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(54) Title: TARGETING SOMATIC GENE THERAPY TO JOINTS

(57) Abstract

A method of transfecting a cell in a structure of a joint is disclosed, wherein a DNA vector containing a nucleic acid cassette encoding, for example, a cell ablation agent or a therapeutic agent, is directly injected into the joint. The invention also includes a transfected synovial cell.

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TARGETING SOMATIC GENE THERAPY TO JOINTS

FIELD OF THE INVENTION

The present invention relates generally to the introduction of genetic material into the joints and cells comprising the joint structures for the purpose of somatic gene therapy. Further, it relates to methods for introducing novel genetic material into cells within the joint and achieving expression of gene products with therapeutic potential.

BACKGROUND OF INVENTION

Somatic gene therapy involves the treatment of genetic or acquired disease by the introduction of recombinant genes into somatic cells. Many different organs have been traditionally considered targets for somatic gene therapy including the bone marrow, liver, endothelial cells, epithelial cells, fibroblasts, and muscle. In general, somatic gene therapy has traditionally been considered for those organs and cells which can be studied in vitro or ex vivo. One of the reasons for this focus is that many schemes for somatic gene therapy involve the harvest of cells from an organ by surgical means, growing these cells in the laboratory, introducing genes into these cells using viral vectors, and then reimplanting these cells into the body using a cellular transplantation procedure. Broad applications of ex vivo schemes for gene therapy, particularly the transplantation of fibroblasts into many sites, have been proposed.

More recently attention has turned to schemes for somatic gene therapy which involve in vivo delivery of recombinant genes to essential tissues by direct injection or targeted delivery. The present invention describes a novel approach to somatic gene therapy

involving the direct delivery of recombinant genes to joint spaces and cells comprising essential structures of the joint for the purposes of treating various forms of arthritis or other diseases of the joints. Major joints may be targeted for somatic gene therapy by injection of genetic material into the joint space. This therapy would be intended to prevent the often crippling and painful manifestations of inflammatory or degenerative arthritis as well as to enhance the process of repair or regeneration after illness, injury, or surgery.

SUMMARY OF INVENTION

An object of the present invention is the transformation of cells comprising essential structures of the joint.

An additional object of the present invention is a method of direct delivery of genetic material to the cells comprising essential structures of the joint.

A further object of the present invention is the in vivo introduction of genetic material into cells comprising essential structures of the joint.

Another object of the present invention is a method of injecting genetic material into the joint space for uptake by cells constituting structures of the joint.

An additional object of the present invention is the coupling of genetic materials to non-genetic materials termed vehicles which enhance the uptake, stability, and expression of genetic material into cells of the joint.

A further object of the present invention is a method for treating pathophysiological conditions resulting from inflammatory processes.

Another object of the present invention is a method for treating hypertrophy or inappropriate proliferation of cellular elements of the joint.

A further object of the present invention is a method for enhancing repair, regeneration, and recovery of essential structures comprising the joint after surgery or injury.

An additional object of the present invention is a method for ablating certain cells in the joint.

A further object of the present invention is a method for generating animal models of arthritis.

In accomplishing the foregoing objects there is provided in accordance with one aspect of the present invention a method of transfecting a cell in a structure of a joint comprising the step of introducing a DNA vector directly into the joint. The vector is comprised of elements required for directing transcription of the genetic material and expression of a specific gene product encoded by a nucleic acid cassette within the vector. The following elements are linked sequentially at appropriate distance for allowing functional expression: a promoter; a 5′ mRNA leader sequence; an initiation site; a nucleic acid cassette containing the sequence to be expressed; a 3′ untranslated region; and a polyadenylation signal. One or more of these elements may be eliminated for specific applications.

In an embodiment of this invention the vector is comprised of DNA sequences which can be inserted into cells of the joint and can transform these cells to produce novel gene products.

In a preferred embodiment, the nucleic acid cassette encodes a protein, polypeptide, or anti-sense RNA. The protein can be selected from the group including enzymes, proteins constituents of the extracellular matrix, as well as cytokines, interleucins, growth factors, toxins, as well as receptors for these ligands and also natural or genetically modified receptors for steroid hormones.

In an alternative embodiment, the nucleic acid cassette encodes a protein, polypeptide, or anti-sense RNA for the purpose of generating animal models of joint disease. The protein can be selected from the group including growth factors, tissue

transplantation antigens (histocompatibility antigens), viral antigens, non-viral antigens, interferon, or toxins.

In preferred embodiments the genetic material is administered in conjunction with non-genetic material termed vehicles which comprise a solutions or suspensions. This non-genetic material can be selected from the group including sucrose, protamine, polybrene, spermidine, polylysine, other polycations, proteins, $CaPO_4$ precipitates, soluble or insoluble particles, or matrices for slow release of genetic material. The proteins may be selected from the group including lactoferrin, histone, natural or synthetic DNA binding proteins, natural or synthetic DNA binding compounds, viral proteins, non-viral proteins or any combinations of these. In addition, vehicles may be comprised of synthetic compounds which bind both to DNA and function as ligands for receptors on cells comprising structures of the joint.

In specific embodiments of the present invention, the nucleic acid cassette is transiently expressed by cells of the joints or is persistently expressed by cells of the joint as an episome. Alternatively, the nucleic acid cassette is stably expressed by cells of the joint.

This approach can be used for the treatment of pathophysiological conditions resulting from inflammatory diseases affecting the joints, degenerative diseases of the joints, proliferative diseases of the joints, repair and regeneration of essential joint structures as well as other acquired diseases of the joints. This approach can also be used to generate animal models of arthritis.

Other and further objects, features and advantages will be apparent from the following descriptions of the presently preferred embodiments in the invention which are given for the purpose of disclosure and when taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows expression of E. coli β-galactosidase in synovial structures of the rabbit knee after DNA mediated gene transfer into the joint. Arrows indicate the regions of synovium stained with X-gal.

A. Experimental animal after injection with CMV-B-gal.

B. Control animal injected with different DNA construct.

C. Close-up of experimental animal injected with CMV-B-gal.

D. Close-up of control animal.

Figure 2 is a schematic of one proposed mechanism for DNA mediated gene transfer into synovial cells. Synovial cells form the boundary between the synovial fluid and adjacent tissues. The synovial fluid is synthesized by synovial cells and is constantly remodelled by pinocytosis. DNA injected into the joint can be taken up during the process of pinocytosis.

Figure 3 is a schematic of one proposed mechanism for DNA mediated gene transfer into inflammatory cells invading the joint. Packaging DNA in a form which is preferentially taken up by phagocytes (such as particulate DNA) can lead to preferential ingestion of DNA by phagocytosis. This can be used to alter gene expression in phagocytes or ablate these cells.

Figure 4 is a schematic of one proposed mechanism for targeting DNA to different cells such as chondrocytes and synovial cells as a complex with a vehicle that is capable of receptor mediated uptake, membrane fusion, or enhanced pinocytosis.

The drawings are not necessarily to scale and certain features of the invention may be exaggerated in scale and shown in schematic form in the interest of clarity and conciseness.

DETAILED DESCRIPTION OF THE INVENTION

It will be readily apparent to one skilled in the art that various substitutions and modification may be made to the invention

disclosed herein without departing from the scope and spirit of the invention.

The terms "structures comprising the joint" and "cells of the joint" refer to all of the cellular and non-cellular materials which comprise the joint and are involved in normal function of the joint or are present within the joint due to pathological conditions. These include materials associated with: the joint capsule such as, synovial membranes, synovial fluid, synovial cells; the cartilaginous components of the joint such as chondrocytes, extracellular matrix of cartilage; the bony structures such as bone, periosteum of bones, periosteal cells, osteoblasts, osteoclasts; the immunological components such as inflammatory cells, lymphocytes, mast cells, monocytes, eosinophils; other cells like fibroblasts; or combinations thereof.

The term "transformed" as used herein refers to transient or permanent changes in the characteristics (expressed phenotype) of a cell by the mechanism of gene transfer. Genetic material is introduced into a cell in a form where it expresses a specific gene product or alters the expression or effect of endogenous gene products.

The term "transfection" as used herein refers to the process of introducing a DNA expression vector into a cell. Various methods of transfection are possible including microinjection, $CaPO_4$ precipitation, liposome fusion (e.g. lipofection) or use of a gene gun.

The term "transient" as used herein relates to the introduction of genetic material into a cell to express specific proteins, peptides, or RNA etc. The introduced genetic material is not integrated into the host cell genome or replicated and is accordingly eliminated from the cell over a period of time.

The term "stable" as used herein refers to the introduction of genetic material into the chromosome of the targeted cell where it integrates and becomes a permanent component of the genetic material

in that cell. Gene expression after stable transduction can permanently alter the characteristics of the cell leading to stable transformation.

The term "persistent" as used herein refers to the introduction of genes into the cell together with genetic elements which enable episomal (extrachromosomal) replication. This can lead to apparently stable transformation of the characteristics of the cell without the integration of the novel genetic material into the chromosome of the host cell.

The term "ligand" refers to a protein capable of binding to a receptor on a cell.

The term "nucleic acid cassette" as used herein refers to the genetic material of interest which can express a protein, or a peptide, or RNA to incorporate it transiently, permanently or episomally into the structures comprising the joint. The nucleic acid cassette is positionally and sequentially oriented in a vector with other necessary elements such that the nucleic acid in the cassette can be transcribed and, when necessary, translated in the cells of the joint.

The term "vector " as used herein refers to a construction comprised of genetic material designed to direct transformation of a targeted cell. A vector contains multiple genetic elements positionally and sequentially oriented with other necessary elements such that the nucleic acid in a nucleic acid cassette can be transcribed and when necessary translated in the transfected cells. In the present invention the preferred vector comprises the following elements linked sequentially at appropriate distance for allowing functional expression: a promoter; a 5′ mRNA leader sequence; an initiation site; a nucleic acid cassette, containing the sequence to be expressed; a 3′ untranslated region; and a polyadenylation signal.

The term "genetic material" as used herein refers to contiguous fragments of DNA or RNA. The genetic material which is

introduced into cells comprising essential structures of the joint according to the methods described herein can by any DNA or RNA. For example the nucleic acid can be: 1) normally found in cells of the joints, 2) normally found in cells of the joints but not expressed at physiologically appropriate levels, 3) normally found in cells of the joints but not expressed at optimal levels in certain pathological conditions, 4) novel fragments of genes normally expressed or not expressed in structures of the joint, 5) synthetic modifications of genes expressed or not expressed within structures the joints, 6) any other DNA which may be modified for expression in cells of the joint, and 7) any combination of the above.

The term "pharmacological dose" as used herein refers to a dose of vector and level of gene expression resulting from the action of the promotor on the nucleic acid cassette when introduced into the appropriate cell type which will produce sufficient protein, polypeptide, or antisense RNA to either (1) increase the level of protein production, (2) decrease or stop the production of a protein, (3) inhibit the action of a protein, (4) inhibit proliferation or accumulation of specific cell types, (5) induce proliferation or accumulation of specific cell types. As an example, if a protein is being produced which causes the accumulation of inflammatory cells within the joint, the expression of this protein can be inhibited, or the action of this protein be interfered by expression of appropriate regulatory proteins. The dose will depend on the protein being expressed, the promoter, uptake and action of the protein RNA. Given any set of parameters, one skilled in the art will be able to determine the dose.

The term "ablation agent" as used herein refers to an agent which is capable of destroying the cell in which it is present. Examples of ablation agents used in the present invention include diphtheria toxin and herpes thymidine kinase.

The term "vehicle" as used herein refers to non-genetic material combined with the vector in a solution or suspension which enhance the uptake, stability, and expression of genetic material into cells of the joint. Examples of a vehicle include: sucrose, protamine, polybrene, spermidine, polylysine, other polycations, proteins, $CaPO_4$ precipitates, soluble and insoluble particles, or matrices for slow release of genetic material. The proteins may be selected from the group including lactoferrin, histone, natural or synthetic DNA binding proteins, natural or synthetic DNA binding compounds, viral proteins, non-viral proteins or any combinations of these. In addition, vehicles may be comprised of synthetic compounds which bind both to DNA and function as ligands for receptors on cells comprising structures of the joint.

Examples of proteins or polypeptides which can be expressed by the vector in the transformed cells of the joint for the purposes of somatic gene therapy include enzymes, extracellular matrix elements, cytokines, interleucins, growth factors, toxins, as well as receptors for these ligands and also natural or genetically modified receptors for steroid hormones.

Examples of proteins or polypeptides which can be expressed by the vector in the transformed cells of the joint for the purposes of generating animal models of arthritis include growth factors, tissue transplantation antigens (histocompatibility antigens), viral antigens, non-viral antigens, interferon, or fragments of diphtheria toxin.

One specific embodiment of the present invention is a method of transfecting a cell in a structure of a joint comprising the step of introducing a DNA vector directly into the joint, said vector comprised of the following elements linked sequentially at appropriate distances for allowing functional gene expression: a promotor; a 5′ mRNA leader sequence, an initiation site, a nucleic acid cassette containing the nucleic acid sequence to be expressed, a 3′ untranslated region, and a polyadenylation signal. One skilled

in the art will readily recognize how to order and position these elements such that the nucleic acid sequence is expressed. Further, one skilled in the art will readily recognize that certain elements of this DNA vector may be eliminated for specific applications. In preferred embodiments, the DNA vector without the nucleic acid cassette will contain a restriction site for insertion of the specific cassette. Once the specific cassette is inserted, the restriction site may no longer be present.

In the present invention the nucleic acid cassette is activated with a promotor. The promotor is any of the wide variety of promotors known in the art. Examples of the promotors which are used in the present invention are the retroviral LTR promotor, cytomegalovirus promotor, dihydrofolate reductase promotor, viral promotors, or non-viral promotors. Alternatively, the promotor can be selected from those shown to specifically express in the select cell types which may be found associated with the structures of the joint such as synovial cells, fibroblasts, lymphocytes, periosteal cells, chondrocytes, osteoblasts, osteoclasts, or more than one of these cell types.

One skilled in the art will recognize that the selection of the promotor will depend on the vector, the nucleic acid cassette, the cell type to be targeted, and the desired biological effect. One skilled in the art will also recognize that in the selection of a promotor the parameters can include: achieving sufficiently high levels of gene expression to achieve a physiological effect, maintaining a critical level of gene expression, achieving temporal regulation of gene expression, achieving cell type specific expression, achieving pharmacological, endocrine, paracrine, or autocrine regulation of gene expression, and preventing inappropriate or undesirable levels of expression. Any given set of selection requirements will depend on the conditions but can be readily determined once the specific requirements are determined.

Those promotors which naturally occur in the cells comprising structures of the joint will be preferred.

Introduction of a vector into cells comprising structures of the joint can be used to treat a variety of pathological conditions. These conditions can result from the abnormal production of a protein or a polypeptide, for example too little or too much of a protein or polypeptide, the production of an abnormal protein or polypeptide, atopic production of a protein or peptide, or abnormal regulation of production of a protein or polypeptide. Introduction of a vector into cells comprising structures of the joint can also be used to provide proteins or peptides or genetic elements (DNA or RNA) with therapeutic actions. One skilled in the art will recognize that even complex pathophysiological events such as inflammatory diseases, degenerative, diseases, injury and regeneration can be understood as comprising a series of molecular interactions between proteins and can be treated according to the embodiment of this invention.

For example, in the treatment of a pathological condition the vector in a vehicle will be introduced into cells comprising structures of the joint by injecting a pharmacological dose of the vector and vehicle into a joint.

The preferred embodiments of this invention involves transient or persistent expression within the joint. This is preferable to stable expression since it enables adjustment of the level of expression in response to the evolution of the disease process.

Stable expression may presently be achieved by the use of viral vectors or the transplantation of cells which are stably transformed with viral or DNA vectors.

Specific diseases which can be treated by administration of vectors to cells within the joint include various arthritis, avascular necrosis, or injuries requiring repair and regeneration of structures comprising the joint. Various types of arthritis can be treated. A list of these various types are shown below. This list

is based on a classification of joint disorders: (after table 358-1, p. 2048 Harrison's Principles of Internal Medicine, Thorn, G. W., Adams, R. D., Braunwald, E., Isselbacher, K. J., Petersdord, R. G., McGraw Hill Book Company, 1977)

I. Periarticular
- A. Tendinitis
- B. Bursitis
- C. Fibrositis
- D. Bone lesions
- E. Soft Tissue inflammation

II. Articular
- A. Cartilage and ligaments
  1. Degenerative joint disease
  2. Traumatic disorders
  3. Neuropathic arthropathy
  4. Metabolic disorders
- B. Synovium
  1. synovial tumors
  2. pigmented villonodular synovitis
  3. hemorrhagic disorders
  4. septic disorders
  5. crystal induced disorders (gout)
     - a. Immune complex disease and vasculitis
     - b. Systemic lupus erythematosis
     - c. rheumatoid arthritis
     - d. Reiter's syndrome
     - e. Psoriasis
     - f. ankylosing spondylitis
     - g. scleroderma
     - h. arthritis of intestinal disease

The method of treating a pathophysiological condition or repairing and regenerating structures of the joint comprises the steps of injecting a joint of a human with a pharmacological dose of

a DNA vector in a vehicle, wherein the nucleic acid cassette in the vector encodes a protein, polypeptide or RNA. The vector is taken up by appropriate cells within the joint and expresses the protein, polypeptide or RNA. Specific embodiments of the invention include treatment of disorders listed above.

Specific embodiments of the present invention include a method of ablation of inflammatory cells in a joint comprising the step of introducing a vector into the joint, said vector comprised of the following elements linked sequentially at appropriate distances for allowing function expression: a promoter which is specifically expressed in leucocytes; a 5' mRNA leader sequence; an initiation site; a nucleic acid cassette containing the sequence for an ablation agent; a 3' untranslated region; and a polyadenylation signal; wherein the vector is targeted for selective uptake by phagocytic cells in the joint. In the preferred embodiment, the vector is directly targeted by attaching the vector to a molecule which attaches to the surface of the phagocytic cell.

In specific embodiments of the present invention for the treatment of arthritis, a soluble receptor for cytokines can be used. Examples of a soluble receptor include IL-1 or IL-6.

An alternative embodiment of the present invention includes a method of treating arthritis in humans comprising the step of injecting an inflamed joint of a human with a pharmacological dose of a DNA vector in a vehicle, wherein the nucleic acid cassette in the vector encodes a sequence for a steroid receptor. This can be the normal receptor or it can be a genetically modified receptor.

When treating pathophysiological conditions or repairing or regenerating structures of the joint, it is found that a useful nucleic acid cassette encodes antisense RNA to prostaglandin synthase.

Another embodiment of the prevent invention is a method of making an animal model for inflammatory arthritis comprising the step of injecting the joint of an animal with a functional DNA

vector in a vehicle. The nucleic acid cassette in the vector encodes a transplantation antigen, cell adhesion antigen, antigen from micro-organisms, viral antigen, synthetic antigen or recombinant antigen. Examples of the transplantation antigen include the histocompatibility antigens, Class I transplantation antigen, Class II transplantation antigen and other transplantation antigens, allogeneic transplantation antigen and xenogeneic transplantation antigen.

Another embodiment of the present invention is a transformed synovial cell comprised of nucleic acid incorporated into a synovial cell by gene transfer. In specific embodiments, the nucleic acid can be any of the genetic materials described above. Synovial cells are capable of expressing either protein, polypeptide or antisense RNA. Generally, synovial cells can express cytokines, interleucins, receptors for natural ligands, genetically modified receptors for natural ligands, inhibitors of natural ligands, steroid receptors, genetically modified steroid receptors, cell adhesion molecules, genetically modified adhesion molecules, enzymes affecting prostaglandin metabolism, enzymes involved in extracellular matrix, RNA molecules inhibiting production of transplantation antigens, cell adhesion molecules, receptors, or enzymes involved in prostaglandin metabolism. The gene in the transformed synovial cells can specifically encode a steroid receptor, IL-1, IL-6, IL-8, or soluble IL-1 receptor, the transplantation antigen or an antisense RNA to prostaglandin synthase.

Another embodiment of the present invention is a method of targeting a DNA vector to joints comprising the steps of identifying human antibodies from patients with autoimmune arthritis, cloning the antibodies to develop monoclonals with the same epitope binding determinants and coupling DNA vectors to the monoclonals; wherein the DNA/monoclonal complex is delivered to the cells of the joint. In the preferred embodiment the identified antibodies have the property of binding specifically to proteins on cells within the

joint. One skilled in the art recognizes that a variety of methods are available for identifying antibodies.

An alternative method of gene therapy is a method of introducing DNA into an extracellular space enclosed by a cellular membrane. For example, this method of gene therapy can be used in both humans and animals and it comprises the step of introducing a pharmacological dose of a DNA vector in a vehicle into an extracellular space in the human or animal. The extracellular space is enclosed by a cellular membrane. An example of this type of treatment would be the introduction of a DNA vector into the synovium of the joint.

Another method for gene therapy in humans and animals is introduction of a pharmacological dose of DNA vector in a vehicle into a fluid space in the animal or human. This fluid space is reabsorbed or remodelled by surrounding cells. Examples of this type of gene therapy would be the introduction of a DNA vector into a follicle of the thyroid, the synovium of the joint or the vitreous of the eye.

Another embodiment of the present invention is a method for gene therapy in humans and animals, comprising the step of introducing a pharmacological dose of a DNA vector into a fluid of the animals or humans. The flow and removal of this fluid is by endocytosis or pinocytosis from the surrounding cells. Examples of this occurs in the thyroid follicles, synovium of the joint, and in the vitreous of the eye.

Another embodiment of the present invention is a method of gene therapy in animals or humans comprising the step of introducing a pharmacological dose of DNA vector in a vehicle into a fluid space of the animal or human. The flow and removal of the fluid through a membrane causes the fluid to be filtered and the cells of the membrane then uptake the filtered DNA. An example of this is in the kidney and in the central nervous system, including the spinal column and brain.

One skilled in the art will readily recognize that a variety of other tissues, fluid space and extracellular spaces may be used. As long as they meet the specific criteria of being surrounded by a cellular membrane or the uptake in removal of the fluid or the filtering of the fluid.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

Example 1

Introduction of a Marker Gene into Cells of the Joint

A vector comprising the cytomegalovirus immediate early promotor, 5' untranslated sequences and an intron from SV40, 3' untranslated sequences from SV40 including the polyadenylation signal and a nucleic acid cassette containing the E. coli beta-galactosidase gene was combined with a vehicle containing 20% sucrose at pH 7.4. This solution was injected into the knee joint of rabbits using a transpatellar injection. As a control, an identical vector containing the E. coli chloramphenicol acetyltransferase gene in place of the beta-galactosidase gene was injected into joints on the opposite leg using identical methods. Three days after injection animals were sacrificed, the joint was disarticulated, and stained in a solution containing X-gal at pH 7.2. Under these conditions cells taking up and expressing the E. coli beta-galactosidase gene will be stained blue. Cells not expressing beta-galactosidase do not stain with this dye under these conditions.

The analysis showed that there was diffuse blue staining in regions of the joint representing the synovial cells of joint injected with the beta-galactosidase containing vector (figures 1A, 1C). No staining was observed over the tendons of bony surfaces of the joints. No blue staining was apparent in joints injected with the control vector (figures 1B, 1D).

These results demonstrate that cells of the joint are capable of taking up DNA vectors and are capable of expressing gene products encoded by the vector.

Example 2

Expression of anti-inflammatory factors to prevent inflammatory arthritis

The inflammatory attack on joints in animal models and human diseases may be mediated, in part, by secretion of cytokines such as IL-1 and IL-6 which stimulate the local inflammatory response. The inflammatory reaction may be modified by local secretion of soluble fragments of the receptors for these ligands. The complex between the ligand and the soluble receptor prevents the ligand from binding to the receptor which is normally resident on the surface of cells, thus preventing the stimulation of the inflammatory effect. Therapy consists of the construction of a vector containing the soluble form of receptors for appropriate cytokines (for example Il-1 or IL-6) together with promotors capable of inducing high level expression in structures of the joint and a vehicle which enables efficient uptake of this vector. This DNA is injected into affected joints where the secretion of an inhibitor for IL-1 such as a soluble IL-1 receptor or natural IL-1 inhibitor modifies the local inflammatory response and resulting arthritis.

This method is useful in treating episodes of arthritis which characterize many "autoimmune" or "collagen vascular" diseases. This method can also prevent disabling injury of large joints by inflammatory arthritis.

Example 3

Induction of "steroid response" by gene transfer of steroid receptors into cells of the joint

Current therapy for severe arthritis involves the administration of pharmacological agents including steroids to

depress the inflammatory response. Steroids can be administered systemically or locally by direct injection into the joint space.

Steroids normally function by binding to receptors within the cytoplasm of cells. Formation of the steroid-receptor complex changes the structure of the receptor so that it becomes capable of binding to specific sequences within the genome of the cell and altering the expression of specific genes. Genetic modifications of the steroid receptor can be made which enable this receptor to bind naturally occurring steroids with higher affinity, bind pharmacological forms of steroid at higher affinity, or bind to synthetic steroids. Other modifications can be made to create steroid receptor which is "constitutively active" meaning that it is capable of binding to DNA and regulating gene expression in the absence of steroid altogether.

One approach to treating arthritis is to introduce a vector in which the nucleic acid cassette expresses a genetically modified steroid receptor into cells of the joint. Expression of a constitutively active steroid receptor within cells of the joint induces the therapeutic effects of steroids without the profound systemic toxicity of these drugs. Of particular importance is the ability to target these genes differentially to specific cell types (for example synovial cells versus lymphocytes) to affect the activity of these cells.

Alternatively, steroid receptors which have a higher affinity for natural steroids can be introduced into the joint. These receptors exert an increased anti-inflammatory effect when stimulated by physiological concentrations of steroids or lower doses of pharmacologically administered steroids. Alternatively, constitution of a steroid receptor which binds a novel steroid enables the use of drugs which would affect only cells taking up this receptor. These strategies obtain a therapeutic effect from steroids on arthritis without the profound systemic complications associated with these drugs.

Example 4

Inhibition of prostaglandin synthase

Drugs which inhibit the enzyme prostaglandin synthase are important agents in the treatment of arthritis. This is due, in part, to the important role of certain prostaglandin in stimulating the local immune response. Salicylates are widely used drugs but can be administered in limited doses which are often inadequate for severe forms of arthritis.

Gene transfer is used to inhibit the action of prostaglandin synthase specifically in affected joints by the expression of an antisense RNA for prostaglandin synthase. The complex formed between the antisense RNA and mRNA for prostaglandin synthase interferes with the proper processing and translation of this mRNA and lowers the levels of this enzyme in treated cells.

Alternatively, genes encoding enzymes which alter prostaglandin metabolism can be transferred into the joint. These have an important anti-inflammatory effect by altering the chemical composition or concentration of inflammatory prostaglandin.

Example 5

Generating an animal model of inflammatory arthritis

Inflammatory arthritis is thought to result from an autoimmune attack on cells comprising essential structures of the joint. The association of various forms of arthritis with certain tissue transplantation (Histocompatibility) antigens, suggests that these antigens, or dysregulation of these antigens, may play an important role in the genesis of these diseases.

Animal models of inflammatory arthritis can be generated by gene transfer of vectors capable of expressing heterogenic (not self) or xenogeneic (other species) transplantation antigens within the joint, thus mimicking the effect of dysregulation of tissue transplantation antigens. This induces an autoimmune attack not only on the transplantation antigens themselves, but on other

antigens within the joint which are presented to the immune system in an abnormal manner.

Alternatively, overproduction of interferon in tissues is thought to induce dysregulation of class I antigens and potentiate a inflammatory reaction. This can be modeled in animals by introduction of vectors expressing interferon into cells of the joint.

Example 6

Gene transfer to enhance repair or regeneration of joints

The regenerative capacity of the joint is limited by the fact that chondrocytes are not capable of remodelling and repairing cartilaginous tissues such as tendons and cartilage. Further, collagen which is produced in response to injury is of a different type -- lacking the tensile strength of normal collagen. Further, the injury collagen is not remodeled effectively by available collagenase.

Gene transfer using promoters specific to chondrocytes (i.e., collagen promoters) is used to express different collagens or collagenase for the purpose of improving the restoration of function in the joints and prevent scar formation. Gene transfer into fibroblasts or muscle cells in the environment of the joint is used to locally secrete growth factors such as IGF-1. The growth factors maintain muscle mass and proliferation and enhance the strength of the damaged joint.

Gene transfer for these purposes is affected by direct introduction of DNA into the joint space where it comes into contact with chondrocytes and synovial cells. further, the genes infiltrate into the environment of the joint where they are taken up by fibroblasts, myoblasts, and other constituents of periarticular tissue. Additionally, the gene gun can be used interoperatively or via arthroscopy.

Example 7

Treatment of gouty arthritis by gene transfer

Gout is caused by the accumulation of uric acid in joints. This remains a common and painful disorder in the aging population despite medical management. Gene transfer into the joints is used to express products (for example enzymes) capable of degrading uric acid to non-toxic products. These products include urease or urate oxidase which are capable of metabolizing uric acid or urate binding globulins which render this compound more soluble and thus prevent crystalline formation within the joint.

Example 8

Persistent expression using episomal vectors

In each of the foregoing examples, transient expression of recombinant genes induces the desired biological response. In some diseases more persistent expression of recombinant genes is desirable. This is achieved by adding elements which enable extrachromosomal (episomal) replication of DNA to the structure of the vector. Vectors capable of episomal replication are maintained as extrachromosomal material and can replicate. These sequences will not be eliminated by simple degradation but will continue to be copied. Episomal vectors provide prolonged or persistent expression, though not necessarily stable or permanent, expression of recombinant genes in the joint. Persistent as opposed to stable expression is desirable to enable adjustments in the pharmacological dose of the recombinant gene product as the disease evolves over time.

Example 9

Gene delivery using the gene gun

The repair and regeneration of tissues comprising the joint following injury or surgery is enhanced by directly delivering recombinant genes to specific sites at the time of surgery. This is

done in conjunction with surgical reconstructions or arthroscopy using the "gene gun" (Yang, N.S. et al.; In vivo and in vitro gene transfer to mammalian somatic cells by particle bombardment; Proc. Natl. Acad. Sci. USA 87:9568-72 (1990)) to deliver particles coated with DNA to specific regions of the joint. The use of the gene gun and particle bound DNA enables constitution of gene expression at highly specific locations.

Example 10

Vehicles for gene delivery into cells of the joint

Initial experiments used DNA in solution for gene transfer into cells of the joint. This DNA is taken up by synovial cells during the process of these cells continually resorbing and remodeling the synovial fluid by secretion and pinocytosis. Gene delivery is enhanced by packaging DNA into lipophilic particles (for example lipofectin) which binds nonspecifically to hydrophobic membranes resulting in a fusion of the particle with the membrane and release of the DNA into the cytoplasm. Similarly, complexes of DNA and proteins which bind to receptors on the surface of cells in the joint enhances uptake and expression. Alternatively, particulate DNA complexed with $CaPO_4$ or polycations can be efficient substrates for phagocytosis by monocytes or other inflammatory cells.

Example 11

Ablation of inflammatory cells invading the joint.

Inflammatory cells invading the joint are ablated using vectors which contain the diphtheria toxin gene under the control of a promotor which is expressed only in leukocytes. These vectors are delivered to the joint as particles and are selectively taken up by phagocytotic cells. The uptake and expression of this vector results in expression of diphtheria toxin which is lethal to that specific cell. Alternatively, a vector which expressed herpes

thymidine kinase gene under the control of a specific promoter could be introduced into cells, and these cells are then ablated by administration of acytovin.

All patents and publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents an publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples of DNA vectors along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

What we claim is:

CLAIMS

1. A method of transfecting a cell in a structure of a joint comprising the step of introducing a DNA vector directly into the joint, said vector comprised of the following elements linked sequentially at appropriate distance for allowing functional expression: a promoter; a 5′ mRNA leader sequence; an initiation site; a nucleic acid cassette containing the sequence to be expressed; a 3′ untranslated region; and a polyadenylation signal.

2. The method of claim 1, wherein the cell is stably transformed.

3. The method of claim 2, wherein the cell is persistently transformed.

4. The method of claim 3, wherein the cell is transiently transformed.

5. The method of claim 1, further comprising injecting a vehicle with the DNA vector.

6. The method of claim 5, wherein the vehicle is selected from the group consisting of sucrose, protamine, polybrene, polylysine, polycations, proteins, $CaPO_4$, spermidine, soluble or insoluble particles, and matrices for slow release.

7. A method for ablation of inflammatory cells in a joint comprising the step of introducing a vector into the joint said vector comprised of the following elements linked sequentially at appropriate distances for allowing functional expression: a promoter which is specifically expressed in leukocytes; a 5′ mRNA leader sequence; an initiation site; a nucleic acid cassette containing the sequence for an ablation agent; a 3′ untranslated region; and a polyadenylation signal;

Wherein said vector is targeted for selective uptake by phagocytic cells in the joint.

8. The method of claim 7, wherein the vector is selectively targeted by attaching the vector to a molecule which attaches to the surface of a phagocytic cell.

9. The method of claim 7, wherein the ablation agent is diphtheria toxin.

10. The method of claim 7, wherein the vector is coupled to protein with trophic properties.

11. A method for treating a pathophysiological condition in humans, comprising the step of injecting a joint of a human with a pharmacological dose of a DNA vector in a vehicle; wherein a nucleic acid cassette in said vector encodes a protein, polypeptide or RNA.

12. The method of claim 11, wherein the pathophysiological condition is selected from the group consisting of periarticular arthritis, articular arthritis of cartilage and ligaments and articular arthritis of synovium.

13. The method of claim 11, wherein the nucleic and cassette contains sequences selected from the group consisting of cytokines, interleucins, receptors for natural ligands, genetically modified receptors for natural ligands, inhibitors of natural ligands, steroid receptors, genetically modified steroid receptors, cell adhesion molecules, genetically modified adhesion molecules, enzymes affecting prostaglandin metabolism, enzymes involved in extracellular matrix, RNA molecules inhibiting production of transplantation antigens, cell adhesion molecules, receptors involved in prostaglandin metabolism and enzymes involved in prostaglandin metabolism.

14. The method of claim 11, wherein the sequences are selected from the group consisting of a steroid receptor. IL-1, IL-6, a transplantation antigen and an antisense RNA to prostaglandin synthase.

15. A method for repairing and regenerating structures of the joints, comprising the step of injecting a joint of a human with a pharmacological dose of a DNA vector in a vehicle; wherein a nucleic acid cassette in said vector encodes a protein, polypeptide or RNA.

16. The method of claim 15, wherein the pathophysiological condition is selected from the group consisting of periarticular arthritis, articular arthritis of cartilage and ligaments and articular arthritis of synovium.

17. The method of claim 15, wherein the nucleic and cassette contains sequences selected from the group consisting of collagens, growth factors, extracellular matrix proteins, inhibitory growth factors, enzymes involved in modifying extracellular matrix and enzymes involved in remodelling extracellular matrix.

18. A method for treating arthritis in humans comprising the step of injecting an arthritis joint of a human with a pharmacological dose of a DNA vector in a vehicle; wherein a nucleic acid cassette in said vector encodes the sequence for a soluble receptor.

19. The method of 18, wherein the sequence is a soluble receptor for a cytokine.

20. The method of 19, wherein the cytokine is IL-1 or IL-6.

21. A method of treating arthritis in humans comprising the step of injecting an inflamed joint of a human with a pharmacological dose of a DNA vector in a vehicle; wherein a nucleic acid cassette in said vector encodes the sequence for a steroid receptor.

22. The method of claim 21, wherein the vector contains a genetically modified receptor.

23. The method of claim 21, wherein the nucleic acid cassette encodes an antisense RNA to prostaglandin synthase.

24. A method of making an animal model for inflammatory arthritis comprising the step of injecting a joint of an animal with a functional DNA vector in a vehicle; wherein a nucleic acid cassette in said vector encodes an antigen selected from the group consisting of a transplantation antigen, cell adhesion

antigen, antigen from a micro-organism, viral antigen, synthetic antigen and recombinant antigens.

25. The method of claim 24, wherein the transplantation antigen is selected from the group consisting of histocompatibility antigen, allogeneic transplantation antigen, and xenogeneic transplantation antigen.

26. The method of claim 25, wherein the histocompatibility antigen is selected from Class I and Class II transplantation antigens.

27. A transformed synovial cell, comprising nucleic acid incorporated into the synovial cell by gene transfer.

28. The synovial cell of claim 27, wherein said nucleic acid is selected from:

nucleic acid not normally found in synovial cell;

nucleic acid normally found in synovial cell but not express at physiologically significant levels;

nucleic acid normally expressed at physiologically desirable levels in synovial cell;

nucleic acid which may be modified for expression in synovial cell; and any combination thereof.

29. The synovial cell of claim 27, wherein the nucleic acid is capable of expressing a protein, polypeptide or antisense RNA.

30. The synovial cell of claim 29, wherein the nucleic acid encodes a steroid receptor.

31. The synovial cell of claim 29, wherein the nucleic acid encodes IL-1, IL-6, IL-8 or soluble IL-1 receptor.

32. The synovial cell of claim 28, wherein the nucleic acid encodes a transplantation antigen.

33. The synovial cell of claim 2[illegible] wherein the nucleic acid encodes an antisense RNA to pr[illegible]aglandin synthesis.

34. A method of targeting DNA vectors to joints comprising the steps of:

identifying human antibodies from patients with autoimmune arthritis, wherein said identified antibodies have the property of binding specifically to proteins on cells within the joint;

cloning the antibodies to develop monoclonals with the same epitope binding determinants; and

coupling DNA vectors to said monoclonals, wherein said DNA/monoclonal complex is delivered to the cells of the joint.

35. A method of treating gouty arthritis comprising the step of injecting a joint of a human with a pharmacological dose of a DNA vector in a vehicle, wherein a nucleic acid cassette in said vector encodes a product capable of degrading uric acid.

36. The method of claim 35, wherein said cassette encodes the nucleic acid sequence for urease or urate oxidase.

37. A method for gene therapy in a human or animal, comprising the steps of introducing a pharmacological dose of a DNA vector in a vehicle into an extracellular space in said human or animal, wherein said extracellular space is enclosed by a cellular membrane and wherein the nucleic acid cassette in said vector encodes a protein, polypeptide or RNA used in gene therapy.

38. The method of claim 37, wherein the extracellular space is the synovium of the joint.

39. A method for gene therapy in a human or animal, comprising the step of introducing a pharmacological dose of a DNA vector in a vehicle into a fluid space in said human or animal, wherein said fluid space is reabsorbed or remodelled by surrounding cells and wherein the nucleic acid cassette in said vector encodes a protein, polypeptide or RNA used in gene therapy.

40. The method of claim 39, wherein the fluid space is selected from the group consisting of a follicle of the thyroid, synovium of the joint and vitreous of the eye.

41. A method for gene therapy in a human or animal, comprising the step of introducing a pharmacological dose of a DNA vector

into a fluid of the animal or human, wherein the flow and removal of said fluid involved endocytosis or pinocytosis of surrounding cells and wherein the nucleic acid cassette in said vector encodes a protein, polypeptide or RNA used in gene therapy.

42. A method for gene therapy in an animal or human, comprising the step of introducing a pharmacological dose of a DNA vector in a vehicle into a fluid space of the animal or human, wherein the flow or removal of fluid causes said fluid to be filtered through a membrane comprised of cells capable of taking up the filtered DNA.

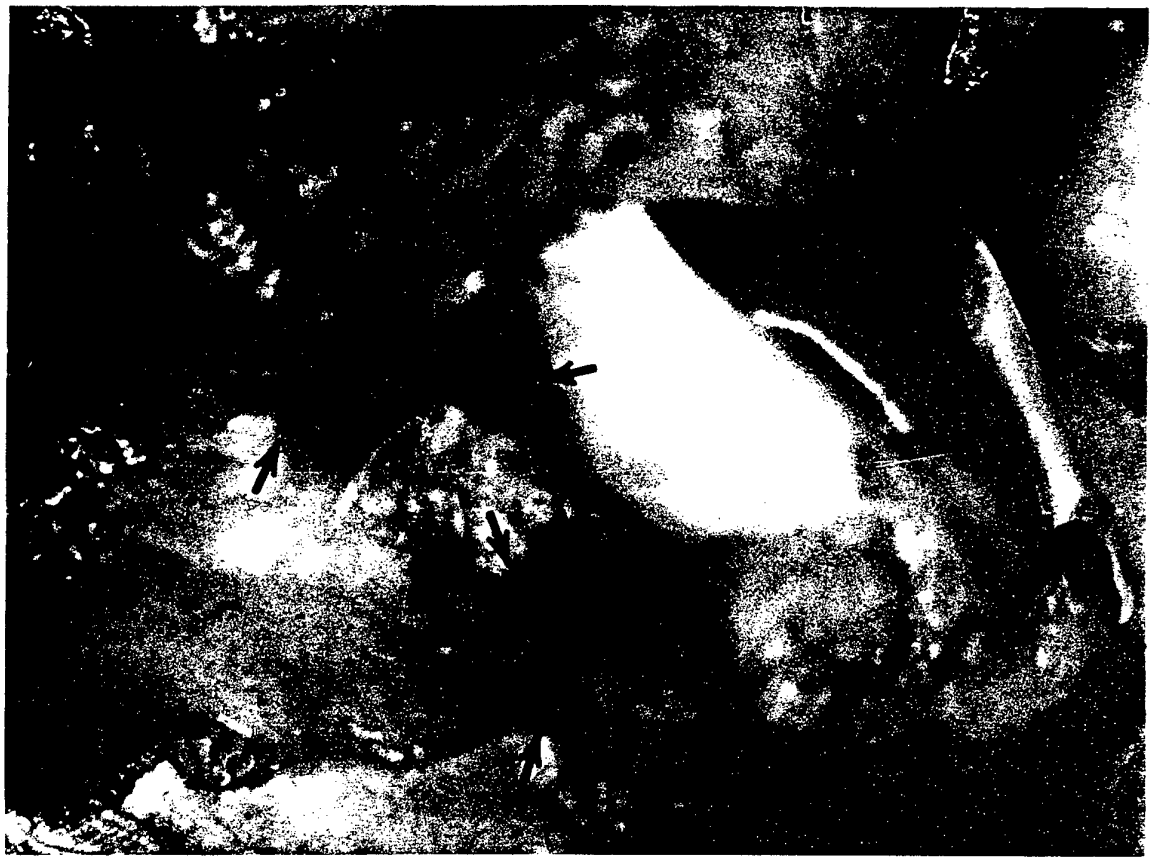

Figure 1A

Figure 1B

Figure 1C

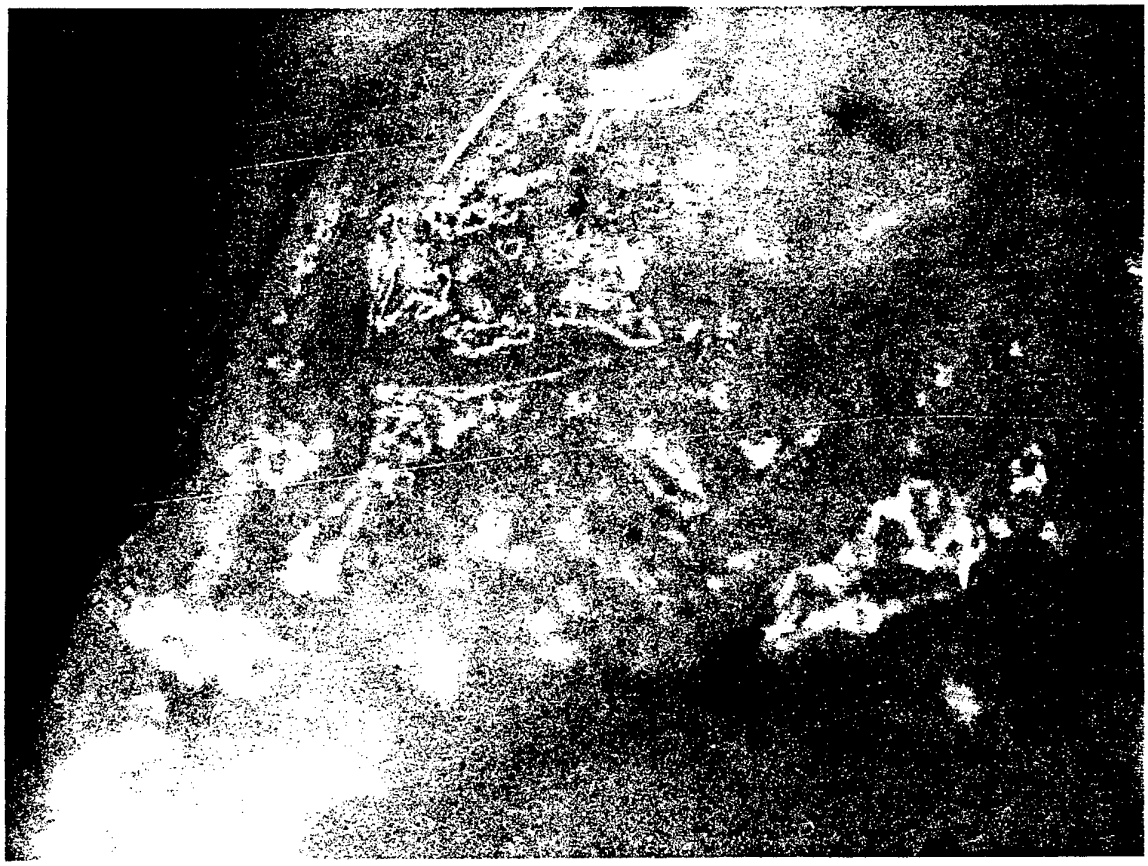

Figure 1D

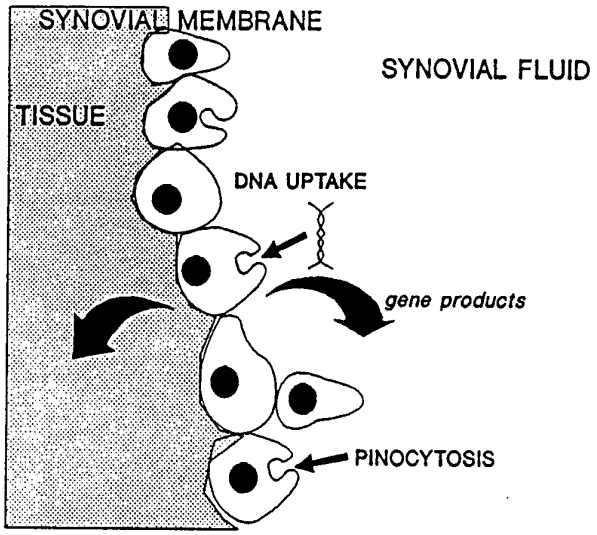

Figure 2

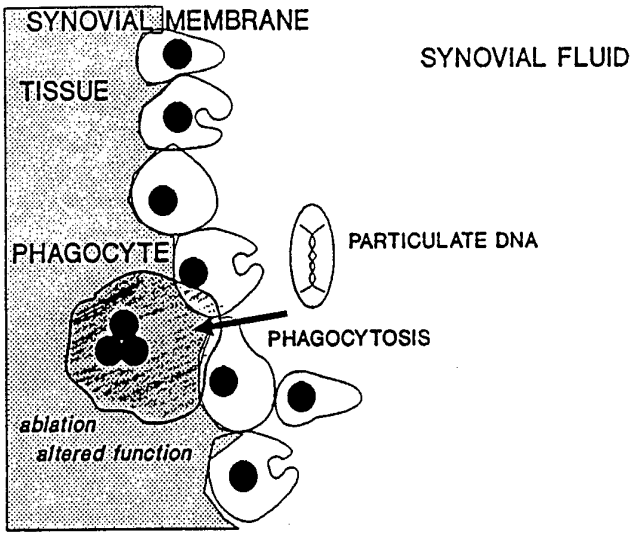

Figure 3

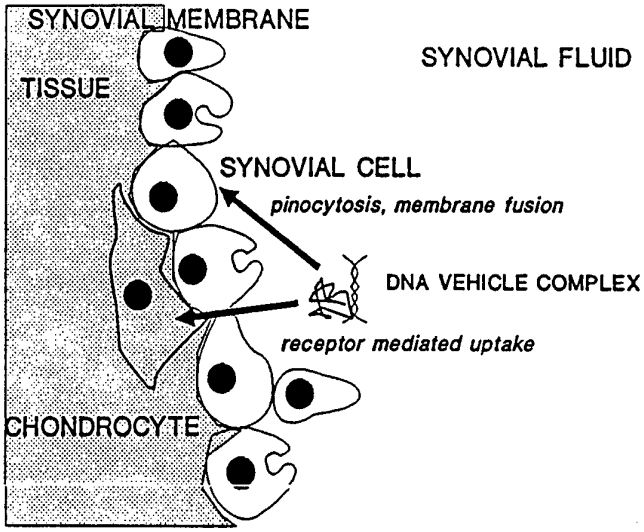
SYNOVIAL MEMBRANE
SYNOVIAL FLUID
TISSUE
SYNOVIAL CELL
pinocytosis, membrane fusion
DNA VEHICLE COMPLEX
receptor mediated uptake
CHONDROCYTE

Figure 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06479

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 48/00, 39/395; C12N 5/10, 15/85, 15/90
US CL :514/44; 424/85.8; 435/240.2, 172.3, 320.1; 935/62
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 424/85.8; 435/240.2, 172.3, 320.1; 935/62; 536/23.5, 24.1, 24.5; 530/388.1, 388.2, 391.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, FILE REGISTRY, BIOSIS, MEDLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	The Journal of Immunology, Volume 145, No. 11, issued 01 December 1990, Case et al, "IL-1 Regulation of Transin/Stromelysin Transcription in Rheumatoid Synovial Fibroblasts Appears to Involve Two Antagonistic Transduction Pathways, an Inhibitory, Prostaglandin-Dependent Pathway Mediated by cAMP, and a Stimulatory, Protein Kinase C-Dependent Pathway", pages 3755-3761, see page 3756.	27-29 30-33
X Y	Journal of Cellular Biochemistry, Supplement 16F, April 1992, Evans et al, "Gene Transfer to Joints for Arthritis Therapy", abstract V207, page 46, see entire abstract.	27-29 1-26, 30-42

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
19 AUGUST 1993

Date of mailing of the international search report
01 SEP 1993

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Ir. ational application No.
PCT/US93/06479

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,620,923 (Laboureur et al) 16 November 1971, col. 1, lines 4-20, col. 9, line 26-col. 10, line 26.	35-36
X,P Y	US, A, 5,166,320 (Wu et al) 24 November 1992, see entire document.	41-42 1-26, 34-40
Y	WO, A, 88/08450 (Finlayson) 03 November 1988, see abstract only.	35-36
X Y	EP, A, 273,085 (Myers) 06 July 1988, see entire document.	41-42 1-40
X Y	Chemical Abstracts, Volume 117, No. 1, issued 06 July 1992, Bandara et al, "Gene transfer to synoviocytes: prospects for gene treatment of arthritis", abstract 97w, page 105, see entire abstract.	1-6, 11, 12, 15, 16, 27-29, 37-42 7-10, 13, 14, 17-26, 30-36

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06479

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(Telephone Practice)
Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06479

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-23 and 35-42, drawn to a method of direct vector injection, classified in Class 514, Subclass 44;
II. Claims 24-26, drawn to a method of making an animal model, classified in Class 514, subclass 44;
III. Claims 27-33, drawn to transformed synovial cells; classified in class 435, subclass 240.2;
IV. Claim 34, drawn to a method of antibody targeting of DNA vectors to joints, classified in class 424, subclass 85.8.

The inventions listed as Groups I-IV do not meet the requirements for Unity of Invention for the following reasons:

The methods represented by Groups I, II and IV are not directed to a single inventive concept since Group I requires transfection of cells for the purpose of effecting a biological or pharmacological effect, whereas Group II requires the creation of an animal model which would have industrial applicability for the testing of putative antiarthritic drugs, and Group IV requires a diagnostic step, the development of monoclonal antibodies and the coupling of the antibodies to a DNA vector. Group III is distinct from the other groups as it is a product which is not used to carry out any of the claimed methods, but rather has industrial applicability apart from the environment of the joint; e.g., in cell culture for the generation of products which encode desirable proteins. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.