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REVIEW ARTICLE

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Gene therapy for arthritis

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Abstract An accumulating body of evidence shows that gene therapy can be successfully used to treat animal models of arthritis. Based on this success, a clinical trial of gene therapy for rheumatoid arthritis has been initiated. We review the methods and genes used for the previous gene transfer experiments, including our own. Retroviral ex vivo gene transfer and adenoviral in vivo gene transfer were utilized most frequently. Most of the gene transfer strategies aimed at suppression of synovial inflammation, while our study attempted to convert a phenotype of synovial cells. Gene transfer could be used for part of the future therapy for RA. In basic research studies, gene transfer is of great help in defining new target molecules to treat arthritis.

Key words Arthritis · Gene therapy · Cell cycle · Cyclin-dependent kinase inhibitor

Introduction

Beyond this millennium, the clinical application of gene therapy will expand to many human diseases. This modern technology was first applied to the treatment of congenital disorders. Expression of the transferred genes compensated for inherited defects caused by genetic anomalies. It was also applied to the treatment of neoplasm, which is fatal if treated only by conventional methods. The application was limited to these diseases because of potential risks associated with the introduction of foreign genes and the disruption of intrinsic genomes. The results of gene transfer experiments with animals and humans have convinced us that gene therapy can be carried out safely if its protocol is designed carefully. This prompted researchers to test the application of gene therapy to nonlethal, retractable diseases, such as rheumatoid arthritis (RA) and atherosclerosis.

RA is characterized by the chronic inflammation of multiple joints. It is never benign, because it can cause the early death of the patient and seriously affects their quality of life. Thus, it is one of the diseases that require state-of-the-art medicine, such as gene therapy. We review the theory and practice of gene therapies used to treat RA and its animal models.

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Pathology of RA

Synovial tissue affected by RA is filled with granulation tissues which consist of activated macrophages, fibroblasts, and T and B lymphocytes. Through mutual interactions of these cells, macrophages produce a large quantity of proinflammatory cytokines and chemokines, while B cells secrete immunoglobulins, including IgM and IgG rheumatoid factors. Although cytokines derived from T lymphocytes are not abundant in the synovial tissues, T lymphocytes must play a critical role in the inflammation. This view is supported by the efficacy of antirheumatic drugs that inhibit signaling molecules of T lymphocytes, and by the HLA-DRB1 epitope, which is shared by a majority of the patients and is possibly associated with disease severity. In response to the proinflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6, the synovial fibroblasts proliferate vigorously and release tissue-degrading enzymes. These cytokines also activate local osteoclasts. The resulting hyperplastic and destructive synovial membrane, termed a pannus tissue, irreversibly damages the cartilage and bone of the affected joints.

These multiple steps involved in the pathology of RA can be divided into two general aspects: the inflammatory and proliferative phases. In the inflammatory phase, unknown (auto)antigen(s) elicit T cell response in the

synovial tissues. This is followed by an influx of macrophages and other immunocompetent cells into the tissue. The leukocyte migration is promoted by neovascularization and the up-regulated function of adhesion molecules on the vascular endothelium. The accumulated leukocytes, especially synovial macrophages, produce proinflammatory mediators, which promote synovial fibroblasts to escape the differentiated state and to proliferate. This proliferative phase appears to be less dependent on initial T lymphocyte activation because macrophage-derived cytokines mostly account for the inflammatory events in the joints. Therapeutic intervention in the arthritis could be designed to aim at each of these steps.

Gene transfer techniques into the joints

Although RA could affect various organs, the primary target is the joint. However, conventional ways of drug administration, e.g., oral, intravenous, and intramuscular routes, have poor access to the joints. Exposure of the drugs to the synovial leukocytes and fibroblasts depends primarily on passive diffusion through the synovial capillaries. Inevitably, circulating leukocytes are exposed to a higher concentration of the drugs, which is sometimes responsible for adverse effects. On the other hand, joint space is easily accessible by intraarticular injection. This method is preferred in order to avoid systemic effects of the therapeutic reagents, but its efficacy is limited by the relatively short half-life of materials administered by this route. Repeat injections are usually required for the effects to continue.

Theoretically, intraarticular gene transfer should circumvent all of these problems. Therapeutic genes can be transferred directly into the joints. Transferred genes are expressed primarily by the local cells. Once the genes are incorporated by the synovial cells, they are expressed in situ, ensuring a local presence of the gene products for a long time. In this setting, the closed synovial space is preferred in order to avoid systemic adverse effects. If the genes are stably transferred, repeat administration is not required.

For gene transfer to treat arthritis models, both direct and indirect methods have been employed. Direct gene transfer involves intraarticular injection of the therapeutic genes. Genes are inserted either in viral vectors or in nonviral plasmid vectors to allow their expression. The only viral vector that has been employed for direct gene therapy of arthritis is a replication-defective adenovirus. This vector can deliver inserted genes to a wide variety of cells with high efficacy. The wild-type adenoviruses enter the cells via integrins or its specific receptors, replicate in the cells, and eventually lyse the cells for further reinfection. The adenovirus vector for gene therapy has gene deletion to disable viral replication, sparing a space for the insertion of exogenous genes. Thus, the replication-defective adenoviruses should express the inserted genes without injuring the host. However, entry of a large amount of viruses damages the cell membranes. Exposure of viral particles to the host immune system provokes immunity against the virus, which could injure the host tissues when the viruses are delivered repeatedly. These are especially troublesome because adenovirally transferred genes stay in the episome and are not integrated into the genome. Repeat viral transfer is generally required for long-term expression.

Adeno-associated virus is a vector that also delivers the genes into a wide variety of host cells. Although, unlike the adenovirus, the viral DNA are stably integrated into the genome in a site-specific manner, the technical difficulties of large-scale preparation have been hampering this application for the treatment of arthritis. Herpes simplex and human immunodeficiency virus vectors can deliver exogenous genes specifically to neurons and lymphocytes, respectively. As a human immunodeficiency virus vector could modify immune reactions, it is an attractive candidate for future use in arthritis treatment.

To avoid immune reactions against gene products of vector viruses, DNA per se has been used for gene transfer. This "naked DNA" technique utilizes the ability of cells to take up exogenous DNA. It was demonstrated that expression of DNA delivered via intradermal and intramuscular routes was detectable, and sufficient to induce host immunity against the gene products. However, transfer efficiency was far lower than the virus-mediated methods. Unfortunately, intraarticular injection of naked DNA was ineffective in transferring genes to the joint tissue. The efficiency was not improved with help of cationic liposomes, which are widely used to introduce foreign genes into cultured cells. A high concentration of the liposome even damaged the joint tissues. A hemagglutinating virus of Japan (HVJ: Sendai virus)–liposome suspension was developed to increase the transfer efficiency without increasing cytotoxicity. The viral membrane components fused in the liposomes facilitate entry of the exogenous gene to the target cells. This unique technique was successfully used to transfer the genes to the synovial tissues and articular cartilage.[1,2]

In indirect gene transfer, some of the synovial tissues have to be removed from the joints of the patients. Synovial fibroblasts were isolated and the genes of interest were introduced. The fibroblasts expressing the exogenous genes are then selected, expanded in culture, and transferred back into the joints affected by arthritis. Since the genes have to be stably expressed, retroviral vectors have been preferentially employed. The gene transfer efficiency of the retroviruses is relatively low, but a selection procedure overcomes this problem. Although this technique warrants the expression of the transferred genes, it requires a series of laborious procedures, and therefore is not suitable for wide clinical application.

Genetic transfer of anti-inflammatory molecules for treatment of arthritis

Several genes have been used for the experimental treatment of arthritis. The gene of IL-1 receptor antagonist (IL-1ra) was used in the earliest series of experiments. IL-1

is found abundantly in joints affected by RA and osteoarthritis (OA).[3] It appears to be of primary importance in inducing protease synthesis, and thus in cartilage and bone degradation. IL-1ra is a natural antagonist of IL-1, inhibiting IL-1α and IL-1β from binding to their specific receptors. Administration of the recombinant IL-1ra protein inhibited rodent models of RA.[4] Subsequently, the IL-1ra gene was retrovirally introduced into synovial fibroblasts, which were replaced in the inflamed joints of the animals. This gene therapy successfully ameliorated staphylococcal cell wall-induced arthritis (SCWA),[5] collagen-induced arthritis (CIA) in mice,[6] and antigen-induced arthritis in rabbits.[7] The same technique was also used to treat an animal model of OA,[8] and to reduce the pathology of human rheumatoid synovial tissues transplanted in severe combined immunodeficient mice.[9] It was first used in the human gene therapy trial of RA.[10]

Another gene of anti-inflammatory molecules used successfully in the treatment of arthritis models is viral IL-10.[4,11–13] IL-10 is produced primarily by type II T lymphocytes, i.e., Th2 and Tc2 cells, and down-regulates macrophage functions and cytokine production by type I T cells. Thus it suppresses release of proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α, and interferon (INF)-γ. Simultaneously, it activates B lymphocytes for MHC class II expression and synthesis of immunoglobulins, including rheumatoid factors. It also co-stimulates T cells for differentiation. It was reported that IL-10 is elevated in the serum and synovial fluid of patients with RA.[14,15] However, since IL-10 could be pro- or anti-inflammatory, its overall effect on the pathology of RA is still unclear. Viral IL-10, a product encoded by the Epstein–Barr virus, is highly homologous to human and murine IL-10. It shares anti-inflammatory functions with IL-10, but down-regulates B cells for MHC class II expression and does not stimulate T cells. This gene was incorporated in replication-defective adenoviruses, and injected intravenously or intraarticularly to mice with CIA or rabbits with antigen-induced arthritis. The viral IL-10 inhibited the inflammatory processes and ameliorated the pathology of the arthritis. It should be noted that the effect was observed in contralateral joints: injection of the recombinant viral IL-10 adenoviruses into one paw inhibited the arthritis of the noninjected paw of the same animal. It is not clear if viral IL-10-expressing cells migrated to the other joints, or if viral IL-10 exerted its effect via the systemic circulation. Nevertheless, the report encouraged us to apply intraarticular injection of adenoviruses in a clinical setting, since injection to each of the affected joints appears unnecessary.

Use of other anti-inflammatory cytokines for gene therapy of arthritis was described in a few reports. A plasmid containing transforming growth factor (TGF)β cDNA was administered intramuscularly into rats with SCWA.[16] Presumably, the expressed TGFβ suppressed systemic immune reactions since intraarticular injection of TGFβ protein induced synovial inflammation.[17] Systemic gene transfer experiments to treat arthritis have limited developmental value for clinical applications. Most of the genes used were those encoding proteins which had already been shown to be effective when administered systemically. Although it was reported that TNF-α blockade by intravenous injection of recombinant adenoviruses containing soluble TNF-α receptor gene ameliorated CIA in rats,[18] soluble TNF-α receptor protein (etanercept) is already administered to treat actual RA patients in clinics. Considering the potential hazard associated with systemic gene transfer, it is more realistic to inject the gene products repeatedly or continuously to the patients.

It is interesting to note that there are no reports of the success of IL-4 gene therapy. This cytokine belongs to type II cytokines, and is essential for the differentiation and effector function of type II T lymphocytes. It antagonizes type I T lymphocyte differentiation strongly. Transfer of the IL-4 gene was used successfully to treat experimental allergic encephalomyelitis, an animal model of multiple sclerosis. This disease model is mediated purely by Th1 cells, and can be suppressed by the administration of IL-4. The failure of arthritis treatment with IL-4 gene transfer suggests that arthritis is not simply mediated by Type I T cells.

Gene therapy trials to suppress subcellular responses of synovial cells under the inflammatory milieu have also been reported. C-terminal Src kinase inhibits kinase activity of Src-family kinases. Since Src-family kinases participate in the activation processes of a wide variety of cells, including fibroblasts, T cells, B cells, and osteoclasts, its inhibition should suppress inflammation in arthritis. This was shown to be the case by adenoviral gene therapy of adjuvant arthritis in rats.[19] Another targeted intracellular molecule was NF-κB, which is important for proinflammatory cytokine release as well as proliferation of the synovial cells. Antisense oligonucleotides were successfully introduced into the synovial tissues to treat mice with CIA.[2] Although oligonucleotide administration does not fall within the area of gene therapy in a strict sense, this report showed that the HVJ-liposome technique developed in Japan is useful for arthritis gene therapy.

An ultimate “anti-inflammatory” effect can be achieved by removing synovial cells from the inflamed joints. Synovial fibroblasts as well as activated lymphocytes express Fas receptors, and are sensitive to Fas-inducing apoptotic cell death. Thus, ectopic expression of Fas ligands (FasL) in the synovial tissues induces extensive apoptosis in rheumatoid synovial tissues.[20] In vivo adenoviral transfer of FasL gene ameliorated CIA.[21] However, it remains unclear whether the effect would last very long if the inflammatory milieu persists. This cell death-inducing strategy is probably more effective in combination with other anti-inflammatory therapies. Another apoptosis-inducing treatment was tried with synovial fibroblasts transfected with a gene of galectin-1, which induces apoptosis of activated T lymphocytes.[22] Intraperitoneal injection of the transformed fibroblasts into mice with CIA suppressed pathology of the arthritis. Again, the effect of this systemic gene transfer was also observed by intraperitoneal administration of recombinant galectin-1 protein.

Promoting differentiation of synovial cells by gene transfer

Most approaches to the suppression of inflammation required the gene products to be liberated from the cells or displayed on the cell surface to exert their effects. As stated earlier, in the case of nonlethal diseases, it is safer and more realistic to administer the gene products continuously. Indeed, IL-1ra or IL-10 administration was shown to be effective before the gene transfer experiments were carried out.[4,23,24] In addition, inhibition of a certain pathway of inflammation might be compensated in the long run by the activation of other pathways unless factors responsible for the initiation of inflammation are eradicated.

Using a gene transfer technique, we attempted to convert synovial cells to those resistant to proinflammatory stimuli. It is reported that apoptotic cell death of synovial cells is common in rheumatoid synovial tissues,[25] suggesting that synovial hyperplasia is a result of rapid cell cycle progression of the synovial cells rather than impairment of cell death. Thus, we assumed that suppression of the cell cycle of synovial fibroblasts should effectively prevent joint destruction.

Cell cycle turnover is controlled largely by kinase activity of cyclin-dependent kinase (CDK)-cyclin complexes. The complexes phosphorylate proteins of the retinoblastoma family and activate transcription factors such as E2F. The E2F family of transcription factors are essential for DNA synthesis in the S phase. The CDK inhibitors (CDKIs) are intracellular proteins that specifically bind to and inactivate CDK-cyclin complexes, and play a crucial role in the inhibition of cell cycle progression.[26]

Our study demonstrated that the rheumatoid synovial fibroblasts, unlike nonrheumatoid synovial or other fibroblasts, express $p16^{INK4a}$, a member of the CDKIs, when their growth is inhibited in culture.[27] The endogenous $p16^{INK4a}$ protein is maximally expressed by senescent fibroblasts and differentiated neurons, which are incapable of further proliferation.[28,29] The induction of the $p16^{INK4a}$ gene in proliferating cells suppresses cellular doubling and induces the phenotype characteristic of senescent cells.[30–32] Thus, it is termed "a senescence gene." The selective expression of the $p16^{INK4a}$ gene in synovial cells suggested that its in vivo induction of the senescence gene could be used as a new therapeutic strategy for arthritis treatment. This was substantiated by adenoviral gene transfer of the $p16^{INK4a}$ gene.[27] The artificial senescence induction inhibited in vitro proliferation of synovial fibroblasts stimulated by proinflammatory cytokines. The gene was subsequently transferred into the synovial tissues of rats with adjuvant arthritis. The treatment strongly suppressed the pathology of the arthritis.

This report utilized gene transfer, for the first time, to convert the phenotype of synovial fibroblasts. It also established the new principle that cell cycle control is effective in treating arthropathy with proliferative synovitis. Thus, gene transfer could be used as a tool to probe new therapeutic strategies. In this regard, recombinant adenoviruses were used to introduce a gene of IκBα, which blocks NK-κB function, to rheumatoid synovial cells in vitro.[33,34] These experiments clearly showed that NF-κB is one of the molecules that should be targeted for the treatment of RA.

Future treatment

Several blueprints for gene therapy of RA have been reported. However, since the pathology of RA involves multiple aberrations of immunocompetent cells and mesenchymal cells, it is assumed that no single treatment will be powerful enough to achieve a complete cure of RA. Exposure to exogenous antigen(s) eliciting initial inflammation, if any, should be avoided. Lymphocyte migration into the synovial tissues should be inhibited by blocking neovascularization and/or lymphocyte tracking. Interactions among the recruited lymphocytes should be inhibited to suppress the release of proinflammatory mediators. Antagonizing the mediators should also be effective. Our study showed that promoting the differentiation of synovial fibroblasts by the senescence gene is useful to prevent joint destruction. Osteoclast activation should be inhibited for the same purpose. A combination of these strategies will probably be necessary to cure RA without eliciting serious adverse effects. Gene therapy could contribute in some aspects to the ultimate treatment of RA, although conventional pharmaceuticals will be preferred for clinical use. Nevertheless, gene transfer in basic laboratories will help us to define molecular targets to research develop the ultimate treatment.

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