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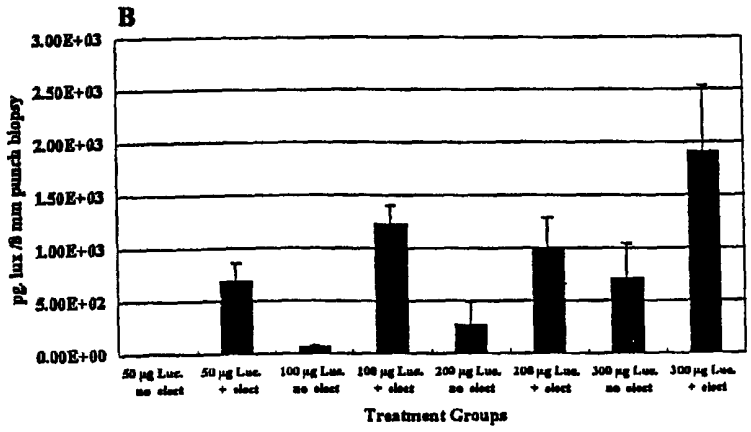
(54) Title: METHOD FOR GENE TRANSFECTION AND VACCINATION THROUGH SKIN USING ELECTROPERMEABILIZATION



(57) Abstract: Cells in the skin or mucosal tissue of an individual can be transfected with a polynucleotide or gene by injecting the polynucleotide into the skin or mucosal tissue and applying a pulsating electrical field in the vicinity of the injection. This approach is suitable for vaccinating an individual to the expressed polypeptide, thereby generating antibodies or to engendering cell-mediated immunity. For example, antibodies generated against the expression product of a gene cloned from genomic or cDNA libraries can be used to characterize the product and, hence, illuminate the function of the gene. The approach also can be employed, in an animal or human, for treating a disease condition or for modifying a skin condition that affects the appearance of the skin. Kits are included for carrying out the methods of the invention.

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METHOD FOR GENE TRANSFECTION AND VACCINATION THROUGH SKIN USING ELECTROPERMEABILIZATION

FIELD OF THE INVENTION

The invention relates generally to delivery of a polynucleotide to cells in epithelial and adipose tissue, to the generation of an immune response against a product of the delivered polynucleotide upon expression thereof by the cells, and to the generation of a therapeutic benefit.

BACKGROUND OF THE INVENTION

Genetic therapy involves the clinical administration of recombinant viruses or nucleic acid formulations containing genes that encode therapeutic proteins. The goal is to deliver and express the therapeutic gene within appropriate cells. Clinical benefit may result directly such as from the biological activity of the expressed gene product or indirectly such as when the expressed gene product acts elicits antibodies that have therapeutic benefit.

Methods to transfect and express nucleic acid into cells *in vitro* are well known in the art and are generally satisfactory for most purposes. Popular among these methods is electropermeabilization, also known as "electroporation," which achieves nucleic acid uptake through pores induced in the cell membrane by application of an electric field. The effective transfection of cells *in vivo*, however, faces additional barriers making it difficult to achieve clinically useful outcomes by genetic therapy. For example, the extracellular milieu surrounding cells is not a homogeneous fluid. Instead, cells within tissue exist in a complex three-dimensional space that may be viewed as a matrix of capacitors, conductors, and resistors. Furthermore, cells in tissues are bound in a

fixed orientation within this electrochemical space. Therefore, the basic principles learned from electropermeabilization *in vitro* are not directly *in vivo.*

Introduction of DNA vaccines by injection into muscle has been previously shown to elicit an immune response to protein encoded by the vector. However, because muscle cells do not appear to be effective at antigen presentation, such approach is not believed to provide a strong immune response. Also, attempts to generate an immune response using iontophoresis (continuous electrical current) in conjunction with topical skin administration of vector have been reported, but with limited success.

Thus, it would be useful if there were more effective methods to deliver and express genes administered *in vivo*, for direct therapeutic benefit and for the goal of eliciting antibodies to the expressed gene product. The present invention satisfies these advantages and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method for transfecting cells in the skin or mucosal tissue of an individual with a polynucleotide comprising injecting the polynucleotide below the stratum corneum layer of the skin of the individual and applying a pulsating electrical field to the skin in the vicinity of the injection.

The present invention also provides a method of vaccinating an individual, comprising: injecting an expression vector into the skin or mucosal tissue of the individual, the expression vector having a gene encoding a polypeptide to which an immune response is desired, the expression vector including expression control elements operably linked to the gene for expressing the gene product in the individual; and

applying a pulsating electrical field to the skin in the vicinity of the injection.

The present invention further provides a method of obtaining antibodies to a vector encoded gene product, comprising: injecting an expression vector into the skin or mucosal tissue of the individual, the expression vector having a gene encoding a polypeptide to which an immune response is desired, the expression vector including expression control elements operably linked to the gene for expressing the gene product in the individual; applying a pulsating electrical field to the skin in the vicinity of the injection; and obtaining the antibodies from the individual. In a further embodiment, the gene is from a genomic or cDNA library. In another embodiment, the antibodies are used to identify a characteristic of the gene product.

The present invention also provides a method of treating a human or animal disease condition or otherwise providing a desired medical or cosmetic benefit, comprising injecting an effective amount of an expression vector encoding a polypeptide associated with the disease condition in the skin or mucosal tissue of the individual and applying an effective pulsating electrical field to the skin in the vicinity of the injection so as to transfect cells of the dermis resulting in the generation of antibodies to the polypeptide which ameliorates or abrogates the disease condition.

In further embodiment of the above described methods, the pulsating electrical field applied to the skin comprises between 4 to 20 pulses of 1,000 to 2,000 V/cm for a period of 10 to 100 microseconds, or comprises six pulses of 1,750 V/cm for a period of 100 microseconds.

The present invention also provides a method of modifying a skin condition which affects the appearance of the skin, comprising injecting an expression vector below the stratum corneum layer of the skin, the

expression vector having a gene encoding a polypeptide that, when expressed, modifies the skin condition; and applying a pulsating electric field to the skin in the vicinity of the injection.

The present invention further provides kits for transfecting cells of the skin or mucosal tissue of an individual. The kit includes a set of electrodes suitable for administering an electric field to the skin or mucosal tissue of the individual, a power supply pulse generator for connecting electrical power to the electrodes and a polynucleotide or vector to be transfected. In a further embodiment, the kits include a device for injecting the polynucleotide into the skin or mucosal tissue of the individual.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the efficiency of intradermal injection of PND2LUX plasmid +/- electropermeabilization in mouse skin.

Figure 2 shows the efficiency of intradermal injection of PND2LUX plasmid +/- electropermeabilization in pig skin.

Figure 3 compares the efficiency of transfection using different electrode designs in the Yorkshire pig model.

Figure 4 shows IgG ELISA average titers of reactive mice for Hbs DNA plasmid +/- electropermeabilization.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is based on the discovery that injection of a polynucleotide into the skin or mucosal tissue, followed by electropermeabilization with a pulsating electrical field, applied to the site of the injection, results in high-level and pervasive uptake of the polynucleotide and the expression of the gene encoded product in cells of the skin or mucous tissue. Following such treatment, the skin is

characterized in having transfected cells in the dermis, the subdermis (*e.g.*, hypodermis) including adipose tissue, as well as the epidermis. In contrast, injection without electropermeabilization results mainly in expression of the gene product in the cells of the epidermis, primarily in the stratum corneum. By the approach, moreover, the inventors discovered that one can engender an antibody response to the expressed protein or polypeptide, and that such response exceeds that seen in the absence of electropermeabilization.

In accordance with the present invention, therefore, dermal cells or adipocytes of an individual can be transfected with a polynucleotide by injecting the polynucleotide below the stratum corneum layer and applying, in the vicinity of the injection, a pulsating electrical field to the skin. Further, the present invention comprehends the generation of an immune response to a polypeptide, by injecting an expression vector, containing the segment that codes for the polypeptide, below the stratum corneum of an individual and then applying a pulsating electrical field to the skin, in the vicinity of the injection. The expression vector thus employed would include one or more expression control elements, operably linked to the coding segment, to effect expression of the polypeptide in cells transfected with the vector.

These same methods also can be used to deliver a polynucleotide or vector into mucosal tissue or engender and antibody response to the encoded polypeptide by injecting the polynucleotide or vector into the mucosal layer and applying an electric field, as described above for the skin. Local dendritic cells in the mucosa become transfected and express the encoded polypeptide, generating a systemic and/or mucosal immune response by the host.

"Mucosal tissue" is used here to denote tissues of the gastrointestinal tract, respiratory tract or urogenital tract. Mucosal tissue herein includes

the epithelial layer, and submucosa including cells resident therein or traveling therethrough. Mucosal tissue also includes mucosa associated lymphoid tissue or MALT. The muscle layer, however, is not intended to be included within the meaning of mucosal tissue as this term is used herein.

Electropermeabilization can be performed by attaching a set of electrodes near to the site of the injection and preferably directly over the injection site. Conventional non-penetrating (*e.g.*, caliper) and penetrating electrode designs are suitable for applying an electrical field to the skin, in accordance with the present invention.

Caliper electrodes generally consist of two flat electrode plates (0.6 x 1.0 cm plates) preferably attached to a micrometer for measuring tissue thickness (preferably between about 0.25 to 0.6 cm) for the applied electrical field. For a flat plate electrode configuration, two copper plates measuring approximately 3 mm by 15 mm are attached to the jaws of a non-conducting digital caliper, and these copper plates can be attached to the pulse generating device. The skin then is "pinched" between the electrodes, and the pulsed electrical field administered as described. It will be appreciated by one skilled in the art that many different caliper electrodes designs are possible and can be used in the methods of the present invention.

Penetrating electrodes have an arrangement of pins, such as acupuncture needles, typically comprising fourteen pins in two rows of seven. These are placed into the tissue at a fixed distance and depth, which usually is about 2.5 mm. (The fixed distance for penetrating electrodes may vary, and other parameters may be adjusted to maintain the same field strength.) Spacing between pins can be from 1 cm to as low as 0.5 mm. The design of a preferred embodiment of a penetrating electrode comprises two linear arrays of five pins (preferably, silver

accupuncture needles) spaced approximately 3 mm apart, with the two arrays separated by a distance of 0.54 cm. While the penetrating caliper design can be varied considerably, the same effective electrical field may be applied by adjusting other parameters, such as voltage, pulse duration, and pulse number.

For electropermeabilization of skin, the preferred treatment parameters include electrical field strengths in the range of 1000 to 2000 V/cm administered in trains of pulses, ranging from 4 to 20 pulses, ranging in duration of from 10 microseconds to 20 milliseconds, at a rate of from one to five Hertz. In a preferred embodiment, treatment conditions include the administration of six 100 microsecond pulses of 1750V/cm field strength, administered at pulse interval of 0.125 seconds. Pulse is preferably unipolar.

The polynucleotide or vector to be transfected may be injected into the skin via traditional means such as using a syringe and needle, or by a needle-free or needle-less injection device. Such latter devices are well known and, generally, involve pressure-assisted delivery through a tiny orifice held against the skin. For gas- powered, disposable, needle-less hypodermic jet injectors, see U.S. patents No. 4,596,556 to Morrow et al., No. 4,913,699 to Parsons, No. 5,730,723 to Castellano et al. Needle-free, gas powered injectors also are commercially available; for instance, see the BIOJECT® device of Bioject Medical Technologies, Inc. (Portland, Oregon). Another needle-free device is a biolistic delivery device that uses pressurized gas to deliver small particles (*e.g.*, gold particles) to targeted regions of the skin, as a function of the gas pressure. An example of a biolistic delivery device is the PDS-1000 "gene gun" of Dupont (Wilmington, Delaware).

In accordance with the present invention, the polynucleotide or vector injected into the skin is optimally injected below the layer of the skin

known as the "stratum corneum." The injection preferably is into the dermis of the skin, but also may be in the subdermal layer (e.g., hypodermis) or in the portion of the epidermal layer below the stratum corneum. The "dermis" as used herein includes all cells resident in the dermis as well as cells travelling therethrough such as lymphocytes, monocytes, and the like.

The polynucleotide or vector to be injected generally will be suspended in a suitable liquid solution prior to injection, while, in biolistic delivery, the polynucleotide is absorbed to the surface of particles of gold or other suitable material. Techniques for affixing polynucleotides to particles, for purposes of biolistic injection, are well known.

The term "polynucleotide" is used here to denote polymers of deoxyribonucleotides or ribonucleotides, in either single- or double-stranded form. Unless otherwise indicated, the term polynucleotide is used interchangeably with gene, cDNA, mRNA encoded by a gene, and the like.

The phrases "gene encoding" or "polynucleotide encoding," used here in reference to an expression vector, means that the expression vector contains a sequence of the codons that correspond, via the genetic code, to the amino acid sequence of a particular polypeptide. Thus, a "gene" is a polypeptide-encoding nucleotide sequence, which may be a genomic segment or a cDNA molecule. The term "gene" encompasses a full encoding sequence, with and without introns, as well as fragments of the such sequences. Thus, the polypeptide encoded by a gene may be only a few amino acids in length, such as a peptide, or may be a large polypeptide or "protein." A "polypeptide" can have posttranslational modifications involving, for example, the addition of a carbohydrate, a phosphate, a lipid, and the like.

The immune response achieved with the present invention can be directed to one or more epitopes characterizing an expression product, or to one or more epitopes formed by the postranslationally modification of that product, or to one or more epitopes formed as a combination the polypeptide with a particular posttranslational modification.

An "expression vector" can be a recombinant expression cassette, which has a nucleotide sequence that can be transcribed into RNA in a cell. The cell can further translate the mRNA into protein. The expression vector can be a plasmid, virus, or nucleic acid fragment. The expression vector also may be a recombinant transposon, such as the "sleeping beauty" transposon of fish (Izsvak et al., *J. Mol. Biol.* 302:93-102 (2000)) or the Tc1/mariner transposon from *C. elegans* (Li et al., *Somat. Cell Mol. Genet.* 24:363-369 (1998)).

Typically, the recombinant expression cassette portion of an expression vector includes the encoding nucleotide sequence to be transcribed, operably linked to expression control elements such as a promoter and possibly other regulatory sequence functionally linked in *cis*. Promoters may inducible or constitutive. Expression control elements also may include an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats ("LTRs"), or adeno associated viral (AAV) inverted terminal repeats ("ITRs"), and the like.

The term "individual" can refer to any sort of animal, including a human or other mammal, a bird, a reptile, an amphibian, a fish, and the like. The invention is applicable to generating an immune response in any animal that has a functioning immune system.

The dermal, subdermal, and epidermal cells as well as mucosal tissue cells that can be transfected via the present invention include such cells as fibroblasts, endothelial cells, adipocytes, and numerous mononuclear cells of a dendritic morphology, the latter having a high cytoplasmic to

nuclear ratio, relatively large and homogeneous nucleus, and a wide range of elaborate dendritic processes extending from the ample cytoplasm into the surrounding tissue matrix. Such cell morphology is consistent with monocytic/dendritic antigen presenting cells known to populate the dermis.

Without adopting any particular theory, the inventors believe that the presence of transfected cells with dendritic morphology may explain why the inventive methodology enhances the immune response against the expression product. Dendritic cells, such as Langerhans cells of the skin, are important antigen-presenting cells and are known to prime and boost immune responses to foreign antigens. Once transfected, antigen-presenting dendritic cells can migrate to lymph nodes to present the expressed gene product to other immune cells, resulting in a robust cellular and humoral immune response. In this regard, Example 4 shows that transfection of skin using the methods of the present invention methods results in the presence of transfected cells within draining lymphnodes.

The development of an enhanced immune responses to a gene-encoded polypeptide in accordance with the methods of the present invention may also result from expression, processing and display of the polypeptide by traditionally non-immunologic cells such as fibroblasts and adipocytes as well as lymphocytes resident in the dermis. Such non-immunologic and immunologic cells once transfected may provide antigen by any of a number of mechanisms including secretion, exosome blebbing, and the like, to antigen presenting cells nearby in the dermis and subdermis. Furthermore, the trauma and cell permeabilization associated with electropermeabilization may provide additional non-transfection bases for the stimulating an immune response by promoting release of endogenous inflammatory mediators that may in

turn recruit or otherwise differentiate resident antigen-presenting cell precursors.

The immune response to a gene-encoded polypeptide generated by using the methods of the present invention include antibodies specific for the expressed polypeptide which may include antibodies of the IgM class, IgG class, IgA class or IgE class. A cell mediated immune response to the polypeptide also may be generated by this approach. Such cell mediated immune response has been discovered to be predominantly of the Th1 type. As used herein "Th1" response refers to an immune response dominated by CD4 T cells of the Th1 subclass. This response is characterized by the secretion of cytokines and lymphokines associated with Th1 T cells as is well known in the art, typically interferon gamma, interleukin-2, and tumor necrosis factor alpha and beta. In the mouse, the Th1 response can be characterized by humoral immunity with an IgG1:IgG2a antibody subclass ratio of 1.0 or less.

It will be appreciated that the methods of the present invention can be used to vaccinate an individual against any of a variety of infectious microorganisms and viruses. For example, if the polypeptide encoded by the gene or a gene fragment in the expression vector were associated with an infectious agent, the resulting antibodies generated may bind to that agent and protect against infection by the agent or reduce the spread of infection. Also, in the case of intracellular pathogens, expression of the polypeptide on the surface of a host cell can provide a target for T cell killing or apoptosis. Pathogenic agents to which a host may be vaccinated using the methods of the present invention include bacteria, fungi, viruses, protozoa, and the like.

The methods of the present invention for generating an immune response may inject the individual with an expression vector encoding genes other than the gene encoding the polypeptide to which the

immune response is desired. The other genes may encode one or more immunomodulatory proteins or co-stimulatory molecules including a cytokine, chemokine, immunostimulatory adjuvant molecules such as Cholera toxin B chain, an engineered immunomodulator such as CTLA4Ig, CD 40 ligand, or Fas ligand, and a low molecular weight immunomodulatory biomolecule, and the like. Genes encoding one or more such immunomodulatory proteins to be expressed in conjunction with the polypeptide immunogen may enhance the immune response, for example, by improving the antigen-presenting characteristics of the transfected cells. Vectors with two or more expression cassettes for cloning in the different genes are well known to those of skill in the art. Individuals treated with a single round of vector followed by a pulsating electrical field can be considered to have received primary immunization. If this is later followed by one or more of such treatments, these treatments can be referred to as a secondary immunization or "boosting." The time between treatments can vary and may be determined empirically, but generally a one to two week period between treatments can be used. By boosting individuals as described herein, an increased immune response to the expressed polypeptide (*e.g.*, greater antibody titers or class switching from IgM to IgG).

The present invention also contemplates boosting individuals using treatments other than vector and a pulsating electrical field. For example, boosting may comprise the vector encoded polypeptide prepared as a traditional vaccine combined with an appropriate adjuvant. The polypeptide may be obtained from natural sources or may prepared by well known methods of recombinant protein expression and purification. Boosting also may involve administering an attenuated viral vector such as an avipox vector (*e.g.*, vaccinia virus, fowlpox virus and canarypox virus) that expresses the gene encoded polypeptide following

transduction of the vector into cells of the individual. Avipox vectors include replication-defective recombinants, which can infect mammalian cells and express transgenes, but do not replicate, and replication-competent recombinants such as from vaccinia. For example, see U.S. patents No. 5,364,773 and No. 5,776,597, both to Paoletti; and No. 5.156.841 to Rapp. Such recombinant avipox vectors may be used to express, in addition to the polypeptide for which an immune response is desired, a TRIad of Costimulatory Molecules (B7-1, ICAM-1 and LFA-3, designated TRICOM), which is known to result in greater activation of immune cells. See Hodge et al., Cancer Res. 15;59(22):5800-7 (1999). Individuals who have been primed to a polypeptide or other immunogenic substance using conventional vaccination or with attenuated viral vectors and the like, as described above, may be boosted by injecting expression vector encoding the polypeptide followed by application of a pulsating electrical field.

The present invention also contemplates administration of nuclease inhibitors, preferably mixed with the polynucleotide or vector during injection to achieve increased levels of transfection and increased immune responses to the expressed polypeptide. Nuclease inhibitors including direct and indirect inhibitors are described in detail in WO 00/11217 to Malone et al. Preferred inhibitors for use with the present invention include a DNAse inhibitor, a polyclonal nuclease antibody, an actin or an actin derivative, aurin tricarboxylic acid (ATA), and the like. Although the agent need not be a specific nuclease inhibitor, it is preferable that the agent have activity against those nucleases found in the target cells and tissues of the host organism. A preferred nuclease inhibitor is ATA, which is typically used at between 25-200 μg/100 μl of injectate.

The present invention also provides a method of obtaining antibodies to a vector encoded gene product, comprising: obtaining an expression vector having a gene encoding a polypeptide to which an immune response is desired, the expression vector including expression control elements operably linked to the gene for expressing the gene product in the individual; injecting the vector below the stratum corneum layer of the skin of the individual; applying a pulsating electrical field to the skin in the vicinity of the injection; and obtaining the antibodies from the individual. In a preferred embodiment, the gene may be from a genomic library or a cDNA library. In this case, vector containing the individual genes or gene fragments are used to generate antibodies to the expressed gene product by injection of the vector and pulsed electropermeabilization as described herein. The antibodies isolated from the immunized individual can then be used to identify a characteristic of the gene product, such as its distribution of expression tissues of a particular animal, is molecular weight by Western blotting, is isoelectric point by isoelectric focusing, its interaction with other proteins by protein-protein binding assays, and the like. These and other useful antibody assays are well known to those of skill in the art.

"Antibodies" as used herein generally comprise two heavy chains and two light chains that associate to form two binding sites in each antibody molecule. This term also includes fragments of antibodies such as Fab'2 fragments and fragments with a single binding site such as Fab,' Fv, sFv, single chain or single domain antibody, and the like. For example, see Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, New York (1988).

Antibodies generated using the methods of the present invention exhibit binding specificity for polypeptide encoded by the gene or gene fragment present in the expression vector. The phrase "binding specificity " used

in relationship to an antibody that binds to a polypeptide or protein refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibody binds to a particular protein and does not bind significantly to other proteins present in the sample. An antibody can be a monoclonal antibody, a polyclonal antibody, or a collection of polyclonal antibodies such as is present in the antiserum of an immunized animal.

Polyclonal sera from immunized individuals are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross-reactivity against protein related or unrelated to the immunogen, using, for example, a competitive binding immunoassay. Polyclonal antibodies or antiserum selected to have low crossreactivity against other unrelated proteins can be removed by immunoabsorbtion prior to use in immunoassay.

Methods of making monoclonal antibodies are well known, as evidence, for example, by Harlow and Lane, *supra*, Kohler & Milstein, *Nature* 256:495 (1975), and Coligan *et al.*, sections 2.5.1-2.6.7. Briefly, a source of immune cells from the skin or from lymphnodes draining the areas of the skin where electropermeabilization had been performed are removed and fused to myeloma cells to produce hybridomas. The hybridomas secreting specific antibodies to the polypeptide are selected, subcloned and established as permanent cell stocks. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion

chromatography, ion-exchange chromatography, and the like. See Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, "Purification of Immunoglobulin G (IgG)" *in* 10 METHODS IN MOLECULAR BIOLOGY 79-104 (Humana Press, 1992). Polynucleotides with coding sequences that correspond to antibody light- and heavy-chain gene sequences, obtained from immune cells of immunized individuals as described above, can be cloned and expressed to obtain a library of Fab' or sFv antibody fragments, displayed on the surface of filamentous phage. See U.S. patents No. 5,885,793 to Griffiths and No. 5,969,108 to McCafferty.

Thus, human as well as other animal species of monoclonal antibodies can be prepared using the methods of the invention. In addition, it will be appreciated by one skilled in the art that antibodies to self antigens such as antibodies to human self antigens may be prepared by, for example, using the methods of the invention where the vector encodes a human polypeptide and the individual injected with the vector is a human.

A variety of immunoassay formats also are available to select antibodies having the appropriate specificity for a given polypeptide immunogen. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. For a description of immunoassay formats and conditions that can be used to determine binding specificity, see generally Harlow and Lane (1988), *supra*; BASIC AND CLINICAL IMMUNOLOGY 7th ed. (1991); Tijssen, P., ENZYME IMMUNOASSAY (1985), CRC Press (Boca Raton, Florida;"Practice and Theory of Enzyme Immunoassays," *in* LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, Elsevier Science Publishers B.V. (Amsterdam). For example, immunoassays in the competitive binding format can be used for crossreactivity determination.

Antibodies produced against vector encoded polypeptides as described herein also may be used to generate proteomics information in high throughput solid phase formats such as microarray or macroarray formats. The encoded gene product, or other target or the antibody specific thereto may be attached immobilized to organic or inorganic solid support, or a combination of any of these, in the form of particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, etc. Typical supports are made of glass or nylon. Such solid support preferably is flat but may take on alternative surface configurations. For example, the solid support may contain raised or depressed regions on which the antibody, polypeptide product or other target is attached. In some embodiments, the solid support will be chosen to provide appropriate light-absorbing characteristics. Thus, the support may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, or combinations thereof. Other suitable solid support materials will be readily apparent to those of skill in the art.

The surface of the solid support can contain reactive groups, which could be carboxyl, amino, hydroxyl, thiol, or the like suitable for conjugating to a reactive group associated with the antibody or encoded gene product or other target. Alternatively, or in addition, such attachment can be by physical means such as through ionic, covalent or other forces well known in the art.

Attachment to the support can be accomplished by carbon-carbon bonds, using supports having, for instance, (poly)trifluorochloroethylene surfaces or, preferably, by siloxane bonds, employing glass or silicon oxide as the solid support, for example. Siloxane bonding can be formed

by reacting the support with trichlorosilyl or trialkoxysilyl groups of the spacer. Aminoalkylsilanes and hydroxyalkylsilanes, bis(2-hydroxyethyl)-aminopropyltriethoxysilane, 2-hydroxyethylaminopropyltriethoxysilane, aminopropyltriethoxysilane or hydroxypropyltriethoxysilane are useful are surface attaching groups.

The antibodies or vector encoding polypeptides can be attached, via conventional technology, to a solid support in the form of a microarray or macroarray. In this context, an array is an orderly arrangement of samples that enables the matching of known and unknown samples. "Microarray" and "macroarray" are relative terms, distinguished from each other in terms of the diameter of the sample spots involved. Thus, microarray sample spots typically are about 200 microns in diameter or less, making it possible to array thousands of sample spots on a single chip. These arrays can be prepared by hand but, preferably, are made using specialized robotics and read by means of specialized imaging equipment, particularly with spots with diameters in the lower-end range. By contrast, macroarray sample spots typically are greater than 200 microns in diameter, making them suitable for imaging by gel and blot scanners. Macroarrays may be prepared by hand using standard microplates or standard blotting membranes.

Spotting methods can be used to prepare a microarry biochip with a variety of probes immobilized thereon. In this case, reactants are delivered by directly depositing relatively small quantities in selected regions of the support. In some steps, of course, the entire support surface can be sprayed or otherwise coated with a particular solution. In particular formats, a dispenser moves from region to region, depositing only as much probe or other reagent as necessary at each stop. Typical dispensers include a micropipette, nanopippette, ink-jet type cartridge or pin to deliver the probe containing solution or other fluid to the support

and, optionally, a robotic system to control the position of these delivery devices with respect to the support. In other formats, the dispenser includes a series of tubes or multiple well trays, a manifold, and an array of delivery devices so that various reagents can be delivered to the reaction regions simultaneously.

Spotting methods are well known and include, for example, those described in U.S. patents No. 5,288,514, No. 5,312,233 and No. 6,024,138. In some cases, a combination of flowing channel and "spotting" on predefined regions of the support also can be used to prepare microarrys with immobilized antibody, vector encoded polypeptide or other target.

The present invention also provides a method of treating a human disease condition, comprising injecting an expression vector encoding a polypeptide associated with the disease condition below the stratum corneum layer of the skin of the individual and applying an effective pulsating electrical field to the skin in the vicinity of the injection. This method results in the generation of antibodies in the individual specific for the polypeptide which ameliorates or abrogates the disease condition.

This method is applicable to a wide variety of different human diseases including cancer, allergic reactions, and the like. For example if the disease condition is cancer and the polypeptide encoded by the expression vector is a polypeptide expressed by the cells of the cancer, the immune response generated against the peptide may be an antibody or cytolytic or cytotoxic T cell. If the disease condition is an allergic reaction, the vector may contain a gene that encodes a polypeptide involved in the allergic condition. For example, the gene may encode an allergenic polypeptide and expression may result in sensitization, reducing the allergic reaction to the native allergen.

The production of antibodies to the polypeptide may ameliorate or abrogate the disease condition in different ways. For example, if the effect of the polypeptide is to cause the disease condition, the antibodies may be neutralizing antibodies which my complex with the polypeptide and block its affect. If the polypeptide has therapeutic benefit, the generation of antibodies may be ameliorate or abrogate the disease by complexing with the polypeptide and extending its circulating half-life in the body.

The phrase "an effective amount" as used, for example, in reference to the expression vector or the electrical field, relates to an amount sufficient to provide enough vector and pulsating current, respectively, to achieve transfection of cells in the dermal layer and the elicitation of an immune response which, for example, ameliorates or abrogates the condition. A single treatment may be effective in some cases while additional treatments may be used to if needed. The specific therapeutically effective dose level for any particular subject depends upon a variety of factors including the disorder being treated, the severity of the disorder, the activity of the polypeptide, the time between multiple treatments, the age, body weight, sex, diet and general health of the patient, and like factors well known in the medical arts and sciences.

The presently described approach for achieving pervasise transfection of dermal cells, subdermal cells incuding adipocytes and hair follicles, provides the ability to treat various cosmetic skin conditions resulting in a modification of the appearance of the skin. In this approach, the skin below the stratum corneum is injected with an expression vector having a gene encoding a polypeptide that modifies a characteristic of the skin. Following injection, a pulsating electric field is applied to the skin in the vicinity of the injection.

Skin conditions treatable using these methods include alopecia, obesity, disorders of pigmentation, and the like. For example, a vector encoding Sonic hedgehog protein (*e.g.,* Sato et al., *J. Clin. Invest.* 104:855-864 (1999)) can be used to correct local alopecia. The expression vector encoding Sonic hedgehog may be designed for transient expression to avoid predisposing the individual to basal cell carcinoma. Morbid or cosmetic obesity may be addressed by expressing transcription factors such as PPAR (Zhou et al., PNAS USA 96: 2391-2395 (1999)) in adipocytes, or enzymes involved in fat metabolism. Expression of melanocyte stimulating hormore using the transfection methods of the invention also can be used to address a disorder of pigmentation.

The present invention also provides kits for transfecting dermal cells in the skin of an individual, and for generating an immune response, as described above. A kit of the invention comprises a set of electrodes suitable for administering an electric field to the skin of the individual, a power supply pulse generator for connecting electrical power to the electrodes and a polynucleotide or vector to be transfected. The kit optionally includes a device for injecting the polynucleotide below the stratum corneum layer. Illustrative of such device is a syringe and needle and a need-free injection device, as already described.

EXAMPLES

The examples below detail methods for achieving *in vivo* transfection skin dermal cells by electropermeabilization, and for generating antibody responses to the expressed transgene.

Example 1: Electropmeabilization Animal Models

Animals models are effective for optimizing and testing the methods of the transfection and immunization methods of the present invention. Useful animal models have been described for skin

electropermeabilization with "naked" plasmid include murine (Raz, Carson et al. 1994) and porcine systems (Hengge, Chan et al. 1995; Hengge, Walker et al. 1996; Hengge, Pfutzner et al. 1998).

Each animal model has it advantages and disadvantages. One principal advantage of the murine model is that specific antibody reagents, transgenic animals, and other well defined immunologic reagents and protocols are well established. Additionally, the relatively modest cost associated with purchase, care and maintenance of mice allows the use of large groups for different treatment conditions. Mouse skin, however, is quite thin, requiring skill and patience to perform intradermal injections into the thicker skin at the base of the tail. The difficulty associated with intradermal injection in mice may cause significant variability between treatments. This also may occur if caliper-type electrodes for electrical treatments because the tension of the skin in this region makes it difficult to attach the electrodes. However, the use of large groups of mice somewhat offsets the problems encountered with statistical discrimination.

The skin of the Yorkshire pig, in contrast to the mouse, is similar to human skin, particularly along the abdomen and inner thighs of the pig. The dermis in these areas is robust and easily injected, but lacks the thickness and extensive connective tissue matrix found along the lateral and dorsal surfaces of the animal. Outbred Yorkshire pigs of defined age and weight (preferably 10 Kg) are readily available and inexpensive. One major advantage of the Yorkshire pig model is that multiple treatments may be performed on a single animal (up to 48 treatments), providing for substantial sample sizes and the opportunity to analyze experimental variables within a single animal.

In contrast to mice, reagents needed to perform and evaluate the effectiveness of the methods of the present invention in pigs, include

porcine cDNA clones, immunologic, and immunohistochemical reagents that cross-react with porcine antigens are not readily available. Despite this disadvantage, the low animal cost, tissue histology similar to human, and large number of potential treatments per animal make the Yorkshire pig an excellent model for experimental analysis of skin electropermeabilization.

Example 2: General Methods

Plasmids and DNA preparation. The following CMV promoter-driven plasmids were employed in the experiments: pND2-lux, pEGFP-C1 (Clontech), pND β-gal (LacZ), all purified from transformed bacterial culture (Monster Prep, Bio 101, Carlsbad, CA). Plasmid encoding the hepatitis B surface antigen, pRc/CMV-HBs(S), was purified by Aldevron (Fargo, ND).

Animals. Five- to seven-week BALB/c mice were purchased from Charles River (Wilmington, MA) and were anesthetized by an intraperitoneal injection of ketamine hydrochloride, xylazine hydrochloride, and acepromazine maleate before treatment. Four-week Yorkshire pigs were obtained from Tom Morris, Inc. (Reistertown, MD). Before treatment, pigs were anesthetized intramuscularly with ketamine and acepromazine. To harvest tissues for gene expression, mice were sacrificed by CO_2 inhalation and pigs anesthetized as above followed by lethal pentobarbital injection. All animal work was approved by institutional animal use review board.

Intradermal DNA injection and electroporation. The required concentration of each plasmid DNA (luciferase, pND2Lux, pEGFP, LacZ) was intradermally injected into pig and mouse. Sites on pigs included the ventral abdominal skin and limbs. The sites on mice were limited to the thicker skin just above the base of the tail. Total fluid volume in all experiments was 100 μl in injection-grade water. Either a caliper (plate)

or a pin electrode consisting of two rows of seven 7-mm pins (1 x 5.4-mm gaps) was used to transfer the electric field to the injection site. The pin electrode penetrated about 2.5 mm into the animal skin. In all experiments, a sham treatment in which the electrode was placed on the injection site for 20 s without turning on electricity was performed to eliminate traumatic artifact. All electropermeabilization experiments were performed using PulseAgile Electroporation equipment and software (CytoPulse Sciences, Hanover, MD).

Detection of gene expression. Animals were euthanized 24 or 48 h after treatment and skin biopsies were obtained from the injection sites using an 8-mm disposable biopsy punch. Specific analyses was then carried out to determine gene expression levels for the different plasmid DNA used.

Relative luciferase activity (RLU) was determined (as described by the manufacturer) using the enhanced luciferase assay kit (Cat. No. 556866; Pharmingen, U.S.A.) and a Monolight luminometer 2010 (Analytical Luminescence Laboratories, San Diego, CA). Under ideal conditions with the enhanced luciferase assay kit, the specific activity of luciferase protein in this luminometer is 27,275 RLU/pg luciferase protein. In tissue lysates, measured luciferase specific activity is often substantially lower.

Reagents for LacZ (β-galactosidase) were obtained from Specialty Media (Phillipsburg, NJ) and used according to manufacturer's recommendations. GFP skin sections were imaged fresh and/or fixed in 2% filtered paraformaldehyde solution for 1 h and soaked in 30% sucrose solution overnight (4°C) prior to sectioning. Each biopsy section (LacZ and EGFP) was individually embedded in OCT and frozen in liquid nitrogen. Serial cryostat sections (10 μm; Leica CM1900) were made and placed on polylysine slides (slides briefly soaked in 1:2 solution of

polylysine and deionized water), sections were photographed, and images were analyzed for the respective reporter genes.

Distribution of transgene mRNA expression was characterized by fixing murine skin biopsies in Parafix (1:20 tissue weight/solution). Sections were cut, protease treated, and then hybridized with a ^{35}S-labeled RNA antisense probe designed to recognize HBV sAg transcripts as previously described. See Fox et al. *in* CURRRENT PROTOCOLS IN IMMUNOLOGY (1993) Wiley (New York). For general histological analysis of skin tissues, biopsies were fixed in formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin.

Detection and analysis of transgene expression in draining lymph nodes. One hundred microliters of methylene blue was injected s.q. into the skin at all of the transfection sites in the ventral aspect of the lower extremities of the pig 48 hour after receiving 10 separate plasmid injections of EGFP, pLUX, or control plasmid with electropermeabilization in this area. The pig was euthanized after 30 min to allow for drainage through lymphatics. The draining lymph nodes were identified by their blue color and removed; generally three were identified per limb. They were then analyzed for appropriate transgene expression as described above.

Quantification of antibody response and immunoglobulin subclass analysis. Mice in groups of seven were immunized with i.d. injection of either pRc/CMV-HBs(S) as transgenic antigen or pND2-lux as a negative control followed by electropermeabilization with pin electrode or sham treatment as above. As a positive control, five mice were inoculated with human recombinant hepatitis B vaccine (Recombivax-HB) with a dosing regimen of 0.05 μg in 50 μl per mouse. The human Recombivax-HB-vaccinated mice were on a different calendar schedule. This avoided cross contamination. Some groups were boosted using the same

parameters as on day 0 treatment at week 3 or 9 or both. Antibody determinations were performed every 3 weeks. Antibody levels to hepatitis B surface antigen were determined by ELISA, as previously described (Malone et al., *Behring Instit. Mitt.*, 98:63-72 (1997)), using hepatitis B surface antigen purchased from Biodesign International. IgG subclass analysis was done using HRP-conjugated goat anti-mouse IgG, IgG1, IgG2a, and IgG2b (Southern Biotechnology Associates, Inc.). IgG1:IgG2a ratios were determined for individual animals and compared as previously described to quantitate relative Th1 versus Th2 responses (King et al., *Natl. Med.* 4:1281-1286 (1998); Xin et al., *Biochem. Biophys. Acta*, 1088:131-134 (1998)).

Example 3: Optimization of Electropmerabilization

This example describes optimizing the efficiency of transfection by electropermeabilization into the skin of mice and pigs.

Electroporated mice were treated with a rectangular penetrating electrode array (6 pulses, 1750 V/cm, 100 microsecond duration, 0.125 s pulse interval). Fig. 1 summarizes luciferase expression data obtained by intradermal injection of a CMV/luciferase plasmid, pND2LUX (in 100 microliters water for injection) into the skin at the base of the tail of six week old Balb-c mice, some of which also were exposed to an electric field. The results show marked enhancement of transgene expression associated with skin electroporation. Although 2000 V/cm with six 100 μs pulses was 17 fold better compared to naked DNA injection, this difference leveled out at this range. When the conditions of 1750 V/cm with six pulses were repeated, transgene expression increased still further. The total RLUs went from 1.7 million without electroporation to 45 million with electroporation, 28-fold increase. Because there was little tissue damage seen at 1750 V/cm and six pulses, these treatment

conditions were used for the genetic immunization experiments described below.

To demonstrate the relative enhancement of reporter protein expression obtained with and without electropermeabilization, duplicate intradermal injections of 50 or 100 micrograms of CMV/luciferase plasmid were administered to pigs in 100 microliters (n = 6 per condition) and then 8 mm punch biopsies of treated skin were harvested 24 hours later for luciferase analysis. Luciferase gene expression is quantitatively proportional to the measured relative light units in a luminometer. The results detailed in Figure 2 show almost no luciferase activity in pigs treated with 50 micrograms of plasmid without electropermeabilization. For pigs treated with 50 micrograms of plasmid with electropermeabilization, the level of luciferase activity averaged almost 2.00E+07 RLU per eight mm skin biopsy. Animals treated with 100 micrograms of plasmid without electropermeabilization had less than 5.00E+06 RLU of luciferase activity per eight mm skin biopsy. In contrast, in pigs treated with 100 micrograms of plasmid with electropermeabilization, the level of luciferase activity averaged almost 3.50E+07 RLU per eight mm skin biopsy. Thus, there was a substantial increase in transgene expression of a reporter protein obtained with electropermeabilization over naked plasmid alone, with the increase ranging from 20- to 80-fold.

The levels of luciferase expression obtained in porcine skin with the caliper and penetrating electrode designs were compared using the pND2LUX vector. Also the electrical parameters during treatment with 6 square wave pulses, 1750 V/cm, 100 microsecond duration, 0.125 second (s) pulse interval were monitored. As summarized in Fig. 3, although the electric current through the tissue varies between penetrating and flat plate electrode configurations, the levels of

transgene expressed was virtually identical (1.20E+08). The initial slope on the flat plate oscilloscope tracings also indicated that the flat plate electrodes were "burning" channels through the stratum corneum or other skin structures that resist current flow.

Example 4: Characterization of Transgene Expression in Skin

To characterize the distribution of transgene expression obtained by electropermeabilization of murine skin, 100 micrograms of a CMV plasmid encoding the hepatitis B virus surface antigen (Davis et al., *Hum. Mol. Genet.*, 2:1847-1851 (1993)) was used for electropermeabilization. One day after treatment, skin biopsies were fixed, sectioned, protease and DNAse treated, and hybridized with a ^{35}S-labeled RNA antisense probe designed to recognize HBV sAg transcripts. See Fox et al. (1993), *supra*. After hybridization, the slides were then phosphor imaged (Fuji BAS 5000).

These images demonstrated the expression of the gene in reticular and deep dermis of treated skin. The production of HBV mRNA within transfected cells was not localized in any general area but was evident throughout the dermal tissue. The phoshor imaged figure showed the general distribution of ^{35}S-labeled RNA antisense probe hybridization that in turn corresponds to the distribution of HBV sAg mRNA expression. The above expression distribution pattern differs substantially from the superficial epidermal expression pattern typically observed after treatment without electropermeabilization.

A variety of dermal cell types are transfected by electrical field enhanced naked DNA delivery in the skin of pigs. The initial characterization of the phenotype of skin cells transfected by electropermeabilization was performed by transfecting porcine skin with either a CMV/eGFP plasmid or a plasmid encoding a nuclear localized lac Z gene. Typical images

obtained from either fluorescent imaging (eGFP, whole tissue or frozen sections) or light imaging of X-gal stained tissue sections showed that transfected cells include fibroblasts, endothelial cells, adipocytes, and numerous mononuclear cells with elaborate cytoplasmic processes, possibly dermal dendritic cells.

A typical low power photomicroscopic view shows that the dermal layer of pig skin is the predominant site of successful transfection. At higher power magnification, the morphologic appearance of one of the predominant transgene expressing cell types includes a high cytoplasmic to nuclear ratio, a relatively large and homogeneous nucleus, and a wide range of elaborate dendritic processes that extend form the ample cytoplasm into the surrounding tissue matrix. This cell morphology is consistent with the known appearance of the monocytic/dendritic antigen presenting cells known to populate this tissue layer. Examples of some of the other transfected cell types include fibroblasts, non-dendritic monocytes, and adipose cells.

The migration of transfected cells from the skin to draining lymph nodes, was evaluated in the lower limbs of pigs treated at ten separate intradermal sites (100 microgram DNA in 100 microliter water for injection per site) with electropermeabilization (1750v/cm with six 100 microsecond pulses) comparing positive and negative control DNA. Forty-eight hours later, methylene blue dye was injected into the dermis at the sites of the previous transfection treatment. The dye was allowed to diffuse into the lymph nodes that drain the sites of skin transfection. These lymph nodes were dissected and placed under a fluorescent microscope where transgene-encoded fluorescence was observed. Using luciferase DNA (under the same conditions), there was a small but statistically significant difference between lymph nodes of pigs that received electroporation and pigs that received electroporation with a

control DNA (HBV), while the mean RLU for the pig that received electroporation with luciferase DNA was 252. The RLU mean for the pig that received electroporation with the negative control DNA was 200 relative light units. A Mann-Whitney U test was used to assess the statistical difference and the calculated p value was $p<0.05$.

The above results in both pigs and mice showed that the expression pattern after intradermal injection of plasmid (without electropermeabilization) is limited to the epidermis, and particularly to the stratum corneum. This result predicts a short transfection duration and consequent reduced immune response because the stratum corneum layer of the epidermis typically differentiates and exfoliates in a short time. The ability of the methods of the present invention to obtain pervasive transfection of dermal and even subdermal cells having longer residence in the body that stratum corneum cells explains in part the increased immune responses observed with the inventive approach.

Example 5: Generation of an Immune Response by Electropermeabilization

The potential utility of electropermeabilization for nucleic acid vaccination was examined in groups of Balb-c mice treated by intradermal injection with 100 μg of a HBV sAg expression plasmid with prong electrodes in various conditions. Treatment groups included a luciferase plasmid control, HBV plasmid skin injection without electroporation, HBV plasmid skin injection with electroporation 1750 V/cm x 6 pulses (no boost), HBV plasmid skin injection with electroporation 1750 V/cm x 6 pulses (3 wk boost), and HBV plasmid skin injection with electroporation 1500 V/cm x 6 pulses (3 wk boost). ELISA assays (IgG) for weeks 0,3,6, and 9. Summarized in Figure 4, the results show CTL assays and ELISAs testing for IgM, IgG1A, IgG2A,B, performed when mice reached 16 weeks of age. Mice that received

luciferase encoded DNA and mice that received no electroporation following inoculation did not produce positive titers by week nine. Three out of eleven mice that received HepB DNA with 1750 V/cm electroporation (6 pulses of 100 μs each) (no boost) were positive by week nine. Eight out of fourteen mice were positive for IgG at week 9 in the group that received HBV DNA with 1750 V/cm electroporation (6 pulses at 100 μs each) plus a 3 week boost. Five out of seven mice were positive for IgG at week 9 in the group that received HepB DNA with 1500 V/cm electroporation with 6 pulses (100 μs each) plus a 3 week boost.

Example 6: Use of a Nuclease Inhibitor to Enhance Immune Response Generation Following Electropermeabilization

The Th2 response as indicated by IgG1 production was evaluated in mice treated by intradermal injection a Hepatitis B virus plasmid (100 μg plasmid in 100 μl WFI) combined with or without a direct nucleic nuclease inhibitor, aurin tricarboxylic acid (ATA), and followed by electropermeabilization. The ATA was injected in the amount of 25 μg per injection, and was so administered with the plasmid diluted with WFI; each ID injection was 100 μl total volume. Negative controls consisting of the luciferase plasmid (pND2LUX) alone all were negative for antibody. The HBV plasmid with electroporation with and without boosting (no ATA) had positive titers from 300 to 1068. Titers decreased slightly at week 12 for both boosted and non-boosted animals. The HBV plasmid plus ATA with electroporation, with or without boosting, had titers ranging from 742 to 3080. These titers were substantially higher than for animals not receiving ATA. The highest titers were at week 12 for animals receiving 4-week boosts, ATA and electroporation. The lowest titers were for animals receiving the HBV plasmid and boosting but not electroporation or ATA.

As indicated by IgG2a production, the Th1 response was evaluated in mice treated by intradermal injection a Hepatitis B virus plasmid (100 μg plasmid in 100 μl WFI), with or without a direct nucleic nuclease inhibitor (ATA), followed by electropermeabilization. Negative controls consisting of the luciferase plasmid (pND2LUX) alone all were negative for antibody. The HBV plasmid with electroporation with and without boosting (no ATA) gave positive titers from 37 to 395. Titers decreased slightly at week 12 for both boosted and non-boosted animals. The HBV plasmid plus ATA with electroporation with or without boosting had titers ranging from 88 to 710. These titers were higher than those animals that did not receive ATA. The lowest titers were those animals that received the HBV plasmid and boosting but did not receive electroporation or ATA.

* * * * * * *

The invention thus has been disclosed broadly and illustrated in reference to representative embodiments described above. Those skilled in the art will recognize that various modifications can be made to the present invention without departing from the spirit and scope thereof. For example, the invention can be performed using different voltages, currents, electrode designs, tissue depths, pulse durations, number of pulses and time between pulses than those parameters used herein.

WHAT IS CLAIMED IS:

1. A method of transfecting cells in the skin or mucosal tissue of an individual with a polynucleotide, comprising (A) injecting the polynucleotide in the skin or mucosal tissue of the individual and (B) applying a pulsating electrical field to the skin in the vicinity of the injection.

2. The method of claim 1 wherein said polynucleotide is injected into the dermis of the skin.

3. The method of claim 1, wherein said polynucleotide is a vector.

4. The method of claim 3, wherein said vector is an expression vector having expression control elements operably linked to a polypeptide encoding gene.

5. The method of claim 4, wherein said polypeptide encoding gene is expressed in the transfected cells.

6. The method of claim 5, wherein said gene expression results in the generation of antibodies in the individual to the polypeptide.

7. The method of claim 1, wherein said transfected cells are dermal cells or adipocytes.

8. The method of claim 1, further including the step of administering a nuclease inhibitor with the polynucleotide.

9. The method of claim 1, wherein said pulsating electrical field comprises between 4 to 20 pulses of 1,000 to 2,000 V/cm for a period of 10 to 100 microseconds.

10. The method of claim 1, wherein said pulsating electrical field comprises six pulses of 1,750 V/cm for a period of 100 microseconds.

11. A method of vaccinating an individual, comprising:

a. injecting an expression vector into the skin or mucosal tissue of the individual, said expression vector having a gene encoding a polypeptide to which an immune response is desired, said expression vector including expression control elements operably linked to the gene for expressing the gene product in the individual; and

b. applying a pulsating electrical field to the skin in the vicinity of the injection.

12. The method of claim 11, wherein said vector is injected into the dermis of the skin.

13. The method of claim 11, wherein said step of injecting and applying a pulsating electrical field is repeated one or more subsequent times, to boost the immune response of the individual.

14. The method of claim 11, wherein said individual is a human.

15. The method of claim 11, wherein said immune response is a Th1 immune response.

16. The method of claim 11, wherein said immune response is directed against a polypeptide associated with a pathogenic agent selected from the group consisting of bacteria, fungi, virus, and protozoa.

17. The method of claim 11, wherein said immune response includes the generation of antibodies with binding specificity for the polypeptide.

18. The method of claim 17, further comprising the step of isolating the antibodies from the individual.

19. The method of claim 11, further comprising the step of preparing monoclonal antibodies from immune cells of the individual.

20. The method of claim 11, further including the step of injecting a nuclease inhibitor with the vector.

21. The method of claim 11, wherein said expression vector further encodes a immunoregulatory protein.

22. The method of claim 11, wherein said pulsating electrical field comprises between 4 to 20 pulses of 1,000 to 2,000 V/cm for a period of 10 to 100 microseconds.

23. The method of claim 11, wherein said pulsating electrical field comprises six pulses of 1,750 V/cm for a period of 100 microseconds.

24. A method of obtaining antibodies to a vector encoded gene product, comprising:

a. injecting an expression vector into the skin or mucosal tissue of an individual, said expression vector having a gene encoding a polypeptide to which an immune response is desired, said expression vector including expression control elements operably linked to the gene for expressing the gene product in the individual;

b. applying a pulsating electrical field to the skin in the vicinity of the injection; and

c. obtaining the antibodies from the individual.

25. The method of claim 24, wherein said step of obtaining antibodies from the individual involves obtaining serum or plasma from the individual.

26. The method of claim 24, wherein said step of obtaining antibodies from the individual involves preparing monoclonal antibodies using immune cells from the individual.

27. The method of claim 24, wherein said step of injecting and applying a pulsating electrical field is repeated at one or more subsequent times.

28. The method of claim 24, wherein said gene is obtained from a genomic or cDNA library.

29. The method of claim 28, wherein said antibodies are used to determine a characteristic of the gene product.

30. The method of claim 24, further including the step of administering a nuclease inhibitor with the vector.

31. The method of claim 24, wherein said expression vector further encodes an immunoregulatory protein.

32. The method of claim 24, wherein said pulsating electrical field comprises between 4 to 20 pulses of 1,000 to 2,000 V/cm for a period of 10 to 100 microseconds.

33. The method of claim 24, wherein said pulsating electrical field comprises six pulses of 1,750 V/cm for a period of 100 microseconds.

34. A method of treating a disease condition, comprising injecting an effective amount of an expression vector encoding a polypeptide associated with the disease condition in the skin or mucosal tissue of the individual and applying an effective pulsating electrical field to the skin in the vicinity of the injection so as to transfect cells of the dermis, resulting in the generation of an immune response to the polypeptide which ameliorates or abrogates the disease condition.

35. The method of claim 34, wherein said disease condition is cancer and said polypeptide is expressed by the cells of the cancer.

36. The method of claim 34, wherein said disease condition is an allergic condition, said polypeptide affects the condition.

37. The method of claim 34, further including the step of injecting a nuclease inhibitor with the vector.

38. The method of claim 34, wherein said expression vector further encodes a immunoregulatory protein.

39. The method of claim 34, wherein said pulsating electrical field comprises between 4 to 20 pulses of 1,000 to 2,000 V/cm for a period of 10 to 100 microseconds.

40. The method of claim 34, wherein said pulsating electrical field comprises six pulses of 1,750 V/cm for a period of 100 microseconds.

41. A method of modifying a skin condition which affects the appearance of the skin, comprising injecting an expression vector below the stratum corneum layer of the skin, said expression vector having a gene encoding a polypeptide that, when expressed, modifes the skin condition; and applying a pulsating electric field to the skin in the vicinity of the injection.

42. The method of claim 41, wherein said condition is alopecia.

43. The method of claim 41, wherein said condition is a pigmentation disorder.

44. The method of claim 41, wherein said condition is obesity.

45. The method of claim 41, wherein said pulsating electrical field comprises between 4 to 20 pulses of 1,000 to 2,000 V/cm for a period of 10 to 100 microseconds.

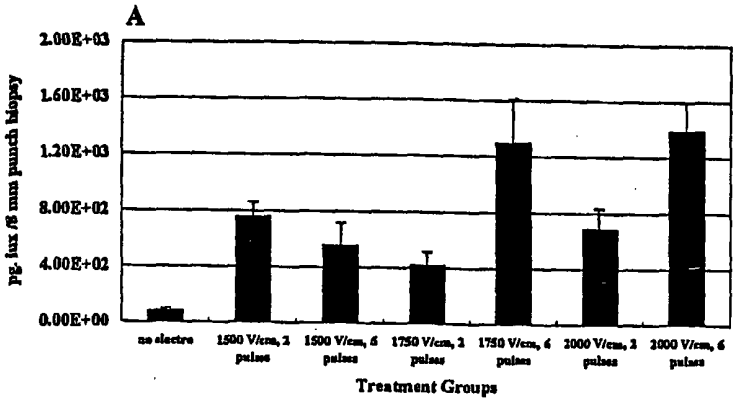
46. A kit for transfecting cells in the skin or mucosal tissue of an individual, comprising a set of electrodes suitable for administering an electric field to the skin or mucosal tissue of the individual, a power supply pulse generator for connecting electrical power to the electrodes and a polynucleotide to be transfected.

47. The kit of claim 46, further including a device for injecting the polynucleotide into the skin or mucosal tissue of the individual.

48. A kit for generating an immune response to a polypeptide in an individual, comprising a set of electrodes suitable for administering an electric field to the skin or mucosal tissue of the individual, a power supply pulse generator for connecting electrical power to the electrodes, and a vector encoding the polypeptide and suitable for expressing the polypeptide in cells of the individual.

49. The kit of claim 48, further including a device for injecting the expression vector into the skin or mucosal tissue of the individual.

Fig. 1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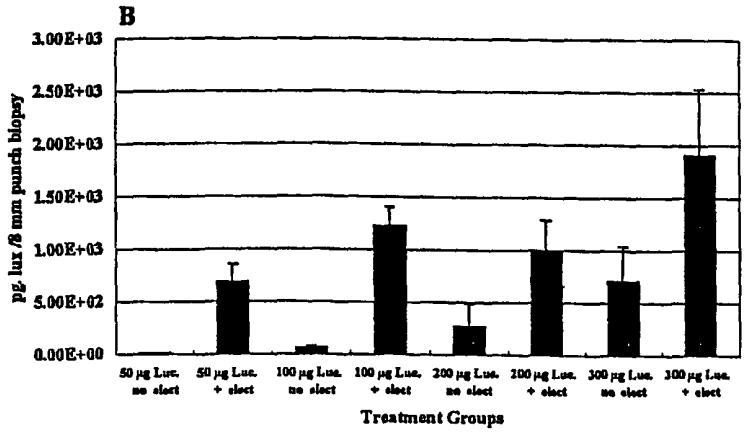
Treatment Groups

Fig. 2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50 µg Luc. + elect
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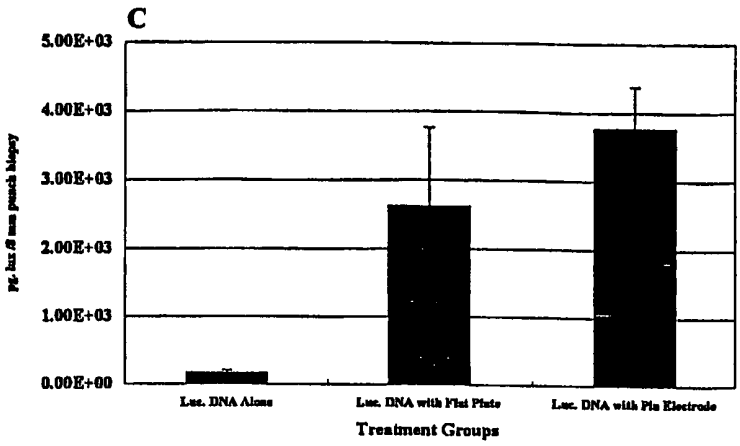
Treatment Groups

Fig. 3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4.00E+03
3.00E+03
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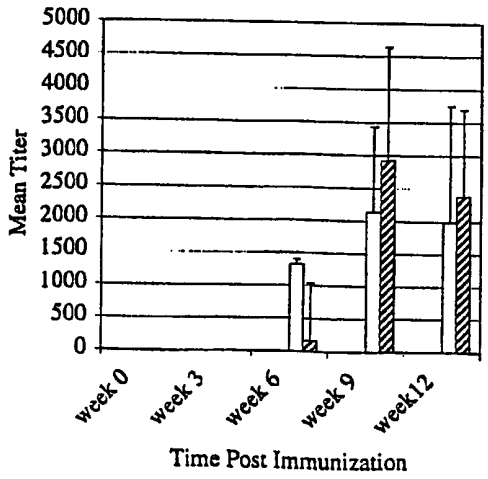
Luc. DNA with Flat Plate
Luc. DNA with Pin Electrode
Treatment Groups

Fig. 4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Mean Titer
week 0
week 3
week 6
week 9
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Time Post Immunization