activity of cholera and *Escherichia coli* heat-labile enterotoxins. *Immunol Today* 20:493–500, 1999.
290. Pizza M, Giuliani MM, Fontana MR, et al. Mucosal vaccines: non toxic derivatives of LT and CT as mucosal adjuvants. *Vaccine* 19:2534–2541, 2001.
291. Peppoloni S, Ruggiero P, Contorni M, et al. Mutants of the *Escherichia coli* heat-labile enterotoxin as safe and strong adjuvants for intranasal delivery of vaccines. *Expert Rev Vaccines* 2:285–293, 2003.
292. Mutsch M, Zhou W, Rhodes P, et al. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *N Engl J Med* 350:896–903, 2004.
293. Iomai Corporation, Gaithersburg, MD 20878. Online. Available at: www.iomai.com.
294. Glenn G, Rao M, Matyas GR, Alving CR. Skin immunization made possible by cholera toxin. *Nature* 391:851, 1998.
295. Glenn GM, Kenney RT, Hammond SA, Ellingsworth LR. Transcutaneous immunization and immunostimulant strategies. *Immunol Allergy Clin N Am* 23:787–813, 2003.
296. Kenney RT, Glenn GM. Transcutaneous immunization using the heat-labile enterotoxin *E. coli* as an adjuvant. In: Schijns VE, O'Hagan D, (eds). *Immunopotentiators in Modern Vaccines*. United Kingdom: Academic Press, Elsevier; 2006, 253–273.
297. Arrington J, Braun RP, Dong L, et al. Plasmid vectors encoding cholera toxin or the heat-labile enterotoxin from *Escherichia coli* are strong adjuvants for DNA vaccines. *J Virol* 76:4536–4546, 2002.
298. Guereña-Burgueño F, Hall ER, Taylor DN, et al. Safety and immunogenicity of a prototype enterotoxigenic *Escherichia coli* vaccine administered transcutaneously. *Infect Immun* 70:1874–1880, 2002.
299. Glenn GM, Taylor DN, Li X, et al. Transcutaneous immunization: a human vaccine delivery strategy using a patch. *Nat Med* 6:1403–1406, 2000.
300. McKenzie R, Bourgeois AL, Frech SA, et al. Transcutaneous immunization with the heat-labile toxin (LT) of enterotoxigenic *Escherichia coli* (ETEC): protective efficacy in a double-blind, placebo-controlled challenge study. *Vaccine* 25:3684–3691, 2007.
- 300a. Glenn G, Frech S, Villar C, et al. Transcutaneous immunization with the heat labile toxin (LT) of enterotoxigenic *Escherichia coli* protects in a phase 2 field trial in travelers to Guatemala (GU) and Mexico (MX) [abstract G-1247A]. 47th Interscience Conference on Antimicrobial Agents and Chemotherapy; 2007 Sep 17–20; Chicago, USA. Washington: American Society for Microbiology; 2007, p. 216.
301. Guebre-Xabier M, Hammond SA, Epperson DE, et al. Immunostimulant patch containing heat-labile enterotoxin from *Escherichia coli* enhances immune responses to injected influenza virus vaccine through activation of skin dendritic cells. *J Virol* 77:5218–5225, 2003.
302. Guebre-Xabier M, Hammond SA, Ellingsworth LR, Glenn GM. Immunostimulant patch enhances immune responses to influenza virus vaccine in aged mice. *J Virol* 78:7610–7618, 2004.
303. Frech SA, Kenney RT, Spyr CA, et al. Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch. *Vaccine* 23:946–950, 2005.
304. Hammond SA, Walwender D, Alving CR, Glenn GM. Transcutaneous immunization: T cell responses and boosting of existing immunity. *Vaccine* 19:2701–2707, 2001.
305. Kenney RT, Yu J, Guebre-Xabier M, et al. Induction of protective immunity against lethal anthrax challenge with a patch. *J Infect Dis* 190:774–782, 2004.
306. Matyas GR, Friedlander AM, Glenn GM, et al. Needle-free skin patch vaccination method for anthrax. *Infect Immun* 72:1181–1183, 2004.
307. Yu J, Cassels F, Scharton-Kersten T, et al. Transcutaneous immunization using colonization factor and heat-labile enterotoxin induces correlates of protective immunity for enterotoxigenic *Escherichia coli*. *Infect Immun* 70:1056–10568, 2002.
308. Weltzin R, Guy B, Thomas WD, Jr, et al. Parenteral adjuvant activities of *Escherichia coli* heat-labile toxin and its B subunit for immunization of mice against gastric *Helicobacter pylori* infection. *Infect Immun* 68:2775–2782, 2000.
309. Gupta PN, Mishra V, Singh P, et al. Tetanus toxoid-loaded transdermal for topical immunization. *J Pharm Pharmacol* 57:295–301, 2005.
310. D'Antonio NF, D'Antonio LF, Wagner JT. Hypodermic fluid dispenser (U.S. Patent no. 6,056,716). Washington, DC: U.S. Patent and Trademark Office; issued May 2, 2000.
311. Sadowski PL, DeBoer DM, Berman CL, et al. Needle assisted jet injector (U.S. Patent no. 6,746,429). Washington, DC: U.S. Patent and Trademark Office; issued June 8, 2004.
312. Glide Pharma™, Abingdon, Oxfordshire OX14 4RU, UK; www.glidepharma.com.
313. Hingson RA, Figge FHJ. A survey of the development of jet injection in parenteral therapy. *Curr Res Anesthesia Analgesia* 31:361–366, 1952.
314. Vorob'ev AA, Nekrasov IL, Bandakov LF. Bezygol'nyi sposob vvedeniya biologicheskikh preparatov v organizm [Russian: Needle-free method for the introduction of biological preparations into organisms]. Moscow: Meditsina; 1972, 1–102.
315. Reis EC, Jacobson RM, Tarbell S, Weniger BG. Taking the sting out of shots: control of vaccination-associated pain and adverse reactions. *Pediatr Ann* 27:375–386, 1998.
316. Pass F, Hayes J. Needle-free drug delivery. In: Rathbone MJ, Hadgraft J, Roberts MS (eds). *Modified-release Drug Delivery Technology*. New York, NY: Marcel Dekker, 2003, 599–606.
317. Mitragotri S. Current status and future prospects of needle-free liquid jet injectors. *Nature Reviews Drug Discovery* 5:543–548, 2006.
318. Centers for Disease Control and Prevention. Needle-free Injection Technology. Online. Available at: www.cdc.gov/nip/dev/jetinject.htm. Accessed December 7, 2006.
319. Guérard A. Présentation, au nom de M. Mathieu, d'un appareil dit à douches filiformes, Séance du 2 mai 1865, Présidence de M. Bouchardat, Vice-Président. *Bulletin de l'Académie Impériale de Médecine (France)* 30:676–677, 1865.
320. Béclard F. Présentation de l'injecteur de Galante, Séance du 18 décembre 1866, Présidence de M. Bouchardat. *Bulletin de l'Académie Impériale de Médecine (France)* 32:321–327, 1866.

321. Lockhart ML. Hypodermic Injector (U.S. Patent no. 2,322,244). Washington, DC: U.S. Patent and Trademark Office; issued June 22, 1943.
322. Hingson RA, Hughes JG. Clinical studies with jet injection. A new method of drug administration. *Curr Res Anesthesia Analgesia* 26:221-230, 1947.
323. Perkin FS, Todd GM, Brown TM, Abbott HL. Jet injection of insulin in treatment of diabetes mellitus. *Proc Amer Diabetes Assoc* 10:185-199, 1950.
324. Activa Brand Products, Mississauga, Ontario L5T 1L4, Canada. Online. Available at: www.advantajet.com/ (successor to Equipement Moniteur, Inc. and Advanced Medical Technologies Inc., Canada).
325. American Jet Injector, Lansdale, PA; 19446-4520, USA; amojet@aol.com (the Am-O-Jet™ is an exact design of the out-of-patent Ped-O-Jet® device).
326. Antares Pharma, Inc., Ewing, NJ; 08618-1433, USA (successor of Medi-Ject, Daystrol-Scientific, and Derata corporations; Vaccijet™ technology acquired in 2001 from Endos Pharma, Laons, France). Online. Available at: www.mediject.com/, www.antarespharma.com/content/products/intro/intro.html. (Avijet™ is Vaccijet électrique design used by Merial³²⁹ for poultry vaccination).
327. Avant Medical Corporation, San Diego, CA 92121, USA. Online. Available at: www.avantmedical.com.
328. Coon W, Hodgson P, Hinerman DL. Fundamental problems in jet injection. *Am J Med Sci* 227:39-45, 1954.
329. Merial Groupe, sanofi-aventis, Lyon, France (Vetjet™ use under license from Bioject, Inc.). Online. Available at: <http://purevax.us.merial.com/vetjet/>, http://purevax.us.merial.com/media/Instructional_256k.wmv.
330. Wang R, Epstein J, Baraceres FM, et al. Induction of CD4(+) T cell-dependent CD8(+) type 1 responses in humans by a malaria DNA vaccine. *Proc Natl Acad Sci USA* 98:10817-10822, 2001.
331. Rao SS, Gomez P, Mascola JR, et al. Comparative evaluation of three different intramuscular delivery methods for DNA immunization in a nonhuman primate animal model. *Vaccine* 24:367-373, 2006.
332. Chemical Automatics [Khimavtomatika] Design Bureau (CADB), Voronezh, Russia; www.khimavtomatika.ru/ (technology developed initially at All-Union Scientific Research Institute of Surgical Equipment and Tools -VNIISKHAI; some technology licensed since 2000 to Felton International).
333. Provotorov VM, Perel'man MI, Strel'tsov VP, et al. Lechenie zabolevanii legkikh vnutrilegocnym ugol'no-struinyim vvedeniem lekarstvennykh veshchestv [Treatment of lung diseases by intrapulmonary jet injection of drugs]. *Klin Med (Moscow)* 69:48-51, 1991.
334. Crossject S.A., 75004 Paris, France. Online. Available at: www.crossject.com.
335. D'Antonio Consultants International, Inc. (DCI), East Syracuse, NY 13057-9325, USA. Online. Available at: www.dantoniiconsultants.com.
336. Carter EW, Kerr DE. Optimization of DNA-based vaccination in cows using green fluorescent protein and protein A as a prelude to immunization against staphylococcal mastitis. *J Dairy Sci* 86:1177-1186, 2003.
337. EMS Electro Medical Systems, CH-1260 Nyon, Switzerland. Online. Available at: www.ems-medical.com (EMS/MPM device from EMS Medical GmbH, 8462 Konstanz, Germany).
338. Cartier R, Ren SV, Walther W, et al. In vivo gene transfer by low-volume jet injection. *Anal Biochem* 282:262-265, 2000.
- 338a. EuroJet Medical Kft., H-1151 Budapest, Hungary. Online. Available at <http://www.ejm.hu>.
339. INJEX – Equidyne Systems, Inc. (wholly owned subsidiary of HNS International, Inc.), Anaheim, CA 92807, USA, Online. Available at: www.injex.com (successor to American Electromedics Corporation; INJEX technology marketed in arrangement with Rösch AG Medizintechnik).
340. Genesis Medical Technologies, Inc., Golden, CO 80401, USA. Online. Available at: www.geocities.com/~genmedtech (predecessor company to PharmaJet).
341. Med-E-Jet D (dba Donald J. Kuch), Olmsted Falls, OH 44138-1958, USA.
342. Felton International, Inc., Felton Medical, Inc., Shawnee Mission, KS 66214, USA. Online. Available at: www.feltonmedical.com, www.hhs.gov/nvpo/meetings/dec2003/Contents/ThursdayPM/Mathews.pdf (purchased Chemical Automatics Design Bureau technology in 2000).
343. H. Galante et Compagnie, Paris, France. See ref. 320.
344. Heng Yang Weida Science Technology, Heng Yang, Hunan, China.
345. Keystone Industries (Ped-O-Jet International), Cherry Hill, NJ 08002, USA. Online. Available at: www.keystoneind.com (Mizzy Division: www.syrjetinc.com). Ped-O-Jet previously manufactured by Scientific Equipment Manufacturing Corporation (SEMCO), Lodi, NJ and Larchmont, NY, and developed by Medicinal Equipment Development Laboratory, United States Army, Fort Totten, NY. See ref. 372.
346. MADA, Inc., Carlstadt, NJ 07072, USA. Online. Available at: www.madamedical.com, www.madainternational.com/us/prod11_us.html.
347. The Medical House PLC, Sheffield S9 2QJ, United Kingdom. Online. Available at: www.themedicalhouse.com, www.insulinjet.com, www.sq-pen.com.
348. Bremseth DL, Pass F. Delivery of insulin by jet injection: recent observations. *Diabetes Technol Ther* 3:225-232, 2001.
349. Medical International Technologies, Inc., Montreal, Quebec H4R 2E7, Canada. Online. Available at: www.mitcanada.ca.
350. Microbiological Research Establishment (now the Defence Science and Technology Laboratory), Ministry of Defense, Porton Down, Salisbury, Wiltshire SP4 0JG, UK. Online. Available at: www.dstl.gov.uk.
351. Nidec Tosok Corporation, Zama-City, Kanagawa 228-8570, Japan. Online. Available at: www.nidec-tosok.co.jp/english/index.html (formerly manufactured by Tokyo Sokuhan Co. Ltd.).
352. National Medical Products, Inc., Irvine, CA 92618-1605, USA. Online. Available at: <http://jtjp.com>.
353. PATH – Program for Appropriate Technology in Health, Seattle, WA 98107, USA. Online. Available at: www.path.org (MEDIVAX™ project in partnership with Vitajet, Inc., subsequently absorbed into Bioject, Inc.).
354. Sanofi Pasteur SA, F-69367 Lyon 07 France. Online. Available at: www.sanofipasteur.com (jet injection technology developed under corporate predecessors: Institut Mérieux, Pasteur Mérieux Serums & Vaccins, and Pasteur Mérieux Connaught).
355. Schlumberger M, Parent du Châtelet J, Lafarge H, et al. Coût de l'injection d'anatoxine tétanique par injecteur sans aiguille (Imule) lors d'une vaccination collective au Sénégal: comparaison avec l'injection par seringues et aiguilles stérilisables. *Santé* 9:319-326, 1999.
356. Galy M, Genet A, Saliou P. Un progrès dans le domaine de l'injection sans aiguille: le système Imule®. *S.T.P. Pharma Pratiques (France)* 4:261-266, 1992.
357. PenJet Inc., a Visionary Medical Products Company, Beverly Hills, CA 90212, USA. Online. Available at: www.penjet.com.
358. PharmaJet Inc, Golden, CO 80401, USA. Online. Available at: www.pharmajet.com (successor entity to Genesis Medical Technologies).
359. Prolitec, SA (Projection Liquide Technologies), 26400 Aouste sur Sye, France (formerly Béarn Mécanique Aviation SA, F-64143 Billère, France).
360. R.P. Scherer Corporation, Detroit, MI, USA; www.rpscherer.com (absorbed in 1998 into drug delivery unit of Cardinal Health. Online. Available at: www.cardinal.com/pts/content/delivery). K3 model was manufactured by Messer Griesheim GmbH (subsequently BIT Analytical Instruments GmbH, 65824 Schwalbach, Germany) and marketed by Behringwerke AG.
361. Schuco International Limited, London N12 0NE, UK. Online. Available at: www.schuco.co.uk.
362. Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan. Online. Available at: www.shimadzu.com.
363. Imoto J-I, Konishi E. Needle-free jet injection of a mixture of Japanese encephalitis DNA and protein vaccines: A strategy to effectively enhance immunogenicity of the DNA vaccine in a murine model. *Viral Immunol* 18:205-212, 2005.
364. SICIM, Medical Jet s.r.l., Romans d'Isongo, GO, Italy. Online. Available at: www.medicaljet.it/sicim.
365. Société AKRA, 64000 Pau, France. Online. Available at: www.dermojet.com.
366. Z. & W. Manufacturing Co., Wickliffe, OH, USA (acquired in 1965 by Parker Hanifin Corporation; www.parker.com); marketed by Scientific Equipment Manufacturing Corporation (SEMCO), Larchmont, NY.
367. Zogenix, Inc., Hayward, CA 94545, USA (technology originated by Weston Medical, plc and then further developed by Aradigm Corporation). Online. Available at: www.zogenix.com.
368. Shergold OA, Fleck NA, King TS. The penetration of a soft solid by a liquid jet, with application to the administration of a needle-free injection. *J Biomech* 39:2593-2602, 2006.
369. Sero International S.A., CH-1211 Geneva 20, Switzerland. Online. Available at: www.serono.com.
370. Merial Limited, Duluth, GA 30096, USA. Online. Available at: purevax.us.merial.com.
371. Warren J, Zihlerl FA, Kish AW, Zihlerl LA. Large-scale administration of vaccines by means of an automatic jet injection syringe. *JAMA* 157:633-637, 1955.
372. Benenson AS. Mass immunization by jet injection. In: *Proceedings of the International Symposium of Immunology, Opatija, Yugoslavia, 28 September – 1 October 1959* (International Committee for Microbiological Standardization, Section of the International Association of Microbiological Societies). Zagreb: Tiskara Izdavačkog zavoda Jugoslavenske akademije; 1959:393-399 [Library of Congress QW 504 I60p 1959].
373. Hingson RA, Davis HS, Rosen M. The historical development of jet injection and envisioned uses in mass immunization and mass therapy based upon two decades' experience. *Military Medicine* 128:516-524, 1963.
374. Hingson RA, Davis HS, Rosen M. Clinical experience with one and a half million jet injections in parenteral therapy and in preventive medicine. *Military Medicine* 128:525-528, 1963.
375. Neufeld PD, Katz L. Comparative evaluation of three jet injectors for mass immunization. *Can J Public Health* 68:513-516, 1977.
376. Barclay EM, Hingson RA, Abram LE, et al. Mass vaccination against smallpox in Liberia. *The Bulletin (Academy of Medicine of Cleveland)* 47(Suppl, August):16-23, 1962.
377. Meyer HM, Hostetler DD, Bernheim BC, et al. Response of Volta children to jet inoculation of combined live measles, smallpox and yellow fever vaccines. *Bull WHO* 30, 783-794, 1964.
378. Kalabus F, Sansarricq H, Lambin P, et al. Standardization and mass application of combined live measles-smallpox vaccine in Upper Volta. *Am J Epidemiol* 86:93-111, 1967.
379. Millar JD, Foege WH. Status of smallpox eradication (and measles control) in West and Central Africa. *J Infect Dis* 120:725-732, 1969.
380. Millar JD, Morris L, Macedo-Filho A, et al. The introduction of jet injection mass vaccination into the national smallpox eradication program of Brazil. *Tropical and Geographical Medicine* 23:89-101, 1971.
381. Ruben FL, Smith EA, Foster SO, et al. Simultaneous administration of smallpox, measles, yellow fever, and diphtheria-pertussis-tetanus antigens to Nigerian children. *Bull WHO* 48:175-181, 1973.
382. Meyer HM Jr. Mass vaccination against measles in Upper Volta. *Arch Gesamte Virusforsch* 16:243-245, 1965.
383. Hendrickse RG, Montefiore D, Peradze T, et al. Measles vaccination. Report of large scale trial of further attenuated measles vaccine in Nigeria. *J Trop Med Hyg* 69:112-116, 1966.
384. de Quadros CA, Hersh BS, Nogueira AC, et al. Measles eradication: experience in the Americas. *Bull WHO* 76(Suppl 2):47-52, 1998.
385. Hingson RA, Davis HS, Bloomfield RA, Brailey RF. Mass inoculation of the Salk polio vaccine

- with the multiple dose jet injector. GP [General Practitioner] 15:94-96, 1957.
386. Mohammed J, Obineche EN, Onyemelukwe GC, Zaruba K. Control of epidemic meningococcal meningitis by mass vaccination. I. Further epidemiological evaluation of groups A and C vaccines in northern Nigeria. *J Infect* 9:190-196, 1984.
 387. Spiegel A, Greindl Y, Lippeveld T, et al. Effet de deux stratégies de vaccination sur l'évolution de l'épidémie de méningite à méningocoque A survenue à N'Djamena (Tchad) en 1988. *Bull WHO* 71:311-315, 1993.
 388. Spiegel A, Moren A, Varaine F, et al. Aspects épidémiologiques et contrôle des épidémies de méningite à méningocoque en Afrique. *Cahiers Santé* May-Jun 4:231-236, 1994.
 389. Anderson EA, Lindberg RB, Hunter DH. Report of large-scale field trial of jet injection in immunization for influenza. *JAMA* 167:549-552, 1958.
 390. Ivannikov IuG, Efimenko IB, Marinich IG, et al. Otsenka effektivnosti massovoi profilaktiki grippa s ispol'zovaniem inaktivirovannoi khromatograficheskoi vaksiny v Leningrade [Evaluation of mass influenza prevention effectiveness using an inactivated chromatographic vaccine in Leningrad]. *Zh Mikrobiol Epidemiol Immunobiol* (11):18-27, 1980.
 391. Meyer HM Jr. Field experience with combined live measles, smallpox and yellow fever vaccines. *Arch Gesamte Virusforsch* 16:365-366, 1965.
 392. Artus JC. Vaccination de masse par le vaccin souche Rockefeller 17 D au Sénégal. Utilisation des 'Ped-o-Jet'. *Médecine Tropicale* 26:527-536, 1966.
 393. Towle RL. New horizon in mass inoculation. *Public Health Rep* 75:471-476, 1960.
 394. Barrett CD. Automated multiple immunization against diphtheria, tetanus and poliomyelitis. *J Sch Health* 32:48-50, 1962.
 395. Veronesi R, Salles Gomes LF, et al. Importancia do 'jet-injector' (injeção sem agulha) em planos de imunização em massa no Brasil: resultados com as vacinas antitetânica e antivaricólica. *Rev Hosp Clin Fac Med São Paulo* 21:92-95, 1966.
 396. Rey M, Triau R, Diop Mar I, et al. Single shot tetanus immunization and its application to mass campaign. In: 3rd International Conference on Tetanus, São Paulo, Brazil, 17-22 August 1970; Scientific Publication No. 253. Washington, DC: Pan American Health Organization; 1972:94-101.
 397. Rey M, Diop Mar I, Gbezo P, Sow A. Vaccination de masse antitetanique en Afrique. *La Nouvelle Presse Médicale* (France) 2:514, 1973.
 398. Ehrengut W, Allerdist H, Erdmann G. Clinical reactions to an adsorbed killed trivalent influenza vaccine (including A/New Jersey 8/76 antigen) with different immunization methods. *Dev Biol Stand* 39:283-287, 1977.
 399. Munshi AK, Hegde A, Bashir N. Clinical evaluation of the efficacy of anesthesia and patient preference using the needle-less jet syringe in a pediatric dental practice. *J Clin Pediatr Dent* 25:131-136, 2001.
 400. Jimenez N, Bradford H, Seidel KD, et al. A comparison of a needle-free injection system for local anesthesia versus EMLA for intravenous catheter insertion in the pediatric patient. *Anesthesia Analgesia* 102:411-414, 2006.
 401. Greenberg RS, Maxwell LG, Zahurak MS, Yaster M. Preanesthetic medication of children with midazolam using the Biojector jet injector. *Anesthesiology* 83:264-269, 1995.
 402. Zsigmond EK, Kovacs V, Fekete G. A new route, jet injection for anesthetic induction in children: I. midazolam dose-range finding studies. *Int J Clin Pharmacol Ther* 33:580-584, 1995.
 403. Hingson RA, Easley EJ, Gray AL, et al. Hypospray administration of penicillin in the treatment of gonorrhea. *J Ven Dis Inform* 29:61-63, 1948.
 404. Hirsh HL, Welch H, Milloff B, Katz S. Administration of penicillin and streptomycin by means of the Hypospray apparatus (jet injection); absorption, toxicity, and stability. *J Lab Clin Med* 33:805-810, 1948.
 405. Black J, Nagle CJ, Strachan CHL. Prophylactic low-dose heparin by jet injection. *Br Med J* 2(6130):95, 1978.
 406. Baer CH, Bennett WM, Folwick DA, Erickson RS. Effectiveness of a jet injection system in administering morphine and heparin to healthy adults. *Am J Crit Care* 5:42-48, 1996.
 407. Harris M, Joy R, Larsen G, et al. Enfvirtide plasma levels and injection site reactions using a needle-free gas-powered injection system (Biojector). *AIDS* 20:719-723, 2006.
 408. Clarke AK, Woodland J. Comparison of two steroid preparations used to treat tennis elbow, using the Hypospray. *Rheumatol Rehabil* 14:47-49, 1975.
 409. Martins JK, Roedel EA. Medijector - A new method of corticosteroid-anesthetic delivery. *J Occup Med* 21:821-824, 1979.
 410. Lawton RL. Jet injection of drugs into malignant neoplasms. *Cancer Chemotherapy Rep* 37:57-58, 1964.
 411. Brodell RT, Bredle DL. The treatment of palmar and plantar warts using natural alpha interferon and a needleless injector. *Dermatol Surg* 21:213-218, 1995.
 412. Resman Z, Metelko Z, Skrabalo Z. The application of insulin using the jet injector DG-77. *Acta Diabetol Lat* 22:119-125, 1985.
 413. Welty TK, Josimovich JB, Gerende JH, Hingson RA. Reduction of variability in the anovulatory period following medroxyprogesterone acetate injection by using jet injectors. *Fertility Sterility* 21:673-682, 1970.
 414. Bareille P, MacSwiney M, Albanese A, et al. Growth hormone treatment without a needle using the Preci-Jet 50 transjector. *Arch Dis Childhood* (London) 76:65-67, 1997.
 415. Dörr HG, Zabransky S, Keller E, et al. Are needle-free injections a useful alternative for growth hormone therapy in children? Safety and pharmacokinetics of growth hormone delivered by a new needle-free injection device compared to a fine gauge needle. *J Pediatr Endocrinol Metab* 16:383-392, 2003.
 416. Kutscher AH, Hyman GA, Zegarelli EV, et al. A comparative evaluation of the jet injection technique (Hypospray) and the hypodermic needle for the parenteral administration of drugs: a controlled study. *Am J Med Sci* 54:418-420, 1962.
 417. Schramm J, Mitragotri S. Transdermal drug delivery by jet injectors: energetics of jet formation and penetration. *Pharm Res* 19:1673-1679, 2002.
 418. Figge FHJ, Barnett DJ. Anatomic evaluation of a jet injection instrument designed to minimize pain and inconvenience of parenteral therapy. *Am Pract* 3:197-206, 1948.
 419. Weller C, Linder M. Jet injection of insulin vs. the syringe-and-needle method. *JAMA* 195:156-159, 1966.
 420. Garbsch H, Pietschmann H. Röntgenologische Darstellung der Gelenks- und Weichteilinfiltation mit dem 'Hypospray Jet Injector'. *Z Rheumaforsch* 25:237-242, 1966.
 421. White WG. Porton Jet injector. *Br Med J* 3:472-473, 1969.
 422. Bennett CR, Mundell RD, Monheim LM. Studies on tissue penetration characteristics produced by jet injection. *J Am Dent Assoc* 83:625-629, 1971.
 423. Patsch C-J, von Büren E, Kühn B, et al. Visualization of injection depot after subcutaneous administration by syringe and needle-free device (Medi-Jector): first results with magnetic resonance imaging. *Eur J Pediatr* 156:893-898, 1997.
 424. Hughes JG, Jordan RG, Hill FS. Jet injection in pediatric practice. *Pediatrics* 3:801-811, 1949.
 425. Schramm-Baxter J, Mitragotri S. Needle-free jet injections: dependence of jet penetration and dispersion in the skin on jet power. *J Control Release* 97:527-535, 2004.
 426. Bioject, Inc. Guide to selection and use of Biojector syringes. Portland, OR: Bioject, Inc.; 1997. Document 171-0134-00 Rev C 5/97.
 427. Cockshott WP, Thompson GT, Howlett LJ, Seeley ET. Intramuscular or intralipomatous injections? *New Engl J Med* 307:356-358, 1982.
 428. Poland GA, Borrud A, Jacobson RM, et al. Determination of deltoid fat pad thickness. Implications for needle length in adult immunization. *JAMA* 277:1709-11, 1997.
 429. Chambon L, Barne M, Tommasi U-B, et al. Étude de l'utilisation d'un injecteur sans aiguille pour la vaccination B.C.G. intradermique. *Médecine Tropicale* (Marseille) 30:809-828, 1970.
 430. British Thoracic and Tuberculosis Association. A comparison of intradermal BCG vaccination by jet injection and by syringe and needle. A report from the Research Committee of the British Thoracic and Tuberculosis Association. *Tubercle* 52:155-165, 1971.
 431. Carnus H. Ped-o-jet et viabilité du BCG. *Médecine Tropicale* (Marseille) 33:20-23, 1973.
 432. Carnus H. Influence du Ped-o-jet sur la viabilité du vaccin BCG intradermique lyophilisé: étude au laboratoire. *Bull WHO* 51:101-102, 1974.
 433. Parker V. Jet gun or syringe? A trial of alternative methods of BCG vaccination. *Public Health London* 98:315-320, 1984.
 434. Paul SS, Nath KR, Chhabra AK, Verma M. Comparison of BCG inoculation by conventional intradermal and jet methods. *Indian Pediatrics* 15:341-347, 1978.
 435. Cockburn TA, Witt MT, Ludlow CE, Macleod KIE. A comparison of jet injection with the mantoux test in mass skin testing with tuberculin. *Am Rev Respir Dis* 92:982-985, 1965.
 436. Hendrix C, Nichols C, Hirsh L. A new method of administering the tuberculin skin test. *Am J Public Health* 56:818-820, 1966.
 437. De Partearroyo R, Ruiz Benítez G. Consideraciones sobre el tuberculino-diagnóstico. Estudio comparativo del Mantoux y la jeringuilla Dermo-Jet. *Rev Clin Esp* (Spain) 100:119-125, 1966.
 438. Bettag OL, Hall C. Mantoux tuberculin testing - Standard method vs. jet injection. *Diseases of the Chest* 51:530-536, 1967.
 439. Morse DC, Hall A, Kaluzny A, Runde RH. Comparative tuberculin testing. Intradermal gun versus intradermal needle. *Amer Rev Resp Dis* 96:107-110, 1967.
 440. Dull HB, Herring LL, Calafiore D, et al. Jet injector tuberculin skin testing: Methodology and results. *Am Rev Respir Dis* 97, 38-45, 1968.
 441. Luby JP, Kaiser RL, Herring LL, Dull HB. Jet injector tuberculin skin testing: a comparative evaluation. Quantitative aspects. *Am Rev Respir Dis* 97:46-53, 1968.
 442. Marsallon, Magnin, Jégo, Richer. Intradermo-réaction tuberculique et vaccination B.C.G. intradermique par injecteur à jet sous pression. *Rev Corps Santé Armées Terre Mer Air* (France) 13:57-61, 1972.
 443. Brólio R, Veronesi R, Mazza CC, et al. Viabilidade da aplicação do teste tuberculínico com o Dermo-jet. *Rev Saúde Pública* (Brazil) 10:219-226, 1976.
 444. Wijsmuller G, Snider DE. Skin testing: A comparison of the jet injector with the mantoux method. *Am Rev Respir Dis* 112:789-798, 1975.
 445. ten Dam HG. Jet-injectors in BCG vaccination. *Clinical Pediatrics* 10:4-5, 1971.
 446. World Health Organization (Milstien J). The immunological basis for immunization series. Module 5: Tuberculosis. Geneva: World Health Organization, Global Programme For Vaccines And Immunization, Expanded Programme On Immunization, 1993, document WHO/EPI/GEN/93.15, 20 pages.
 447. Meyer HM, Bernheim BC, Rogers NG. Titration of live measles and smallpox vaccines by jet inoculation of susceptible children. *Proc Soc Exp Biol Med* 118:53-57, 1965.
 448. Zsigmond EK, Darby P, Koenig HM, Goll EF. Painless intravenous catheterization by intradermal jet injection of lidocaine: A randomized trial. *J Clin Anesth* 11:87-94, 1999.
 449. Pilipenko VG, Miroshnichenko MA, Loktev NA. Immunizatsiia assotsirovannyimi di- i trivaktsinami protiv chumy, tularemii i sibirskoi iazvy pri pomoshchi bezygol'nogo in'ektora. Soobschenie I [Russian: Plague, tularemia and anthrax immunization with associated di- and trivaccines using a jet injector. I]. *Zh Mikrobiol Epidemiol Immunobiol* (5):59-64, 1974.
 450. Loktev NA, Pilipenko VG, Basilova GI, et al. Bezygol'naia immunizatsiia assotsirovannoi vaktsinoy protiv chumy, tularemii i sibirskoi iazvy [Russian: Jet immunization with polyvalent vaccine against plague, tularemia, and anthrax]. *Zh Mikrobiol Epidemiol Immunobiol* (6):109-110, 1980.

451. Diop Mar I, Sarrat H, Robin Y, et al. Vaccination anticholérique par voie intradermique au Pédajet. Réponse clinique et immunologique (d'après une expérience sénégalaise). *Bull Soc Pathol Exot Filiales* 64:663-672, 1971.
452. Parent du Châtelet I, Lang J, Schlumberger M, et al. Clinical immunogenicity and tolerance studies of liquid vaccines delivered by jet-injector and a new single-use cartridge (Imule®): comparison with standard syringe injection. *Vaccine* 15:449-458, 1997.
453. Hoke CH Jr, Egan JE, Sjogren MH, et al. Administration of hepatitis A vaccine to a military population by needle and jet injector and with hepatitis B vaccine. *J Infect Dis* 171(Suppl 1):S53-S60, 1995.
454. Fisch A, Cadilhac P, Vidor E, et al. Immunogenicity and safety of a new inactivated hepatitis A vaccine: a clinical trial with comparison of administration route. *Vaccine* 14:1132-1136, 1996.
455. Williams J, Fox-Leyva L, Christensen C, et al. Hepatitis A vaccine administration: comparison between jet-injector and needle injection. *Vaccine* 18:1939-1943, 2000.
456. Lemon SM, Scott RM, Bancroft WH. Subcutaneous administration of inactivated hepatitis B vaccine by automatic jet injection. *J Med Virol* 12:129-136, 1983.
457. Matheï C, Van Damme P, Meheus A. Hepatitis B vaccine administration: comparison between jet-gun and syringe and needle. *Vaccine* 15:402-404, 1997.
458. Vibes J. Efficacité comparée de deux techniques de vaccination contre la grippe. Taux sérologique obtenus après administration du vaccin par le Porton Jet et la seringue. *Médecine et Maladies Infectieuses* 1:343-348, 1971.
459. Payler DK, Skirrow, MB. Intradermal influenza vaccination. *Br Med J* 2:727, 1974.
460. McIntosh K, Orr I, Andersen M, et al. Response of normal children to influenza A/New Jersey/76 virus vaccine administered by jet injector. *J Infect Dis* 136(Suppl):S584-S587, 1977.
461. Jackson LA, Austin G, Chen RT, et al. Safety and immunogenicity of varying doses of trivalent inactivated influenza vaccine administered by needle-free jet injectors. *Vaccine* 19:4703-4709, 2001.
462. Lipson MJ, Carver DH, Eleff MG, et al. Antibody response to poliomyelitis vaccine administered by jet injection. *Am J Public Health* 48:599-603, 1958.
463. Rey M, Triau R. Essais de primo-vaccination antitétanique en un temps avec une anatoxine concentrée inoculée par injecteurs sans aiguille (Note préliminaire). *Bull Soc Méd Afrique Noire Lang Française* 12:230-239, 1967.
464. Edwards EA, Johnson DP, Pierce WE, Peckinpaugh RO. Reactions and serologic responses to monovalent acetone-inactivated typhoid vaccine and heat-killed TAB when given by jet injection. *Bull WHO* 51:501-505, 1974.
465. Budd MA, Scholtens RG, McGehee RF, Jr, Gardner P. An evaluation of measles and smallpox vaccines simultaneously administered. *Am J Public Health Nations Health* 57:80-86, 1967.
466. Hendrickse RG, Montefiore D. Measles vaccination with reduced dosage. *Brit Med J* 3(622):28-30, 1968.
467. Sarno MJ, Blase E, Galindo N, et al. Clinical immunogenicity of measles, mumps and rubella vaccine delivered by the Injex jet injector: comparison with standard syringe injection. *Pediatr Infect Dis J* 19:839-842, 2000.
468. Elisberg BL, McCown JM, Smael JE. Vaccination against smallpox. II. Jet injection of chorio-allantoic membrane vaccine. *J Immunol* 77:340-351, 1956.
469. Roberto RR, Wulff H, Millar JD. Smallpox vaccination by intradermal jet injection. C. Cutaneous and serological responses to primary vaccination in children. *Bull WHO* 41:761-769, 1969.
470. Agafonov VI, Beliakov VD, Ishkil'din MI, et al. Immunologicheskaja effektivnost' privivok protiv ospy i tularemii bezygol'nym metodom [Russian: Immunological effectiveness of immunization against smallpox and tularemia by the jet injection method]. *Voenno-meditsinskii Zhurnal* (Russia) 4:48-51, 1973.
471. Jackson J, Dworkin R, Tsai T, et al. Comparison of antibody response and patient tolerance of yellow fever vaccine administered by the Biojector® needle-free injection system versus conventional needle/syringe injection. Third International Conference on Travel Medicine, Paris, 25-29 April 1993;264:209.
472. Artenstein MS, Branche WC, Jr, Zimmerly JG, et al. Meningococcal infections. 3. Studies of group A polysaccharide vaccines. *Bull WHO* 45:283-286, 1971.
473. Gotschlich EC, Rey M, Etienne J, et al. The immunological responses observed in field studies in Africa with group A meningococcal vaccines. *Prog Immunobiol Stand* 5:485-491, 1972.
474. Greenwood BM, Wali SS. Control of meningococcal infection in the African meningitis belt by selective vaccination. *Lancet* 1:729-732, 1980.
475. Mohammed I, Zaruba K. Control of epidemic meningococcal meningitis by mass vaccination. *Lancet* 2(8237):80-83, 1981.
476. Binkin N, Band J. Epidemic of meningococcal meningitis in Bamako, Mali: epidemiological features and analysis of vaccine efficacy. *Lancet* 2:315-318, 1982.
477. Rey JL, Soubiran G, Fayet MT, Triau R. Évaluation sérologique d'une campagne de vaccination antiméningococcique de masse au Niger. *Bull Soc Pathol Exot Filiales* (France) 82:248-254, 1989.
478. Amato Neto V, Finger H, Gotschlich EC, et al. Serologic response to serogroup C meningococcal vaccine in Brazilian preschool children. *Rev Inst Med Trop Sao Paulo* 16:149-153, 1974.
479. Taunay AE, Galvao PA, de Moraes JS, et al. Disease prevention by meningococcal serogroup C polysaccharide vaccine in preschool children: Results after eleven months in Sao Paulo, Brazil [abstract]. *Pediatr Res* 8:429, 1974.
480. Taunay AE, Feldman RA, Bastos CO, et al. Avaliação do efeito protector de vacina polissacarídica antimeningocócica da grupa C em crianças de 6 a 36 meses. *Revista do Instituto Adolfo Lutz* 32:77-82, 1978.
481. Mumper RJ, Cui Z. Genetic immunization by jet injection of targeted pDNA-coated nanoparticles. *Methods* 1:255-262, 2003.
482. Marshall JL, Hoyer RJ, Toomey MA, et al. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoma embryonic antigen immune responses. *J Clin Oncol* 18:3964-3973, 2000.
483. Evans LS, Lewinsohn DM, Johnson M, et al. Microsphere encapsulation or Biojector™ delivery enhances adjuvanted DNA vaccines in rhesus macaques. 19th Annual Symposium on Nonhuman Primate Models for AIDS, 8-11 September 2001, Monterey, CA, abstract #128. *J Med Primatol*. 2002;31:298.
484. Timmerman JM, Singh G, Hermanson G, et al. Immunogenicity of a plasmid DNA vaccine encoding chimeric idiotype in patients with B-cell lymphoma. *Cancer Res* 62:5845-52, 2002.
485. Lundholm P, Leanderson A-Ca, Christensson B, et al. DNA mucosal HIV vaccine in humans. *Virus Research* 82:141-145, 2002.
486. Konishi E, Terazawa A, Fujii A. Evidence for antigen production in muscles by dengue and Japanese encephalitis DNA vaccines and a relation to their immunogenicity in mice. *Vaccine* 21:3713-3720, 2003.
487. Hoke CH, Binn LN, Egan JE, et al. Hepatitis A in the US Army: epidemiology and vaccine development. *Vaccine* 10(Suppl 1):S75-S79, 1992.
488. Horn H, Opiz B, Schau G. Investigations into the risk of infection by the use of jet injectors. *Health and Social Serv J* 85:2396-2397, 1975.
489. Agafonov VI, Bulatova TI, Gamleshko KhP, et al. Effektivnost' kompleksnoi immunizatsii briushnotifoznoi vaksinoi s poliana-toksinom v sochetanii s chumnym i ospennym antigenam [Effectiveness of comprehensive immunization with typhoid fever vaccine and polyanatoxin in combination with plague and small pox antigens]. *Voenno-meditsinskii Zhurnal* [Military Medical Journal] (Russia) (10):51-54, 1978.
490. Lenz TR. Foreign body granuloma caused by jet injection of tetanus toxoid. *Rocky Mountain Med J* 63:48, 1966.
491. Schneider U, Birnbacher R, Schober E. Painfulness of needle and jet injection in children with diabetes mellitus. *Eur J Pediatr* 153:409-410, 1994.
492. Kremer MG. Jet vaccination [letter]. *Brit Med J* 4:303, 1970.
493. Eli Lilly and Company. Influenza Virus Vaccine Polyvalent (Types A and B) [vaccine product insert; 03516, 80:12, PA 1787 AMP]. Indianapolis, IN: Eli Lilly and Company; December 28, 1962;102.
494. Salanga VD, Hahn JF. Traumatic ulnar neuropathy from jet injection: Case Report. *J Trauma* 19:283-284, 1979.
495. Harris M, Larsen G, Valyi M, et al. Transient neuropathy after needle-free injection outside of recommended sites [letter]. *AIDS* 20:784-785, 2006.
496. Tabita PV. Side effect of the jet injector for the production of local anesthesia. *Anesthes Prog* 102-104, 1979.
497. Rosenthal SR. Transference of blood by various inoculation devices. *Am Rev Respir Dis* 96:815-819, 1967.
498. Petersen NJ, Bond WW, Carson LA (Special Investigations Section, Hepatitis Laboratories Division). Informal quarterly report of October-December 1977 [memorandum]. Phoenix, Arizona: Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control; 1977:1-3.
499. Darlow HM. Jet vaccination. *Br Med J* 4(734):554, 1970.
500. Abb J, Deinhardt F, Eisenberg J. The risk of transmission of hepatitis B virus using jet injection in inoculation. *J Infect Dis* 144:176-179, 1981.
501. Spiess H. Sterilität von Impfpistolen [Letter: Sterility of vaccination guns]. *Dtsch Med Wochenschr* 100:1445-1446, 1975.
502. Spiess H. Hepatitisübertragung durch Hochdruckinjektion? [Letter: Hepatitis transmission by high pressure injection?]. *Dtsch Med Wochenschr* 100:2465, 1975.
503. Brink PRG, van Loon AM, Trommelen JCM, et al. Virus transmission by subcutaneous jet injection. *J Med Microbiol* 20:393-397, 1985.
504. Centers for Disease Control. Hepatitis B associated with jet gun injection - California. *Morb Mortal Wkly Rep* 35:373-376, 1986.
505. World Health Organization, Expanded Programme on Immunization. Transmission of hepatitis B associated with jet gun injection. *Weekly Epidemiol Rec* 61:309-311, 1986.
506. Canter J, Mackey K, Good LS, et al. An outbreak of hepatitis B associated with jet injections in a weight reduction clinic. *Arch Int Med* 150:1923-1927, 1990.
507. Zachoval R, Deinhardt F, Gurtler L, et al. Risk of virus transmission by jet injection. *Lancet* 1(8578):189, 1988.
508. de Souza Brito G, Chen RT, Stefano IC, et al. The risk of transmission of HIV and other blood-borne diseases via jet injectors during immunization mass campaigns in Brazil. 10th International Conference on AIDS, Yokohama, 7-12 August 1994;10:301 (abstract PC0132. Online. Available at: www.aegis.org/conferences/iac/1994/PC0132.html. Accessed 13 November 2006).
509. Department of Defense. C. Issues of administration, 1. Jet injector use. In: Poland GA, (ed.). *Vaccines in the Military: a Department of Defense-wide Review of Vaccine Policy and Practice. A Report for the Armed Forces Epidemiological Board*, August 1999. Falls Church, VA: Infectious Diseases Control Subcommittee of the Armed Forces Epidemiological Board, 1999;60. Online. Available at: www.ha.osd.mil/afeb/reports/vaccines.pdf. Accessed November 14, 2006.
510. Weintraub AM, Ponce de Leon M. Potential for cross-contamination from use of a needleless injector. *AJIC Am J Infect Control* 26:442-445, 1998.
511. Lukin EP, Evstigneev VI, Makhilai AA, et al. Bezygol'nye in'ektsii i 'shpřitsevy'e' infektsii

- [Russian: Needle-free injections and 'needle-transmitted' infections]. *Voenno-meditsinskii Zhurnal* (Russia) 318:48-52, 1997.
512. Hoffman PN, Abuknesha RA, Andrews NJ, et al. A model to assess the infection potential of jet injectors used in mass immunisation. *Vaccine* 19:4020-4027, 2001.
513. Wenger JD, Spika JS, Smithwick RW, et al. Outbreak of *Mycobacterium chelonae* infection associated with use of jet injectors. *JAMA* 264:373-376, 1990.
514. Souto FJD, Espírito Santo GA, Philippi JC, et al. Prevalência e fatores associados a marcadores do vírus da hepatite B em população rural do Brasil central. *Rev Panam Salud Pública*. 2001 Dec;10:388-394. Online. Available at: www.scielosp.org/scielo.php?script=sci_arttext&pid=S1020-49892001001200004. Accessed November 14, 2006.
515. World Health Organization. Expanded Programme on Immunization, Global Advisory Group. IV. Injection equipment and sterilization practices. *Weekly Epidemiol Rec* 62:8-9, 1987.
516. Centers for Disease Control and Prevention. General Recommendations on Immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morb Mortal Wkly Rep* 43:(RR-1):7-8, 1994.
517. Department of Defense. Subj: MMQC-97-1169 Automatic jet hypodermic injection units/ withdrawal (DPSC 970147). Fort Detrick MD: Quad Service MMQC USAMMA/AFMLO/NMLC; December 5, 1997. Online. Available at: http://usamma.detrack.army.mil/ftp/mmqc_messages/Q971169.txt. Accessed November 14, 2006.
518. Zehrung DL. Pilot safety study to determine the ability of the protector cap jet injector to prevent cross-contamination. Online. Available at: www.clinicaltrials.gov/ct/show/NCT00219453. Accessed November 22, 2006.
- 518a. Program for Appropriate Technology in Health, personal communication, 2007.
519. Alibek K, Handelman S. Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World—Told from Inside by the Man Who Ran It. New York, NY: Dell; 1999.
520. Saltykov RA, Nekrasov IL, Lesniak OT, Ulanova AA. Immunizatsiia zhivoi sibiriazvennoi vaktsinoi STI pri pomoshchi bezyol'nogo in'ektora v eksperimente [Russian: Experimental immunization with live anthrax STI vaccine using a needleless injector]. *Zh Mikrobiol Epidemiol Immunobiol* 48:52-55, 1971.
521. Burgasov PN, Cherkasskii BL, Adilov DA, et al. Immunizatsiia liudei protiv sibirskoi izvzy bezyol'nym metodom [Russian: Immunization against anthrax by a needleless method]. *Zh Mikrobiol Epidemiol Immunobiol* 50:23-26, 1973.
522. Agafonov VI, Babkin EI, Bulatova TI, et al. Bezyol'nyi metod immunizatsii assotsirovannymi sorbirovannymi vaktsinami [Russian: Jet method of immunizing with associated adsorbed vaccines]. *Voenno-meditsinskii Zhurnal* (12):44-48, 1974.
523. Gapochko KG, Vasilenko AZh, Misnikov OP, et al. Kliniko-immunologicheskoe obosnovanie assotsirovannoi immunizatsii [Russian: The clinico-immunological validation of associated immunization]. *Voenno-meditsinskii Zhurnal* (3):35-38, 1992.
524. Djupesland PG, Skretting A, Winderen M, Holand T. Breath actuated device improves delivery to target sites beyond the nasal valve. *Laryngoscope* 116:466-472, 2006.
525. Vijay-Kumar M, Gewirtz AT. Role of epithelium in antigen presentation. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 423-434.
526. Kelsall BL, Leon F, Smythies LE, Smith PD. Antigen handling and presentation by mucosal dendritic cells and macrophages. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 451-486.
527. Stober w, McGhee JR. Inductive and effector tissues and cells of the mucosal immune system. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 371-374.
528. Suman JD, Laube BL, Dalby R. Comparison of nasal deposition and clearance of aerosol generated by a nebulizer and an aqueous spray pump. *Pharm Res* 16:1648-1652, 1999.
529. Pontiroli AE, Cladera A, Pozza G. Intranasal drug delivery- potential advantages and limitations from a clinical pharmacokinetic perspective. *Clin Pharmacokinet* 17:299-307, 1989.
530. Bienenstock J. Mucosal and other mechanisms of resistance in the respiratory tract: An overview. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 1401-1402.
531. Brandtzaeg P, Carlsen HE, Farstad IN. The human mucosal B-cell system. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 617-654.
532. Neutra MR, Kozłowski PA. Mucosal vaccines: the promise and the challenge. *Nat Rev Immun* 6:148-158, 2006.
533. Mestecky J. The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J. Clin. Immunol* 7:265-269, 1987.
534. Mestecky J, Moldoveanu Z, Elson CO. Immune response versus mucosal tolerance to mucosally administered antigens. *Vaccine* 23:1800-1803, 2005.
535. Balmelli C, Demotz S, Acha-Orbea H, et al. Trachea, lung, and tracheobronchial lymph nodes are the major sites where antigen- presenting cells are detected after nasal vaccination of mice with human papillomavirus type 16 virus-like particles. *J Virol* 76:12596-12602, 2002.
536. Laube BL. The expanding role of aerosols in systemic drug delivery, gene therapy and vaccination. *Resp Care* 50:1161-1176, 2005.
537. Dunn C, Curran MP. Inhaled human insulin (Exubera): a review of its use in adult patients with diabetes mellitus. *Drugs* 66:1013-1032, 2006.
538. Wong-Chew RM, Islas-Romero R, Garcia-Garcia Mde L, et al. Immunogenicity of aerosol measles vaccine given as the primary measles immunization to nine-month-old Mexican children. *Vaccine* 24:683-90, 2006.
539. Wong-Chew RM, Islas-Romero R, Garcia-Garcia Mde L, et al. Induction of cellular and humoral immunity after aerosol or subcutaneous administration of Edmonston-Zagreb measles vaccine given as the primary dose to 12-month-old children. *J Inf Dis* 189:254-257, 2004.
540. De Castro JF, Kumate J. La vacunación contra el sarampión. Situación in México and America. Avances en el metodo de inmunización por aerosol. *Bol Med Hosp Infant Mex* 47:449-461, 1990.
541. Dilraj A, Cutts F, de Castro J. Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomized trial. *Lancet* 355:798-803, 2000.
542. Leung K, Louca E, Gray M, et al. Use of the next generation pharmaceutical impactor for particle size distribution measurement of live viral aerosol vaccines. *J Aerosol Med* 18:414-426, 2005.
543. OptiNose AS, N-0349 Oslo, Norway. Online. Available at: www.optinose.no.
544. Bakke H, Samdal HH, Holst J, et al. Oral spray immunization may be an alternative to intranasal vaccine delivery to induce systemic antibodies but not nasal mucosal or cellular immunity. *Scan J Immunol* 63:223-231, 2006.
545. Huo Z, Sinha R, McNeela EA, et al. Induction of protective serum meningococcal bactericidal and diphtheria neutralizing antibodies and mucosal immunoglobulin A in volunteers by nasal insufflations of the *Neisseria meningitidis* serogroup C polysaccharide-CRM197 conjugate vaccine mixed with chitosan. *Infect Immun* 73:8256-8265, 2005.
546. Huang J, Garmise JR, Crowder MT, et al. A novel dry powder influenza vaccine and intranasal delivery technology: introduction of systemic and mucosal immune responses in rats. *Vaccine* 23:794-801, 2004.
547. de Swart RL, Kuiken T, Fernandez-de Castro J, et al. Aerosol measles vaccination in macaques: Preclinical studies of immune responses and safety. *Vaccine* 24:6424-6436, 2006.
548. AerovectRx, Inc. Atlanta, GA 30319, USA. Online. Available at: www.aerovectrx.com.
549. Yuki Y, Kiyono H. New generation of mucosal adjuvants for the induction of protective immunity. *Rev Med Virol* 13:293-310, 2003.
550. Eriksson K, Holmgren J. Recent advances in mucosal vaccines and adjuvants. *Curr Opin Immunol* 14:666-672, 2002.
551. Moyle PM, McGeary RP, Blanchfield JT, Toth I. Mucosal immunization: adjuvants and delivery systems. *Curr Drug Deliv* 1:385-396, 2004.
552. Cox E, Verdonck F, Vanrompay D, Goddeeris B. Adjuvants modulating mucosal immune responses or directing systemic responses towards the mucosa. *Vet Res* 37:511-539, 2006.
553. Holmgren J, Czerkinsky C, Eriksson K, Mharandi A. Mucosal immunization and adjuvants: a brief overview of recent advances and challenges. *Vaccine* 21(Suppl 2):S89-S95, 2003.
554. Hernandez HM, Figueredo M, Garrido N, et al. Intranasal immunisation with a 62 kDa proteinase combined with cholera toxin or CpG adjuvant protects against *Trichomonas vaginalis* genital tract infections in mice. *Int J Parasitol* 35:1333-1337, 2005.
555. Hickey DK, Jones RC, Bao S, et al. Intranasal immunization with C. muridarum major outer membrane protein (MOMP) and cholera toxin elicits local production of neutralizing IgA in the prostate. *Vaccine* 22:4306-4315, 2004.
556. Teloni R, von Hunolstein C, Mariotti S, et al. Antibody classes and subclasses induced by mucosal immunization of mice with *Streptococcus pyogenes* M6 protein and oligodeoxynucleotides containing CpG motifs. *Indian J Med Res* 119:126-130, 2004.
557. Arakawa T, Tsuboi T, Kishimoto A, et al. Serum antibodies induced by intranasal immunization of mice with *Plasmodium vivax* Pvs25 co-administered with cholera toxin completely block parasite transmission to mosquitoes. *Vaccine* 21:3143-3148, 2003.
558. Bowe F, Lavelle EC, McNeela EA, et al. Mucosal vaccination against serogroup B meningococci: induction of bactericidal antibodies and cellular immunity following intranasal immunization with NadA of *Neisseria meningitidis* and mutants of *Escherichia coli* heat-labile enterotoxin. *Infect Immun* 72:4052-4060, 2004.
559. Erume J, Partidos H. Evaluation of the adjuvant effect of *Escherichia coli* heat-labile enterotoxin mutant (LTk63) on the systemic immune responses to intranasally co-administered measles virus nucleoprotein. Part I: antibody responses. *Afr Health Sci* 1:3-8, 2001.
560. Nasal vaccination, *Escherichia coli* enterotoxin, and Bell's palsy. *N Engl J Med* 350:860-861, 2004.
561. National Institute of Allergy and Infectious Diseases. Safety evaluation of toxin adjuvants delivered intranasally. Online. Available at: www.niaid.nih.gov/dmid/enteric/intranasal.htm. Last accessed August 16, 2006.
562. Pimenta FC, Miyaji EN, Areas AP, et al. Intranasal immunization with the cholera toxin B subunit-pneumococcal surface antigen A fusion protein induces protection against colonization with *Streptococcus pneumoniae* and has negligible impact on the nasopharyngeal and oral microbiota of mice. *Infect Immun* 74:4939-4944, 2006.
563. Olive C, Sun HK, Ho MF, et al. Intranasal administration is an effective mucosal vaccine delivery route for self-adjuvanting lipid core peptides targeting the group A streptococcal m protein. *J Infect Dis* 194:316-324, 2006.
564. Dell K, Koesters R, Linnebacher M, et al. Intranasal immunization with human papillomavirus type 16 capsomeres in the presence of non-toxic cholera toxin-based adjuvants elicits increased vaginal immunoglobulin levels. *Vaccine* 24:2238-2247, 2006.
565. Price GA, Russell MW, Cornelissen CN. Intranasal administration of recombinant *Neisseria gonorrhoeae* transferrin binding proteins A and B conjugated to the cholera toxin B subunit induces systemic and vaginal antibodies in mice. *Infect Immun* 73:3945-3953, 2005.

566. Areas AP, Oliveira ML, Miyaji EN, et al. Expression and characterization of cholera toxin B-pneumococcal surface adhesin A fusion protein in *Escherichia coli*: ability of CTB-PsaA to induce humoral immune response in mice. *Biochem Biophys Res Commun* 321:192-196, 2004.
567. Larsson C, Holmgren J, Lindahl G, Bergquist C. Intranasal immunization of mice with group B streptococcal protein Rib and cholera toxin B subunit confers protection against lethal infection. *Infect Immun* 72:1184-1187, 2004.
568. Zhang P, Yang QB, Marciani DJ, et al. Effectiveness of the quillaja saponin semi-synthetic analog GPI-0100 in potentiating mucosal and systemic responses to recombinant HagB from *Porphyromonas gingivalis*. *Vaccine* 21:4459-4471, 2003.
569. Kang SM, Yao Q, Guo L, Compans RW. Mucosal immunization with virus-like particles of simian immunodeficiency virus conjugated with cholera toxin subunit B. *J Virol* 77:9823-9830, 2003.
570. Yasuda Y, Isaka M, Taniguchi T, et al. Frequent nasal administrations of recombinant cholera toxin B subunit (rCTB)-containing tetanus and diphtheria toxoid vaccines induced antigen-specific serum and mucosal immune responses in the presence of anti-rCTB antibodies. *Vaccine* 21:2954-2963, 2003.
571. Singh SR, Hulett K, Pillai SR, et al. Mucosal immunization with recombinant MOMP genetically linked with modified cholera toxin confers protection against *Chlamydia trachomatis* infection. *Vaccine* 24:1213-1224, 2006.
572. Yoshino N, Lu FX, Fujihashi K, et al. A novel adjuvant for mucosal immunity to HIV-1 gp120 in nonhuman primates. *J Immunol* 173:6850-6857, 2004.
573. Egan MA, Chong SY, Hagen M, et al. A comparative evaluation of nasal and parenteral vaccine adjuvants to elicit systemic and mucosal HIV-1 peptide-specific humoral immune responses in cynomolgus macaques. *Vaccine* 22:3774-3788, 2004.
574. De Filette M, Fiers W, Martens W, et al. Improved design and intranasal delivery of an M2e-based human influenza A vaccine. *Vaccine* 2006;Jun 12; [Epub ahead of print].
575. Helgeby A, Robson NC, Donachie AM, et al. The combined CTA1-DD/ISCOM adjuvant vector promotes priming of mucosal and systemic immunity to incorporated antigens by specific targeting of B cells. *J Immunol* 176:3697-3706, 2006.
576. Akhiani AA, Stensson A, Schon K, Lycke N. The nontoxic CTA1-DD adjuvant enhances protective immunity against *Helicobacter pylori* infection following mucosal immunization. *Scand J Immunol* 63:97-105, 2006.
577. Stephenson I, Zambon MC, Rudin A, et al. Phase I evaluation of intranasal trivalent inactivated influenza vaccine with nontoxic *Escherichia coli* enterotoxin and novel biovector as mucosal adjuvants, using adult volunteers. *J Virol* 80:4962-4970, 2006.
578. Baudner BC, Verhoeff JC, Giuliani MM, et al. Protective immune responses to meningococcal C conjugate vaccine after intranasal immunization of mice with the LTK63 mutant plus chitosan or trimethyl chitosan chloride as novel delivery platform. *J Drug Target* 13:489-498, 2005.
579. Kende M, Del Giudice G, Rivera N, Hewetson J. Enhancement of intranasal vaccination in mice with deglycosylated chain A ricin by LTR72, a novel mucosal adjuvant. *Vaccine* 24:2213-2221, 2006.
580. Baudner BC, Giuliani MM, Verhoeff JC, et al. The concomitant use of the LTK63 mucosal adjuvant and of chitosan-based delivery system enhances the immunogenicity and efficacy of intranasally administered vaccines. *Vaccine* 21:3837-3844, 2003.
581. Erikkson AM, Schon KM, Lyke NY. The cholera toxin-derived CTA1-DD vaccine adjuvant administered intranasally does not cause inflammation or accumulate in the nervous tissues. *J Immunol* 173:3310-3319, 2004.
582. Etchart N, Baaten B, Andersen SR, et al. Intranasal immunisation with inactivated RSV and bacterial adjuvants induces mucosal protection and abrogates eosinophilia upon challenge. *Eur J Immunol* 36:1068-1069, 2006.
583. Treanor J, Nolan C, O'Brien D, et al. Intranasal administration of a proteosome-influenza vaccine is well-tolerated and induces serum and nasal secretion influenza antibodies in healthy human subjects. *Vaccine* 24:254-262, 2006.
584. Sardinias G, Reddin K, Pajon R, Gorrige A. Outer membrane vesicles of *Neisseria lactamica* as a potential mucosal adjuvant. *Vaccine* 24:206-214, 2006.
585. Chabot S, Brewer A, Lowell G, et al. A novel intranasal Protollin-based measles vaccine induces mucosal and systemic neutralizing antibody responses and cell-mediated immunity in mice. *Vaccine* 23:1374-1383, 2005.
586. Perez O, Bracho G, Lastré M, et al. Novel adjuvant based on a proteoliposome-derived cochleate structure containing native lipopolysaccharide as a pathogen-associated molecular pattern. *Immunol Cell Biol* 82:603-610, 2004.
587. Jones T, Cyr S, Allard F, et al. Protollin: a novel adjuvant for intranasal vaccines. *Vaccine* 22:3691-3697, 2004.
588. Wimer-Mackin S, Hinchcliffe M, Petrie CR, et al. An intranasal vaccine targeting both the *Bacillus anthracis* toxin and bacterium provides protection against aerosol spore challenge in rabbits. *Vaccine* 24:3953-3963, 2006.
589. Pinczewski J, Zhao J, Malkevitch N, et al. Enhanced immunity and protective efficacy against SIVmac251 intrarectal challenge following ad-SIV priming by multiple mucosal routes and gp120 boosting in MPL-SE. *Viral Immunol* 18:236-243, 2005.
590. Borsutzky S, Ebensen T, Link C, et al. Efficient systemic and mucosal responses against the HIV-1 Tat protein by prime/boost vaccination using the lipopeptide MALP-2 as adjuvant. *Vaccine* 24:2049-2056, 2006.
591. Luhrmann A, Tschernig T, Pabst R, Niewiesk S. Improved intranasal immunization with live-attenuated measles virus after co-inoculation of the lipopeptide MALP-2. *Vaccine* 23:4721-4726, 2005.
592. Honko AN, Sriranganathan N, Lees CJ, Mizel SB. Flagellin is an effective adjuvant for immunization against lethal respiratory challenge with *Yersinia pestis*. *Infect Immun* 74:1113-1120, 2006.
593. Lee SE, Kim SY, Jeong BC, et al. A bacterial flagellin, *Vibrio vulnificus* FlaB, has a strong mucosal adjuvant activity to induce protective immunity. *Infect Immun* 74:694-702, 2006.
594. Tafaghodi M, Jaafari MR, Sajadi Tabassi SA. Nasal immunization studies using liposomes loaded with tetanus toxoid and CpG-ODN. *Eur J Pharm Biopharm* 64:138-145, 2006.
595. Agger EM, Rosenkrands I, Olsen AW, et al. Protective immunity to tuberculosis with Ag85B-ESAT-6 in a synthetic cationic adjuvant system IC31. *Vaccine* 24:5452-5460, 2006.
596. Kodama S, Abe N, Hirano T, Eto M, Suzuki M. Safety and efficacy of nasal application of CpG oligodeoxynucleotide as a mucosal adjuvant. *Laryngoscope* 116:331-335, 2006.
597. Shi T, Liu WZ, Gao F, et al. Intranasal CpG-oligodeoxynucleotide is a potent adjuvant of vaccine against *Helicobacter pylori*, and T helper 1 type response and interferon-gamma correlate with the protection. *Helicobacter* 10:71-79, 2005.
598. Abe N, Kodama S, Hirano T, et al. Nasal vaccination with CpG oligodeoxynucleotide induces protective immunity against nontypeable *Haemophilus influenzae* in the nasopharynx. *Laryngoscope* 116:407-412, 2006.
599. Heikenwalder M, Polymenidou M, Junt T, et al. Lymphoid follicle destruction and immunosuppression after repeated CpG oligodeoxynucleotide administration. *Nat Med* 10:187-192, 2004.
600. Wozniak TM, Ryan AA, Triccas JA, Britton WJ. Plasmid interleukin-23 (IL-23), but not plasmid IL-27, enhances the protective efficacy of a DNA vaccine against *Mycobacterium tuberculosis* infection. *Infect Immun* 74:557-565, 2006.
601. Bermudez-Humaran LG, Cortes-Perez NG, Lefevre F, et al. A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. *J Immunol* 175:7297-7302, 2005.
602. Toka FN, Rouse BT. Mucosal application of plasmid-encoded IL-15 sustains a highly protective anti-Herpes simplex virus immunity. *J Leukoc Biol* 78:178-186, 2005.
603. Bertley FM, Kozlowski PA, Wang SW, et al. Control of simian/human immunodeficiency virus viremia and disease progression after IL-2-augmented DNA-modified vaccinia virus Ankara nasal vaccination in nonhuman primates. *J Immunol* 172:3745-3757, 2004.
604. Lynch JM, Briles DE, Metzger DW. Increased protection against pneumococcal disease by mucosal administration of conjugate vaccine plus interleukin-12. *Infect Immun* 71:4780-4788, 2003.
605. Lee S, Gierynska M, Eo SK, et al. Influence of DNA encoding cytokines on systemic and mucosal immunity following genetic vaccination against herpes simplex virus. *Microbes Infect* 5:571-578, 2003.
606. Boyaka PN, McGhee JR. Cytokines as adjuvants for the induction of mucosal immunity. *Adv Drug Deliv Rev* 51:71-79, 2001.
607. Bracci L, Canini I, Venditti M, et al. Type I IFN as a vaccine adjuvant for both systemic and mucosal vaccination against influenza virus. *Vaccine* 24(Suppl 2):56-57, 2006.
608. Illum L. Chitosan and its use as a pharmaceutical excipient. *Pharm Res* 15:1326-1331, 1998.
609. Illum L, Jabbal-Gill I, Hinchcliffe M, et al. Chitosan as a novel nasal delivery system for vaccines. *Adv Drug Deliv Rev* 51:81-96, 2001.
610. McNeela EA, Jabbal-Gill I, Illum L, et al. Intranasal immunization with genetically detoxified diphtheria toxin induces T cell responses in humans: enhancement of Th2 responses and toxin-neutralizing antibodies by formulation with chitosan. *Vaccine* 22:909-914, 2004.
611. Sasaki S, Sumino K, Hamajima K, et al. Induction of systemic and mucosal immune responses to human immunodeficiency virus type 1 by a DNA vaccine formulated with QS-21 saponin adjuvant via intramuscular and intranasal routes. *J Virol* 72:4931-4939, 1998.
612. Michalek SM, O'Hagen DT, Childers NK, et al. Antigen delivery systems I: Non-living microparticles, liposomes, and Immune Stimulating Complexes (ISCOMs). In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 987-1008.
613. Curtiss R. Antigen delivery systems II: Development of live recombinant attenuated bacterial antigen and DNA vaccine delivery vector vaccines. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 1009-1038.
614. Rosenthal KL. Recombinant live viral vectors as vaccines for mucosal immunity. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 1039-1052.
615. Mestecky J, Michalek SM, Moldoveanu Z, Russell MW. Routes of immunization and antigen delivery systems for optimal mucosal immune responses in humans. *Behring Inst Mitt* 98:33-43, 1997.
616. Kersten G, Hirschberg H. Antigen delivery systems. *Expert Rev Vaccines* 3:453-462, 2004.
617. Cusi MG, Correale P, Valassina M, et al. Comparative study of the immune response in mice immunized with four live attenuated strains of mumps virus by intranasal or intramuscular route. *Arch Virol* 146:1241-1248, 2001.
618. Luminova NV, Krasnova VP, Liashenko VA. The specific activity and immunological safety of a live mumps vaccine from the Leningrad-3 strain in intranasally revaccinated adult subjects. *Vopr Virusol* 39:113-116, 1994.
619. Krasnova VP, Luminova NV, Liashenko VA. An intranasal method of revaccination against mumps. *Vopr Virusol* 39:24-26, 1994.
620. Ogra PL, Chiba Y, Beutner KR, Morag A. Vaccination by non-parenteral routes: characteristics of immune response. *Dev Biol Stand* 33:19-26, 1976.
621. Terada K, Niizuma T, Ogita S, Kataoka N. Responses of varicella zoster virus (VZV)-specific

- immunity in seropositive adults after inhalation of inactivated or live attenuated varicella vaccine. *Vaccine* 20:3638–3643, 2002.
622. Tsuji T, Shiraki K, Sato H, et al. Humoral immunoreponse to varicella-zoster virus pernasally coadministered with *Escherichia coli* enterotoxin in mice. *Vaccine* 18:2049–2054, 2000. Not used.
624. Parker JN, Pfister LA, Quenelle D, et al. Genetically engineered herpes simplex viruses that express IL-12 or GM-CSF as vaccine candidates. *Vaccine* 24:1644–1652, 2006.
625. Lin YH, Deatly AM, Chen W, et al. Genetic stability determinants of temperature sensitive, live attenuated respiratory syncytial virus vaccine candidates. *Virus Res* 115:9–15, 2006.
626. Nolan SM, Surman SR, Amaro-Carambot E, et al. Live-attenuated intranasal parainfluenza virus type 2 vaccine candidates developed by reverse genetics containing L polymerase protein mutations imported from heterologous paramyxoviruses. *Vaccine* 23:4765–4774, 2005.
627. Not used.
628. Valosky J, Hishiki H, Zaoutis TE, Coffin SE. Induction of mucosal B-cell memory by intranasal immunization of mice with respiratory syncytial virus. *Clin Diagn Lab Immunol* 12:171–179, 2005.
629. Belshe RB, Newman FK, Anderson EL, et al. Evaluation of combined live, attenuated respiratory syncytial virus and parainfluenza 3 virus vaccines in infants and young children. *J Infect Dis* 190:2096–2103, 2004.
630. Choi AH, McNeal MM, Basu M, et al. Intranasal or oral immunization of inbred and outbred mice with murine or human rotavirus VP6 proteins protects against viral shedding after challenge with murine rotaviruses. *Vaccine* 20:3310–3321, 2002.
631. Enose Y, Ui M, Miyake A, et al. Protection by intranasal immunization of a nef-deleted, nonpathogenic SHIV against intravaginal challenge with a heterologous pathogenic SHIV. *Virology* 298:306–316, 2002.
632. Parr EL, Parr MB. Immune responses and protection against vaginal infection after nasal or vaginal immunization with attenuated herpes simplex virus type-2. *Immunology* 98:639–645, 1999.
633. Niedrig M, Stolte N, Fuchs D, et al. Intra-nasal infection of macaques with Yellow Fever (YF) vaccine strain 17D: a novel and economical approach for YF vaccination in man. *Vaccine* 17:1206–1210, 1999.
634. Belyakov IM, Isakov D, Zhu Q, et al. Enhancement of CD8+ T cell immunity in the lung by CpG oligodeoxynucleotides increases protective efficacy of a modified vaccinia Ankara vaccine against lethal poxvirus infection even in a CD4-deficient host. *J Immunol* 177:6336–6343, 2006.
635. Phelps AL, Gates AJ, Hillier M, et al. Comparative efficacy of modified vaccinia Ankara (MVA) as a potential replacement smallpox vaccine. *Vaccine* Aug 1; 2006 [Epub ahead of print].
636. Meseda CA, Garcia AD, Kumar A, et al. Enhanced immunogenicity and protective effect conferred by vaccination with combinations of modified vaccinia virus Ankara and licensed smallpox vaccine Dryvax in a mouse model. *Virology* 339:164–175, 2005.
637. MedImmune, Inc., Gaithersburg, MD 20878, USA. Online. Available at: www.medimmune.com.
638. Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis* 181:1133–1137, 2000.
639. Nichol KL, Mendelman PM, Mallon KP, et al. Effectiveness of live attenuated intranasal influenza virus vaccine in healthy working adults: a randomized trial. *JAMA* 282:137–145, 1999.
640. Ashkenazi S, Vertruyen A, Aristegui J, et al. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. *Pediatr Infect Dis J* 25:870–879, 2006.
641. Fleming DM, Crovari P, Wahn U, et al. Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. *Pediatr Infect Dis J* 25:860–869, 2006.
- 641a. Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med* 356(7):685–696, 2007.
- 641b. Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. *Pediatrics* 118(6):2298–2312, 2006.
- 641c. Tam JS, Capeding MR, Lum LC, et al. Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. *Pediatr Infect Dis J* 26(7):619–628, 2007.
642. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr* 136:168–175, 2000.
643. Piedra PA, Gaglani MJ, Kozinetz CA, et al. Herd immunity in adults against influenza-related illnesses with use of the trivalent-live attenuated influenza vaccine (CAIV-T) in children. *Vaccine* 23:1540–1548, 2005.
644. McCrumb F. Studies with live attenuated measles virus vaccine: clinical and immunologic responses in institutionalized children. *Am J Dis Child* 101:45–56, 1961.
645. McCrumb F, Bulkeley J, Hornick R, et al. Clinical trials with living attenuated measles virus vaccines. *Am J Public Health* 52:11–15, 1962.
646. Black F, Sheridan S. Studies on an attenuated measles virus vaccine. *N Engl J Med* 263:165–169, 1960.
647. Cernescu C, Cahal N. Antimeasles vaccination by natural routes- experimental background and practical consequences. *Rev J Med Virol* 35:259–271, 1984.
648. Whittle H, Rowland M, Mann G. Failure of measles vaccine sprayed into the oropharynx of infants. *Lancet* 1:1045, 1983.
649. Terskikh A, Danilov A, Shel'tchov G, et al. Theoretical substantiation and effectiveness of immunization with aerosols of liquid measles vaccine. *Vest Akada Med Narek* 26:84–90, 1971.
650. Kress S, Schluederberg A, Hornick R, et al. Studies with live attenuated measles virus vaccine. *Am J Dis Child* 101:57–63, 1961.
651. Simasathien S, Migasena S, Bellini W, et al. Measles vaccination of Thai infants by intranasal and subcutaneous routes: possible interference from respiratory infections. *Vaccine* 15:329–334, 1997.
652. Beck M, Smerdel S, Dedic I, et al. Immune response to Edmonston-Zagreb measles virus strain in monovalent and combined MMR vaccine. *Dev Biol Standard* 65:95–100, 1986.
653. Okuno Y, Ueda S, Hosai H, et al. Studies on the combined use of killed and live measles vaccine. II. Advantages of the inhalation method. *Biken J* 8:81–85, 1965.
654. Ueda S, Hosai H, Minekawa, et al. Studies on the combined use of killed and live measles vaccine. III. Conditions for the 'take' of live vaccine. *Biken J* 9:97–101, 1966.
655. Sabin AB, Fernandez de Castro J, Flores Arechiga A, et al. Clinical trials of inhaled aerosol of human diploid and chick embryo measles vaccine. *Lancet* 2:602, 1982.
656. Sabin AB, Flores Arechiga A, Fernandez de Castro J, et al. Successful immunization of children with and without maternal antibody by aerosolized measles vaccine. I. Different results with undiluted human diploid cell and chick embryo fibroblast vaccines. *JAMA* 249:2651–2662, 1983.
657. Sabin AB, Flores Arechiga A, Fernandez de Castro J, et al. Successful immunization of infants with and without maternal antibody by aerosolized measles vaccine. II. Vaccine comparisons and evidence for multiple antibody response. *JAMA* 251:2362–2371, 1984.
658. Sabin AB, Albrecht P, Takeda AK. High effectiveness of aerosolized chick embryo fibroblast measles vaccine in seven month old and older infants. *J Inf Dis* 152:1231–1237, 1985.
659. De Castro JF, Valdespino Gomez JL, Diaz Ortega JL, Zarate Aquino ML. Diploid cell measles vaccine. *JAMA* 256:714, 1986.
660. Torigoe S, Biritwum RB, Isomura S, et al. Measles in Ghana: A trial of an alternative means of administration of measles vaccine. *J Trop Peds* 32:304–309, 1986.
661. Sepúlveda-Amor J et al. A randomized trial demonstrating successful boosting responses following simultaneous aerosols of measles and rubella vaccines in school age children. *Vaccine* 20:2790–2795, 2002.
662. Bennett JV, Fernandez de Castro J, Valdespino-Gomez JL, et al. Aerosolized measles and measles-rubella vaccines induce better measles antibody booster responses than injected vaccines: randomized trials in Mexican schoolchildren. *Bull WHO* 80:806–812, 2002.
663. Bellanti JA, Zelig BJ, Mendez-Inocencio J, et al. Immunologic studies of specific mucosal and systemic immune responses in Mexican school children after booster aerosol or subcutaneous immunization with measles vaccine. *Vaccine* 22:1214–1220, 2004.
664. Dilraj A, Cutts F, Bennett J, et al. Persistence of measles antibody two years after revaccination by aerosol or subcutaneous routes. *Ped Infect Dis J* 12:1211–1213, 2000.
665. Dilraj A, Sukhoo R, Cutts FT, Bennett JV. Aerosol and subcutaneous measles vaccine: measles antibody responses 6 years after re-vaccination. *Vaccine* 25(21):4170–4174, 2007.
666. De Castro JF, Kumate-Rodriguez J, Sepúlveda J, et al. La vacunación antirarampionosa en Mexico por el metodo de aerosol. *Sal Pub Mex* 39:53–60, 1997.
667. Khanum S, Uddin N, Garelick H, et al. Comparison of Edmonston-Zagreb and Schwarz strains of measles vaccine given by aerosol or subcutaneous injection. *Lancet* 1:150–153, 1987.
668. Ekunwe EO. Immunization by inhalation of aerosolized measles vaccine. *Ann Trop Peds* 10:145–149, 1990.
669. Fernandez Bracho JG, Roldan Fernandez SG. Reacciones tempranas en escolares vacunados con antisarampionosa en aerosol. *Sal Pub Mex* 32:653–657, 1990.
670. Taylor-Robinson CH, Mallinson H. Risk of contact infection after intranasal rubella vaccination. *Lancet* 2:1128–1129, 1979.
671. Al-Nakib W, Best JM, Banatvala JE. Rubella-specific serum and nasopharyngeal immunoglobulin responses following naturally acquired and vaccine-induced infection. Prolonged persistence of virus-specific IgM. *Lancet* 1:182–185, 1975.
672. Ganguly R, Ogra PL, Regas S, Waldman RH. Rubella immunization of volunteers via the respiratory tract. *Infect Immun* 8:497–502, 1973.
673. Moffat MA, Gould JJ, Forbes FA, et al. Studies with rubella vaccine (RA 27-3) using the subcutaneous and intranasal routes. *Scott Med J* 17:140–142, 1972.
674. Puschak R, Young M, McKee TV, Plotkin SA. Intranasal vaccination with RA 27-3 attenuated rubella virus. *J Pediatr* 79:55–60, 1971.
675. Ingalls TH, Horne HW Jr. Immunisation of women with rubella (RA27-3) vaccine administered intranasally. *Lancet* 1:1830–832, 1971.
676. Ingalls TH, Plotkin SA, Philbrook FR, Thompson RF. Immunisation of schoolchildren with rubella (RA27-3) vaccine. Intranasal and subcutaneous administration. *Lancet* 1:99–101, 1970.
677. Saidi S, Naficy K. Subcutaneous and intranasal administration of RA 27-3 rubella vaccine. Alone and in conjunction with live attenuated measles vaccine. *Am J Dis Child* 118:209–212, 1969.
678. Kuck D, Lau T, Leuchs B, et al. Intranasal vaccination with recombinant adeno-associated virus type 5 against human papillomavirus type 16 L1. *J Virol* 80:2621–2630, 2006.
679. Zhang J, Wu X, Qin C, et al. A novel recombinant adeno-associated virus vaccine reduces behavioral impairment and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Neurobiol Dis* 14:365–379, 2003.

680. Xin KQ, Urabe M, Yang J, et al. A novel recombinant adeno-associated virus vaccine induces a long-term humoral immune response to human immunodeficiency virus. *Hum Gene Ther* 12:1047-1061, 2001.
681. Xing Z, Lichty BD. Use of recombinant virus-vectored tuberculosis vaccines for respiratory mucosal immunization. *Tuberculosis* 86:211-217, 2006.
682. Palin A, Chattopadhyay A, Park S, et al. An optimized vaccine vector based on recombinant vesicular stomatitis virus gives high-level, long-term protection against *Yersinia pestis* challenge. *Vaccine* Aug 22; 2006. [Epub ahead of print]
683. Jiang P, Liu Y, Yin X, et al. Elicitation of neutralizing antibodies by intranasal administration of recombinant vesicular stomatitis virus expressing human immunodeficiency virus type 1 gp120. *Biochem Biophys Res Commun* 339:526-532, 2006.
684. Egan MA, Chong SY, Megati S, et al. Priming with plasmid DNAs expressing interleukin-12 and simian immunodeficiency virus gag enhances the immunogenicity and efficacy of an experimental AIDS vaccine based on recombinant vesicular stomatitis virus. *AIDS Res Hum Retroviruses* 21:629-643, 2005.
685. Tan GS, McKenna PM, Koser ML, et al. Strong cellular and humoral anti-HIV Env immune responses induced by a heterologous rhabdoviral prime-boost approach. *Virology* 331:82-93, 2005.
686. Schlereth B, Buonocore L, Tietz A, et al. Successful mucosal immunization of cotton rats in the presence of measles virus-specific antibodies depends on degree of attenuation of vaccine vector and virus dose. *J Gen Virol* 84:2145-2151, 2003.
687. Roberts A, Buonocore L, Price R, et al. Attenuated vesicular stomatitis viruses as vaccine vectors. *J Virol* 73:3723-3732, 1999.
688. Haglund K, Leiner I, Kerksiek K, et al. High-level primary CD8(+) T-cell response to human immunodeficiency virus type 1 gag and env generated by vaccination with recombinant vesicular stomatitis viruses. *J Virol* 76:2730-2738, 2002.
689. Roberts A, Kretzschmar E, Perkins AS, et al. Vaccination with a recombinant vesicular stomatitis virus expressing an influenza virus hemagglutinin provides complete protection from influenza virus challenge. *J Virol* 72:4704-4711, 1998.
690. Li J, Faber M, Papaneri A, et al. A single immunization with a recombinant canine adenovirus expressing the rabies virus G protein confers protective immunity against rabies in mice. *Virology* Aug 26; 2006. [Epub ahead of print]
691. Santosuosso M, McCormick S, Zhang X, et al. Intranasal boosting with an adenovirus-vectored vaccine markedly enhances protection by parenteral *Mycobacterium bovis* BCG immunization against pulmonary tuberculosis. *Infect Immun* 74:4634-4643, 2006.
692. See RH, Zakhartchouk AN, Petric M, et al. Comparative evaluation of two severe acute respiratory syndrome (SARS) vaccine candidates in mice challenged with SARS coronavirus. *J Gen Virol* 87:641-650, 2006.
693. Santosuosso M, Zhang X, McCormick S, et al. Mechanisms of mucosal and parenteral tuberculosis vaccinations: adenoviral-based mucosal immunization preferentially elicits sustained accumulation of immune protective CD4 and CD8 T cells within the airway lumen. *J Immunol* 174:7986-7994, 2005.
694. Liu X, Yang T, Sun QM, Sun MS. Efficient intranasal immunization of newborn mice with recombinant adenovirus expressing rotavirus protein VP4 against oral rotavirus infection. *Acta Virol* 49:17-22, 2005.
695. Phillipotts RJ, O'Brien L, Appleton RE, et al. Intranasal immunisation with defective adenovirus serotype 5 expressing the Venezuelan equine encephalitis virus E2 glycoprotein protects against airborne challenge with virulent virus. *Vaccine* 23:1615-1623, 2005.
696. Shanley JD, Wu CA. Intranasal immunization with a replication-deficient adenovirus vector expressing glycoprotein H of murine cytomegalovirus induces mucosal and systemic immunity. *Vaccine* 23:996-1003, 2005.
697. Wang J, Thorson L, Stokes RW, et al. Single mucosal, but not parenteral, immunization with recombinant adenoviral-based vaccine provides potent protection from pulmonary tuberculosis. *J Immunol* 173:6357-6365, 2004.
698. Lemiale F, Kong WP, Akyurek LM, et al. Enhanced mucosal immunoglobulin A response of intranasal adenoviral vector human immunodeficiency virus vaccine and localization in the central nervous system. *J Virol* 77:10078-10087, 2003.
699. Shanley JD, Wu CA. Mucosal immunization with a replication-deficient adenovirus vector expressing murine cytomegalovirus glycoprotein B induces mucosal and systemic immunity. *Vaccine* 21:2632-2642, 2003.
700. Xiang Z, Ertl HC. Induction of mucosal immunity with a replication-defective adenoviral recombinant. *Vaccine* 17:2003-2008, 1999.
701. Gallichan WS, Rosenthal KL. Long-term immunity and protection against herpes simplex virus type 2 in the murine female genital tract after mucosal but not systemic immunization. *J Infect Dis* 177:1155-1161, 1998.
702. Baca-Estrada ME, Liang X, Babiuk LA, Yoo D. Induction of mucosal immunity in cotton rats to haemagglutinin-esterase glycoprotein of bovine coronavirus by recombinant adenovirus. *Immunology* 86:134-140, 1995.
703. Lubeck MD, Natuk RJ, Chengalvala M, et al. Immunogenicity of recombinant adenovirus-human immunodeficiency virus vaccines in chimpanzees following intranasal administration. *AIDS Res Hum Retroviruses* 10:1443-1449, 1994. Erratum in: *AIDS Res Hum Retroviruses* 11:189, 1995.
704. Hsu KH, Lubeck MD, Bhat BM, et al. Efficacy of adenovirus-vectored respiratory syncytial virus vaccines in a new ferret model. *Vaccine* 12:607-612, 1994.
705. Gallichan WS, Johnson DC, Graham FL, Rosenthal KL. Mucosal immunity and protection after intranasal immunization with recombinant adenovirus expressing herpes simplex virus glycoprotein B. *J Infect Dis* 168:622-629, 1993.
706. Morin JE, Lubeck MD, Barton JE, et al. Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. *Proc Natl Acad Sci* 84:4626-4630, 1987.
707. Bisht H, Roberts A, Vogel L, et al. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. *Proc Natl Acad Sci* 101:6641-6646, 2004.
708. Goonetilleke NP, McShane H, Hannan CM, et al. Enhanced immunogenicity and protective efficacy against *Mycobacterium tuberculosis* of bacille Calmette-Guérin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. *J Immunol* 171:1602-1609, 2003.
709. Durbin AP, Wyatt LS, Siew J, et al. The immunogenicity and efficacy of intranasally or parenterally administered replication-deficient vaccinia-parainfluenza virus type 3 recombinants in rhesus monkeys. *Vaccine* 16:1324-1330, 1998.
710. Gherardi MM, Pérez-Jiménez E, Nájera JL, et al. Induction of HIV immunity in the genital tract after intranasal delivery of a MVA vector: Enhanced immunogenicity after DNA prime-Modified Vaccinia Virus Ankara boost immunization schedule. *J Immunol* 172:6209-6220, 2004.
711. Loch C. Live bacterial vectors for intranasal delivery of protective antigens. *Pharm Sci Technol Today* 3:121-128, 2000.
- 711a. Pammit MA, Raulie EK, Lauriano CM, et al. Intranasal vaccination with a defined attenuated *Francisella novicida* strain induces gamma interferon-dependent antibody-mediated protection against tularemia. *Infect Immun* 74:2063-2071, 2006.
- 711b. Wu TH, Hutt JA, Garrison KA, et al. Intranasal vaccination induces protective immunity against intranasal infection with virulent *Francisella tularensis* biovar A. *Infect Immun* 73:2644-2654, 2005.
712. Mielcarek N, Alonso S, Loch C. Nasal vaccination using live bacterial vectors. *Advan Drug Del Rev* 51:55-69, 2001.
713. Garmony HS, Leary SE, Griffin KF, et al. The use of live attenuated bacteria as a delivery system for heterologous antigens. *J Drug Target* 11:471-479, 2003.
- 713a. Collins DM, de Lisle GW, Aldwell FE, Buddle BM. A new attenuated *Mycobacterium bovis* vaccine protects brushtail possums (*Trichosurus vulpecula*) against experimental tuberculosis infection. *Vaccine* 25(24):4659-4664, 2007.
714. Reveneau N, Geoffroy MC, Loch C, et al. Comparison of the immune responses induced by local immunizations with recombinant *Lactobacillus plantarum* producing tetanus toxin fragment C in different cellular locations. *Vaccine* (2):1769-1777, 2002.
715. Grangette C, Muller-Alouf H, Goudercourt D, et al. Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*. *Infect Immun* 69:1547-1553, 2001.
716. Medagliani D, Ciabattini A, Spinosa MR, et al. Immunization with recombinant *Streptococcus gordonii* expressing tetanus toxin fragment C confers protection from lethal challenge in mice. *Vaccine* 19:1931-1939, 2001.
717. Mercenier A, Muller-Alouf H, Grangette C. Lactic acid bacteria as live vaccines. *Curr Issues Mol Biol* 2:17-25, 2000.
718. Biet F, Kremer L, Wolowczuk I, Delacre M, Loch C. Immune response induced by recombinant *Mycobacterium bovis* BCG producing the cholera toxin B subunit. *Infect Immun* 71:2933-2937, 2003.
719. Steidler L, Robinson K, Chamberlain L, et al. Mucosal delivery of murine interleukin-2 (IL-2) and IL-6 by recombinant strains of *Lactococcus lactis* co-expressing antigen and cytokine. *Infect Immun* 66:3183-3189, 1998.
720. Tree JA, Williams A, Clark S, et al. Intranasal bacille Calmette-Guérin (BCG) vaccine dosage needs balancing between protection and lung pathology. *Clin Exp Immunol* 138:405-409, 2004.
721. Roberts M, Bacon A, Li J, Chatfield S. Prior immunity to homologous and heterologous *Salmonella* serotypes suppresses local and systemic anti-fragment C antibody responses and protection from tetanus toxin in mice immunized with *Salmonella* strains expressing fragment C. *Infect Immun* 67:3810-3815, 1999.
722. Chen L, Wang J, Zganiacz A, Xing Z. Single intranasal mucosal *Mycobacterium bovis* BCG vaccination confers improved protection compared to subcutaneous vaccination against pulmonary tuberculosis. *Infect Immun* 72:238-246, 2004.
723. Lyadova IV, Vordermeier HM, Eruslanov EB, et al. Intranasal BCG vaccination protects BALB/c mice against virulent *Mycobacterium bovis* and accelerates production of IFN-gamma in their lungs. *Clin Exp Immunol* 126:274-279, 2001.
724. Falero-Diaz G, Challacombe S, Banerjee D, et al. Intranasal vaccination of mice against infection with *Mycobacterium tuberculosis*. *Vaccine* 18:3223-3229, 2000.
725. Nuernberger EL, Yoshimatsu T, Tyagi S, et al. Paucibacillary tuberculosis in mice after prior aerosol immunization with *Mycobacterium bovis* BCG. *Infect Immun* 72:1065-1071, 2004.
726. Corner LA, Buddle BM, Pfeiffer DU, Morris RS. Aerosol vaccination of the brushtail possum (*Trichosurus vulpecula*) with bacilli Calmette-Guérin: the duration of protection. *Vet Microbiol* 81:181-191, 2001.
727. Copenhaver RH, Sepulveda E, Armitige LY. A mutant of *Mycobacterium tuberculosis* H37Rv that lacks expression of antigen 85A is attenuated in mice but retains vaccinogenic potential. *Infect Immun* 72:7084-7095, 2004.
728. Lagranderie M, Winter N, Balazuc AM, et al. A cocktail of *Mycobacterium bovis* BCG recombinants expressing the SIV Nef, Env, and Gag antigens induces antibody and cytotoxic responses in mice vaccinated by different mucosal routes. *AIDS Res Hum Retroviruses* 14:1625-33, 1998.
729. Edelman R, Palmer K, Russ KG, et al. Safety and immunogenicity of recombinant Bacille Calmette-Guérin (rBCG) expressing *Borrelia burgdorferi* outer surface protein A (OspA) lipoprotein in

- adult volunteers: a candidate Lyme disease vaccine. *Vaccine* 17:904-914, 1999.
730. Langermann S, Palaszynski S, Sadziene A, et al. Systemic and mucosal immunity induced by BCG vector expressing outer-surface protein A of *Borrelia burgdorferi*. *Nature* 372:552-555, 1994.
731. Langermann S, Palaszynski SR, Burlein JE, et al. Protective humoral response against pneumococcal infection in mice elicited by recombinant bacille Calmette-Guerin vaccines expressing pneumococcal surface protein A. *J Exp Med* 180:2277-2286, 1994.
732. Mielcarek N, Debrue AS, Raze D, et al. Live attenuated *B. pertussis* as a single-dose nasal vaccine against whooping cough. *PLoS Pathog* 2:e65, 2006.
733. Mielcarek N, Debrue AS, Raze D, et al. Attenuated *Bordetella pertussis*: new live vaccines for intranasal immunisation. *Vaccine* 24(Suppl 2):S2-54-55, 2006.
734. Loch C, Antoine R, Raze D, et al. *Bordetella pertussis* from functional genomics to intranasal vaccination. *Int J Med Microbiol* 293:583-588, 2004.
735. Mielcarek N, Nordstrom I, Menozzi FD, et al. Genital antibody responses in mice after intranasal infection with an attenuated candidate vector strain of *Bordetella pertussis*. *Infect Immun* 68:485-491, 2000.
736. Reveneau N, Alonso S, Jacob-Dubuisson F, et al. Tetanus toxin fragment C-specific priming by intranasal infection with recombinant *Bordetella pertussis*. *Vaccine* 20:926-933, 2002.
737. Alonso S, Willery E, Renaud-Mongenie G, Loch C. Production of non-typeable *Haemophilus influenzae* HtraA by recombinant *Bordetella pertussis* with the use of filamentous hemagglutinin as a carrier. *Infect Immun* 73:4295-4301, 2005.
738. Coppens I, Alonso S, Antoine R, et al. Production of *Neisseria meningitidis* transferrin-binding protein B by recombinant *Bordetella pertussis*. *Infect Immun* 69:5440-5446, 2001.
739. Renaud-Mongenie G, Mielcarek N, Cornette J, et al. Induction of mucosal immune responses against a heterologous antigen fused to filamentous hemagglutinin after intranasal immunization with recombinant *Bordetella pertussis*. *Proc Natl Acad Sci* 93:7944-7949, 1996.
740. Pasetti MF, Salerno-Goncalves R, Szein MB. *Salmonella enterica* serovar Typhi live vector vaccines delivered intranasally elicit regional and systemic specific CD8+ major histocompatibility class I-restricted cytotoxic T lymphocytes. *Infect Immun* 70:4009-4018, 2002.
741. Parida SK, Huygen K, Ryffel B, Chakraborty T. Novel bacterial delivery system with attenuated *Salmonella typhimurium* carrying plasmid encoding Mtb antigen 85A for mucosal immunization: Establishment of proof of principle in TB mouse model. *Ann N Y Acad Sci* 1056:366-378, 2005.
742. Vindurampulle CJ, Cuberos LF, Barry EM, et al. Recombinant *Salmonella enterica* serovar Typhi in a prime-boost strategy. *Vaccine* 22:3744-3750, 2004.
743. Capozzo AV, Cuberos L, Levine MM, Pasetti MF. Mucosally delivered *Salmonella* live vector vaccines elicit potent immune responses against a foreign antigen in neonatal mice born to naive and immune mothers. *Infect Immun* 72:4637-4646, 2004.
744. Morton M, Garmory HS, Perkins SD, et al. A *Salmonella enterica* serovar typhi vaccine expressing Yersinia pestis F1 antigen on its surface provides protection against plague in mice. *Vaccine* 22:2524-2532, 2004.
745. Coste A, Cohen J, Reinhardt M, et al. Nasal immunisation with *Salmonella typhimurium* producing rotavirus VP2 and VP6 antigens stimulates specific antibody response in serum and milk but fails to protect offspring. *Vaccine* 19:4167-4174, 2001.
746. Nardelli-Haeffliger D, Benyacoub J, Lemoine R, et al. Nasal vaccination with attenuated *Salmonella typhimurium* strains expressing the Hepatitis b nucleocapsid: dose response analysis. *Vaccine* 19:2854-2861, 2001.
747. Ward SJ, Douce G, Figueiredo D, et al. Immunogenicity of a *Salmonella typhimurium* aroA aroD vaccine expressing a nontoxic domain of *Clostridium difficile* toxin A. *Infect Immun* 67:2145-2152, 1999.
748. Hopkins S, Kraehenbuhl JP, Schodel F, et al. A recombinant *Salmonella typhimurium* vaccine induces local immunity by four different routes of immunization. *Infect Immun* 63:3279-3286, 1995.
749. Sheoran AS, Timoney JF, Ting SA, et al. Intranasal immunogenicity of a Delta cya Delta crp-pabA mutant of *Salmonella enterica* serotype Typhimurium for the horse. *Vaccine* 19:3787-3795, 2001.
750. Anderson RJ, Pasetti MF, Szein MB, et al. DeltagaaBA attenuated *Shigella flexneri* 2a strain CVD 1204 as a *Shigella* vaccine and as a live mucosal delivery system for fragment C of tetanus toxin. *Vaccine* 18:2193-2202, 2000.
751. Noriega FR, Losonsky G, Wang JY, et al. Further characterization of delta aroA delta virG *Shigella flexneri* 2a strain CVD 1203 as a mucosal *Shigella* vaccine and as a live-vector vaccine for delivering antigens of enterotoxigenic *Escherichia coli*. *Infect Immun* 64:23-27, 1996.
752. Herrmann JE, Robinson HA. DNA vaccines for mucosal immunity to infectious diseases. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 1073-1084.
753. Fynan EF, Webster RG, Fuller DH, et al. DNA vaccines: protective immunizations by parenteral, mucosal and gene gun inoculations. *Proc Natl Acad Sci* 90:11478-11482, 1993.
754. Zavala-Spinetti L, Breslin MB, Correa H, Begue RE. Development and evaluation of a DNA vaccine based on *Helicobacter pylori* urease B: failure to prevent experimental infection in the mouse model. *Helicobacter* 11:517-522, 2006.
755. Wang J, Zhao CA, Wang K, et al. Enhanced immunization after intranasal coadministration of *Escherichia coli* heat-labile enterotoxin B subunit and human papillomavirus 16-L1 DNA vaccine. *Chinese Med J* 119:408-411, 2006.
756. Kent SJ, Dale CJ, Ranasinghe C. Mucosally-administered human-simian immunodeficiency virus DNA and fowlpoxvirus-based recombinant vaccines reduce acute phase viral replication in macaques following vaginal challenge with CCR5-tropic SHIVSF162P3. *Vaccine* 23:5009-5021, 2005.
757. Hatzifoti C, Roussel Y, Harris AG, et al. Mucosal immunization with a urease B DNA vaccine induces innate and cellular immune responses against *Helicobacter pylori*. *Helicobacter* 11:113-222, 2006.
758. Devito C, Zuber B, Schroder U, et al. Intranasal HIV-1-gp160-DNA/gp41 peptide prime-boost immunization regimen in mice results in long-term HIV-1 neutralizing humoral mucosal and systemic immunity. *J Immunol* 173:7078-7089, 2004.
759. Xu W, Shen Y, Jiang Z, et al. Intranasal delivery of chitosan-DNA vaccine generates mucosal S1gA and anti-CVB3 protection. *Vaccine* 22:3603-3612, 2004.
760. Garcia-Diaz A, Lopez-Andujar P, Rodriguez Diaz J, et al. Nasal immunization of mice with a rotavirus DNA vaccine that induces protective intestinal IgA antibodies. *Vaccine* 23:489-498, 2004.
761. Bivas-Benita M, Ottenhoff TH, Junginger HE, Borchard G. Pulmonary DNA vaccination: concepts, possibilities and perspectives. *J Control Release* 107:1-29, 2005.
762. Locher CP, Witt SA, Ashlock BM, et al. Human immunodeficiency virus type 2 DNA vaccine provides partial protection from acute baboon infection. *Vaccine* 22:2261-2272, 2004.
763. Bivas-Benita M, van Meijgaarden KE, Franken KL, et al. Pulmonary delivery of chitosan-DNA nanoparticles enhances the immunogenicity of a DNA vaccine encoding HLA-A*0201-restricted T-cell epitopes of *Mycobacterium tuberculosis*. *Vaccine* 22:1609-1615, 2004.
764. Xin KQ, Hamajima K, Sasaki S, et al. Intranasal administration of human immunodeficiency virus type-1 (HIV-1) DNA vaccine with interleukin-2 expression plasmid enhances cell-mediated immunity against HIV-1. *Immunology* 94:438-444, 1998.
765. Okada E, Sasaki S, Ishii N, et al. Intranasal immunization of a DNA vaccine with IL-12- and granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmids in liposomes induces strong mucosal and cell-mediated immune responses against HIV-1 antigens. *J Immunol* 159:3638-3647, 1997.
766. Tadokoro K, Koizumi Y, Miyagi Y, et al. Rapid and wide-reaching delivery of HIV-1 env DNA vaccine by intranasal administration. *Vir Immunol* 14:159-167, 2001.
767. Okuda K, Ihata A, Watabe S, et al. Protective immunity against influenza A virus induced by immunization with DNA plasmid containing influenza M gene. *Vaccine* 19:3681-3691, 2001.
768. Svanholm C, Bandholtz L, Castanos-Velez E, et al. Protective DNA immunization against Chlamydia pneumoniae. *Scan J Immunol* 51:345-353, 2000.
769. Wang X, Hone DM, Haddad A, et al. M cell DNA vaccination for CTL immunity to HIV. *J Immunol* 171:4717-4725, 2003.
770. D'Souza S, Rosseels V, Denis O, et al. Improved tuberculosis DNA vaccines by formulation in cationic lipids. *Inf Imm* 70:3681-3688, 2002.
771. McCluskie MJ, Brazzot Millan CL, Gramzinski RA, et al. Route and method of delivery of DNA vaccine influence immune responses in mice and non-human primates. *Mol Med* 5:287-300, 1999.
772. Kuklin N, Daheshia M, Karem K, et al. Induction of mucosal immunity against herpes simplex virus by plasmid DNA immunization. *J Virol* 71:3138-3145, 1997.
773. Vecino WH, Morin PM, Agha R, et al. Mucosal DNA vaccination with highly attenuated *Shigella* is superior to attenuated *Salmonella* and comparable to intramuscular DNA vaccination for t cells against HIV. *Immunol Lett* 82:197-204, 2002.
774. Fennelly GJ, Khan SA, Abadi MA, et al. Mucosal DNA vaccine immunization against measles with a highly attenuated *Shigella flexneri* vector. *J Immunol* 162:1603-1610, 1999.
775. Shata MT, Hone DM. Vaccination with a *Shigella* DNA vaccine vector induces antigen-specific CD8(+) T cells and antiviral protective immunity. *J Virol* 75:9665-9670, 2001.
776. Xu F, Hong M, Ulmer JB. Immunogenicity of an HIV-1 gag DNA vaccine carried by attenuated *Shigella*. *Vaccine* 21:644-648, 2003.
777. Pasetti MF, Barry EM, Losonsky G, et al. Attenuated *Salmonella enterica* serovar Typhi and *Shigella flexneri* 2a strains mucosally deliver DNA vaccines encoding measles virus hemagglutinin, inducing specific immune responses and protection in cotton rats. *J Virol* 77:5209-5217, 2003.
778. Hamajima K, Kojima Y, Matsui K, et al. Chitin Micro-Particles (CMP): a useful adjuvant for inducing viral specific immunity when delivered intranasally with an HIV-DNA vaccine. *Vir Immunol* 16:541-547, 2003.
779. Singh M, Vajdy M, Gardner J, Briones M, O'Hagan D. Mucosal immunization with HIV-1 gag DNA on cationic microparticles prolongs gene expression and enhances local and systemic immunity. *Vaccine* 20:594-602, 2001.
780. Cusi MG, Zurbriggen R, Valassina M, et al. Intranasal immunization with mumps virus DNA vaccine delivered by influenza virosomes elicits mucosal and systemic immunity. *Virology* 277:111-118, 2000.
781. Wong JP, Zabielski MA, Schmaltz FL, et al. DNA vaccination against respiratory influenza virus infection. *Vaccine* 19:2461-2467, 2001.
782. Hall MA, Stroop SD, Hu MC, et al. Intranasal immunization with multivalent group A streptococcal vaccines protects mice against intranasal challenge infections. *Infect Immun* 72:2507-2512, 2004.
783. Childers NK, Tong G, Mitchell S, et al. A controlled clinical study of the effect of nasal immunization with a *Streptococcus mutans* antigen alone or incorporated into liposomes on induction of immune responses. *Infect Immun* 67:618-623, 1999.
784. de Jonge MI, Hamstra HJ, Jiskoot W, et al. Intranasal immunisation of mice with liposomes containing recombinant meningococcal OpaB and OpaJ proteins. *Vaccine* 22:4021-4028, 2004.

785. VanCott TC, Kaminski RW, Mascola JR, et al. HIV-1 neutralizing antibodies in the genital and respiratory tracts of mice intranasally immunized with oligomeric gp160. *J Immunol* 160:2000–2012, 1998.
786. Yao Q. Enhancement of mucosal immune responses by chimeric influenza HA/SHIV virus-like particles. *Res Initi Treat Acton* 8:20–21, 2003.
787. Galarza JM, Latham T, Cupo A. Virus-like particle vaccine conferred complete protection against a lethal influenza virus challenge. *Viral Immunol* 18:365–372, 2005.
788. Gluck R, Burri KG, Metcalfe I. Adjuvant and antigen delivery properties of virosomes. *Curr Drug Deliv* 2:395–400, 2005.
789. Huckriede A, Bungener L, Stegmann T, et al. The virosome concept for influenza vaccines. *Vaccine* 23(Suppl 1):S26–S38, 2005.
790. Cusi MG, Del Vecchio MT, Terrosi C, et al. Immune-reconstituted influenza virosome containing CD40L gene enhances the immunological and protective activity of a carcinoembryonic antigen anticancer vaccine. *J Immunol* 174:7210–7216, 2005.
791. Hu KF, Ekstrom J, Merza M, et al. Induction of antibody responses in the common mucosal immune system by respiratory syncytial virus immunostimulating complexes. *Med Microbiol Immunol* 197:191–198, 1999.
792. Abusugra I, Morein B. ISCOM is an efficient mucosal delivery system for *Mycoplasma mycoides* subsp. *Mycoides* (MmmSC) antigens including high mucosal and systemic antibody responses. *FEMS Immunol Med Microbiol* 23:5–12, 1999.
793. Andersen CS, Dietrich J, Agger EM, et al. The combined CTA1-DD/ISCOMs vector is an effective intranasal adjuvant for boosting prior BCG immunity to *Mycobacterium tuberculosis*. *Infect Immun* Oct 30; 2006[Epub ahead of print]
794. Aguila A, Donachie AM, Peyre M, et al. Induction of protective and mucosal immunity against diphtheria by a immune stimulating complex (ISCOMS) based vaccine. *Vaccine* 24:5201–5210, 2006.
795. Hagglund S, Hu KF, Larsen LE, et al. Bovine respiratory syncytial virus ISCOMs-protection in the presence of maternal antibodies. *Vaccine* 23:646–655, 2004.
796. Davis SS, Illum L. Absorption enhancers for nasal drug delivery. *Clin Pharm* 42:1107–1128, 2003.
797. Kopig-Hoggard M, Sanchez A, Alonso MJ. Nanoparticles as carriers for nasal vaccine delivery. *Exp Rev Vacc* 4:285–196, 2005.
798. Vajdy M, O'Hagan DT. Microparticles for intranasal immunization. *Ad Drug Del Rev* 51:127–141, 2001.
799. Van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. Chitosan for mucosal vaccination. *Ad Drug Del Rev* 52:139–144, 2001.
800. Jagannathan KS, Vyas SP. Strong systemic and mucosal immune responses to surface-modified PLGA microspheres containing recombinant hepatitis b antigen administered intranasally. *Vaccine* 24:4201–4211, 2006.
801. Byrd W, Cassels FJ. Intranasal immunization of BALB/c mice with enterotoxigenic *Escherichia coli* colonization factor CS6 encapsulated in biodegradable poly(DL-lactide-co-glycolide) microspheres. *Vaccine* 24:1359–1366, 2006.
802. Kang ML, Kang SG, Jiang HL, et al. In vivo induction of mucosal immune responses by intranasal administration of chitosan microspheres containing *Bordetella bronchiseptica* DNT. *Eur J Pharm Biopharm* 63:215–220, 2006.
803. Carcaboso AM, Hernandez RM, Igartua M, et al. Potent, long lasting systemic antibody levels and mixed Th1/Th2 immune response after nasal immunization with malaria antigen loaded PLGA microparticles. *Vaccine* 22:1423–1432, 2004.
804. Hasegawa H, Ichinohe T, Strong P, et al. Protection against influenza virus infection by intranasal administration of hemagglutinin vaccine with chitin microparticles as an adjuvant. *J Med Virol* 75:130–136, 2005.
805. LiCalsi C, Maniaci MJ, Christensen T, et al. A powder formulation of measles vaccine for aerosol delivery. *Vaccine* 19:2629–2636, 2001.
806. Smith JD, Bot S, Dellamary L, et al. Evaluation of novel aerosol formulations designed for mucosal vaccination against influenza virus. *Vaccine* 21:2805–2812, 2003.
807. de Swart R, LiCalsi C, Quirk AV, et al. Measles vaccination of macaques by dry powder inhalation. *Vaccine* 25(7):1183–1190, 2007.
- 807a. Jiang G, Joshi SB, Peek LJ, et al. Anthrax vaccine powder formulations for nasal mucosal delivery. *J Pharm Sci* 95(1):80–96, 2006.
- 807b. Huang J, Mikszta JA, Ferriter MS, et al. Intranasal administration of dry powder anthrax vaccine provides protection against lethal aerosol spore challenge. *Human Vaccines* 3(3):90–93, 2007.
808. AKTIV-DRY, LLC, Boulder, CO 80301, USA. Online. Available at: www.aktiv-dry.com.
809. Sievers RE, Quinn BP, Cape SP, et al. Near-critical Fluid Micronization of Stabilized Vaccines, Antibiotics, and Anti-virals, 8th Conference on Supercritical Fluids and Their Applications, Ischia, Italy, May 28–31, 2006.
810. Grand Challenges in Global Health. Needle-Free Delivery: Finding New Ways to Give Immunizations. Online. Available at: www.gcgh.org/subcontent.aspx?SecID=419. Accessed December 7, 2006.
811. Hodgins DC, Yuan L, Parreno V, Corbeil LB, Saif LJ. Mucosal veterinary vaccines: Comparative vaccinology. In: Mestecky J, Bienenstock J, Lamm ME, et al, (eds). *Mucosal Immunology*. Burlington, MA: Elsevier; 2005, 1085–1107.
812. Flick-Smith HC, Eyles JE, Hebdon R, et al. Mucosal or parenteral administration of microsphere-associated *Bacillus anthracis* protective antigen protects against anthrax infection in mice. *Infect Immun* 70:2022–2028, 2002.
- 812a. Bukreyev A, Rollin PE, Tate MK, et al. Successful topical respiratory tract immunization of primates against Ebola virus. *J Virol* 81(12):6379–6388, 2007.
813. Ulrich LR, Amemiya K, Waag MD, et al. Aerogenic vaccination with a *Burkholderia mallei* auxotroph protects against aerosol-initiated glanders in mice. *Vaccine* 23:1986–1992, 2005.
814. Lowell HG, Kaminski WR, Grate S, et al. Intranasal and intramuscular proteosome-staphylococcal enterotoxin B (SEB) toxoid vaccines: immunogenicity and efficacy against lethal SEB intoxication in mice. *Inf Imm* 64(5):1706–1713, 1996.
815. Eigelsbach HT, Tulis J, Overholt EL, et al. Aerogenic immunization of the monkey and guinea pig with live tularemia vaccine. *Proc Soc Exp Biol Med* 108:732–734, 1961.
- 815a. Baron SD, Singh R, Metzger DW. Inactivated *Francisella tularensis* live vaccine strain protects against respiratory tularemia by intranasal vaccination in an immunoglobulin A-dependent fashion. *Infect Immun* 75(5):2052–2162, 2007.
816. Hornick RB, Eigelsbach HT. Aerogenic immunization of man with live tularemia vaccine. *Bact Rev* 30:532–538, 1966.
817. Jones T, Adamovicz JJ, Cyr SL, et al. Intranasal protollin/F1-V vaccine elicits respiratory and serum antibody responses and protects mice against lethal aerosolized plague infection. *Vaccine* 24:1625–1632, 2006.
- 817a. Luo F, Feng Y, Liu M, et al. Type IVB pilus operon promoter controlling expression of the Severe Acute Respiratory Syndrome-associated coronavirus nucleocapsid gene in *Salmonella enterica* serovar Typhi elicits full immune response by intranasal vaccination. *Clin Vaccine Immunol* 14(8):990–997, 2007.
- 817b. Suguitan AL Jr, McAuliffe J, Mills KL, et al. Live, attenuated influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. *PLoS Med* 3(9):e360, 2006.
818. Creare Inc. Hanover, NH 03755, USA. Online. Available at: www.creare.com.