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Therapeutic gene transfer for rheumatoid arthritis

La terapia genica nell'artrite reumatoide

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RIASSUNTO

L'artrite reumatoide è una grave malattia sistemica autoimmune caratterizzata da una infiammazione sinoviale cronica che porta alla distruzione articolare. I trattamenti convenzionali finora adoperati non si sono dimostrati sufficientemente efficaci. La terapia genica dell'artrite reumatoide prende in considerazione i maggiori artefici dell'infiammazione o della distruzione articolare: agenti bloccanti il TNF-α o l'IL-1 (quali anticorpi monoclonali anti TNF-α, recettore solubile del TNF-α, recettore solubile dell'IL-1 di tipo II, recettore antagonista dell'IL-1), citochine antinfiammatorie (quali IL-4, IL-10, IL-1), fattori di crescita. In questa malattia poliarticolare, il vettore che esprime la proteina terapeutica può essere somministrato sia localmente (tramite iniezioni intra-articolari) o per via sistemica (con iniezioni extra-articolari). Nei modelli sperimentali sono stati usati tutti i principali vettori, inclusi i più recenti lentivirus e virus adeno-associati. Il trasferimento genico ex vivo è stato effettuato con cellule sinoviali, fibroblasti, cellule T, cellule dendritiche e altri tipi cellulari di origine xenogenica. La terapia genica in vivo è il metodo più semplice, sebbene meno controllato. I trials clinici nell'artrite reumatoide umana sono partiti con retrovirus esprimenti l'antagonista del recettore dell'IL-1 ex vivo, dimostrando la fattibilità della strategia con la terapia genica. Il target principale deve essere stabilito con esattezza per cui, attraverso studi preclinici, saranno necessarie ricerche più approfondite.

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INTRODUCTION

Rheumatoid arthritis (RA) is a common and severe disease. Its prevalence in adults is about 0.5%. It not only causes joint pain and severe disability but also increases mortality. RA is an inflammatory autoimmune disease whose the inciting stimulus is unknown, but the cascade of immunological and inflammatory reactions has been elucidated. These reactions produce inflammatory synovitis promptly followed by irreversible joint and bone destruction (1). Available treatments for RA fail to provide long-lasting control of the symptoms or disease progression. The beneficial effects of conventional second-line therapy are incomplete and usually short-lived, despite the progress brought by the introduction of methotrexate in the 1980s. Recent improvements in our knowledge of the pathophysiology of RA have led to the development of biological treatments. Recently developed agents for biological therapy fall into two categories: TNF-α inhibitors and IL-1 inhibitors. These biological treatments provide significant efficacy in the short and medium term in many patients (2-4). Gene therapy is a new avenue of research that may lead to effective biological treatments. Experimental gene therapy, as recently shown in animal models (Tab. I) has focused on the pivotal mechanisms of inflammation and/or joint destruction.

The strategy of gene therapy needs to define three parameters:

- the gene encoding the molecule used for its therapeutic effect: for instance, the IL-1 receptor antagonist (IL-1Ra);
- the vector to be used to transfer the gene: vectors are frequently viral particules, but may be also of non viral origin (plasmids, or synthetic vectors);
- the targeted tissue: in RA, the choice is first between systemic administration (intra-venously

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Tabella I – Gene therapy in animal models of rheumatoid arthritis:main findings.

Gene	*Vector*	*Local/systemic*	*Experimental Model*	*References*
TGF ß	Plasmid	systemic	Streptoccal cell wall induced arthritis	5
TGF ß	Splenocytes infected ex vivo with retrovirus	systemic	CIA	6
IFN ß	Fibroblasts DBA infected ex vivo with retrovirus	systemic	CIA	7
Viral IL-10	Adenovirus	systemic	CIA	8
Viral IL-10	Adenovirus	Local and systemic	CIA	9
Viral IL-10	Adenovirus	Local	CIA	10
Viral IL-10	Adenovirus	Local	AIA	11
Viral IL-10	Adenovirus	Systemic	CIA	12
IL-10	Plasmid	Systemic	CIA	13
IL-10	Splenic T cells transfected ex vivo with plasmid	Systemic	OVA-induced arthritis	14
IL-10	Adenovirus	Systemic	CIA	15
IL-1Ra	Synovial fibroblasts infected ex vivo with retrovirus	Local	AIA	16
IL-1Ra	Synovial fibroblasts infected ex vivo with HSV	Local	IL-1ß induced arthritis	17
IL-1Ra	Synovial fibroblasts infected ex vivo with retrovirus	Local	IL-1ß induced arthritis	18
IL-1Ra	Synovial fibroblasts infected ex vivo with retrovirus	Local	Bacterial cell-wall (BCW) arthritis	19
IL-1Ra	Synovial fibroblasts infected ex vivo with retrovirus	Local	IL-1ß induced arthritis	20
IL-1Ra	3T3 Fibroblasts infected ex vivo with retrovirus	Local	CIA and ZIA	21
IL-1Ra	Adenovirus	Local	IL-1 induced arthritis	22
Viral IL10 + sTNFR-Ig	Adenovirus	Systemic	CIA	23
sTNFRI	AAV	Local	TNFa dependant arthritis	24
sTNFR	Spleen cells infected ex vivo with retrovirus	Systemic	CIA	25
TNFR-IgG	Adenovirus	Systemic	CIA	26
TNFRI-IgG	Adenovirus	systemic	Infection by Listeria monocytogenes	27
TNFRI-IgG	Adenovirus	Local and systemic	CIA	28
TNFRI-IgG + IL-1R-IgG	Adenovirus	Local	AIA	29
IL-4	Adenovirus	Local	CIA	30
IL-4	Adenovirus	Local	AIA	31
IL-4	Adenovirus	Local	CIA	32
IL-4	Retrovirus	Local	AIA	33
IL-4	AAV	Systemic	CIA	34
IL-4	DC infected ex vivo with retrovirus	Systemic	CIA	35
IL-4	DC infected ex vivo with adenovirus	systemic	CIA	36
IL-4 or IL-13	Encapsulated CHO fibroblasts transfected with plasmid	Systemic	CIA	37
IL-4, IL-10 or IL-13	CHO fibroblasts transfected with plasmid	Systemic	TNF transgenic mice	38
IL-13 or IL-4	CHO fibroblasts transfected with plasmid	Systemic	CIA	39
IL-1RII	Keratinocytes transfected with plasmid	Systemic	CIA	40
Fas L	Adenovirus	Local	CIA	41
Fas L	T lymphoma cells transfected with plasmid	Local	SCID-RA	42
P16INK4a or p21CIP1	Adenovirus	Local	CIA	43
P16INK4a	Adenovirus	Local	AA	44
IKKb	Adenovirus	Local	AA	45
Thymidine kinase	Adenovirus	Local	CIA	46

on intra-muscular) or local administration (ie directly within the joint).

Recent severe side effects observed during gene therapy of children suffering severe combined immune deficiency must focus the medical and scientific community on safety issues of gene therapy. This fascinating strategy have to be strictly evaluated in terms of risk/benefit ratio for patients; it can be concluded from the literature that this analysis is clearly in favor of the strategy of gene therapy, but that pre-clinical studies must be accumulated before clinical trials in non short term lethal) diseases (47).

VECTORS FOR GENE THERAPY OF RA

Several gene delivery systems have been developed during the last decade which include viral and non-viral vectors (48). Each of the vector strategies has

its strengths, as well as weaknesses and differs by its efficiency to deliver a therapeutic gene into a given target tissue.

Non viral vectors

Plasmids can be used for gene therapy. They are fragments of DNA from bacterial origin. One of the main advantage of plasmids in gene transfer is they can integrate large exogenous genes. They are characterized by an excellent safety and low immunogenic properties. These vectors are easy to produce on a large scale for clinical use (49). They may be transfer to cells by simple injection, but this plain method (naked DNA) is poorly efficient. In gene therapy, plasmids are generally used combined to an enhancing technology.

- Plasmids and chemical technology: cationic lipids: cationic lipids form spontaneously liposomes. Plasmidic DNA can form complexes with theses liposomes; this complex is able to penetrate the cell membrane, by endocytosis or fusion of the cell membrane with the lipoplexe. Actually, experimental protocols with this technique in experimental models of arthritis remain to be done.
- Plasmids and physical technology: electrotransfert. Electric pulses has be used to introduce foreign DNA into various cell types. This method, called cell electroporation, has been successfully applied to in vivo models. Our group and others recently reported an efficient method for transferring DNA into muscle fibers, in which an intramuscular injection of plasmid DNA is followed by delivery of low-field-strength, long-duration, square-wave electric pulses through external electrodes (50). Exposure of skeletal muscle to a pulsed electric field increases more than 100-fold the expression of a transgene injected i.m in mice. Moreover, the number of transfected muscle fibers is also increased by a 10 to 50 fold factor. This electric field-mediated transfection of plasmids encoding a gene of interest, also called electrotransfer, ensures not only a high level of transgene expression in the transfected muscle, but also elevated sustained plasma levels of the protein gene product, which is continuously released into the circulation by the highly vascularized muscle cells. Thus, systemic delivery of various proteins, such as factor IX or erythropoetin, has been described. Intramuscular electrotransfer has been used in several animal models.
- Plasmids and cell biology: cell can be considered as a vector. Cultured cells may be used as vectors after transfection. They are able to synthesize and secrete the therapeutic protein, in vitro and in vivo. The transfected cells may be inert or active. If inert, the transfection use them as biologic pumps; fibroblasts or keratonocytes are used in this occurrence. Conversely, the specific activity of the cells to be transfect may be useful in some specific protocols: for tissue repair or immunomodulation.

Viral vectors

Their are the largely most used vectors in clinical protocols of gene therapy. Virus are used because of their capacity to integrate DNA fragments and their natural ability to enter the cells then using the cell machinery to synthesize the proteins encoded by the viral genome. The main advantage of a viral system in gene therapy is the ability to obtain high levels of the therapeutic protein. The main problems encountered with viruses are their immunogenicity and the integration of the viral genome within the genome of the host. Most used vectors are retrovirus, adenovirus, and adeno-associated virus (AAV).

- Retroviral and lentiviral vectors: Moloney Murine Leukemia Viruses (MoMLV)-derived Retroviral Vectors (RV) are the most frequently used vectors in gene therapy studies in both animal models and in clinical trials. Stable transgene integration into dividing cells and absence of immune reaction against vector particles are the main advantages of recombinant RV vectors. In early studies on RA, synoviocytes harvested surgically from the joints of animals could easily be transduced *ex vivo* using even low titers of recombinant RV. Transduced cells expressed the transgene *in vitro* for at least 5 weeks and fell rapidly over time (51). Engraftment of *ex vivo* transduced syngeneic synoviocytes into the rat arthritic joints allowed expression of the transgene for about 2 weeks (19, 52). Human synovial fibroblasts are also transduced efficiently (>70%) with RV vectors encoding IL1Ra, sTNFRp55 or IL10 resulting in secretion of soluble molecules for at least 60 days in culture conditions. Implantation of the IL1Ra, or IL10 transduced human fibroblast into SCID mice has resulted in reduced perichondrocyte degradation as well as synovial cell invasion (53).

As expected, direct *in vivo* transduction of syn-

oviocytes could be achieved only using high-titer (>5.10^7) RV (20, 52). Transgene expression was transient declining in rat after 1 week and in rabbit after 4 weeks following injections (51). RV-derived MFG vectors carrying the IL-1Ra gene have been administered locally into joints and systemically into haematopoietic stem cells (54). Although transient (4 to 6 weeks), efficient intra-articular secretion of human IL-1Ra was observed in several animal models of arthritis exceeding its usually estimated therapeutic level. Recently, the MFG-IL-1Ra vectors were used to transduce human synoviocytes *in vitro* and in two clinical studies for (55).

In contrast to RV-derived vectors, lentivirus-derived vectors enable the stable transduction of both dividing and non-dividing cells**.** Nevertheless, the potential risk of insertional mutations due to integration of additional virus sequences into the human genome is a high concern and should be further studied before the use of retrovirus or lentivirus in a clinical setting.

- Adenoviral vectors: Recombinant vectors derived from different serotypes of human adenovirus (Ad) have been used extensively in animal models of RA. The host range of the Ad vectors can be changed by modifying the viral fibre protein so that they can interact more properly with different cell surface components (56). A dose dependent efficacy has been observed by different groups with concomitant development of synovitis in rabbit (16), rat (57), rhesus monkey (46), and mice in which transgene expression weakened after the first week of transduction (58). Ad vectors transduce very efficiently synoviocytes *ex vivo*. However their use is hampered by enhanced inflammation in the synovium, limited transgene persistence and difficulty of repeated inoculation. Further improvements in producing higher titer of gutted or weak immunogenic Ad vectors are needed for a long-term transgene expression.
- Adeno-associated virus: The adeno associated virus is a small single stranded DNA virus. Vectors derived from AAV have several properties favorable to their use in gene therapy for rheumatoid arthritis. Their natural innocuousness, wide tropism spectrum (59), long-term transgene expression pharmacologically regulable (60) and weak immunogenicity are particularly important in the context of a chronic inflammatory disease such as RA. Several studies demonstrated that AAV vectors efficiently transduced synovial cells (61-63) and human primary chondrocytes *in vitro* (64). The efficacy of AAV-mediated gene transfers in RA models was evaluated after either direct injection into animal joints or injection into muscle (34). Recombinant AAV vectors encoding IL4, IL-10, vIL-10, sTNFR and IL1Ra were evaluated in various rodent models of RA. Joint administration in a LPS induced RA rat model showed persistence of AAV vector. A CMV promoter-mediated inflammation-enhanced transduction of synoviocytes was observed allowing for reactivation of transgene expression (62). rAAV-IL1Ra administration in this model led to improvement of the biological markers of the disease (65). Expression of IL1Ra could be disease-reactivated 80 days after the initial exposure, thus preventing a recurrent arthritic episode. Intra-muscular administration of a rAAV encoding IL-4 in the CIA mouse model showed long-term (129 days) IL-4 expression in muscle and injection within the tarsus improved clinical scores (34). Intra-articular administration of rAAV in a similar CIA mouse model also demonstrated long-term expression (7 months). Intra-articular injection of rAAV encoding sTNFR1 form in a TNF-α transgenic mouse model of RA showed that both synoviocytes and muscle cells were transduced (66) resulting in a noticeable amelioration of the joint score up to 2 months after administration. A disease inducible rAAV transduction was also performed (63). AAV vectors, although exhibiting a good efficiency for RA gene therapy in terms of cell transduction capacity and long lasting *in vivo* gene expression, still need to be further investigated for the pre-existing and induced immune responses prior to clinical application in RA. These inconveniences might be resolved using alternative serotypes of AAV, tissue specific promoters and transient immunosuppression (67, 68)

WHAT ARE THE BEST CANDIDATE MOLECULES FOR GENE THERAPY IN RA?

The choice should be based on the respective role of the various processes involved in RA (Fig. 1). Several molecules may be used simultaneously. This can be achieved either by using gene therapy to produce several products or by combining gene therapy and conventional biological therapy.

The treatment of an autoimmune disease such as

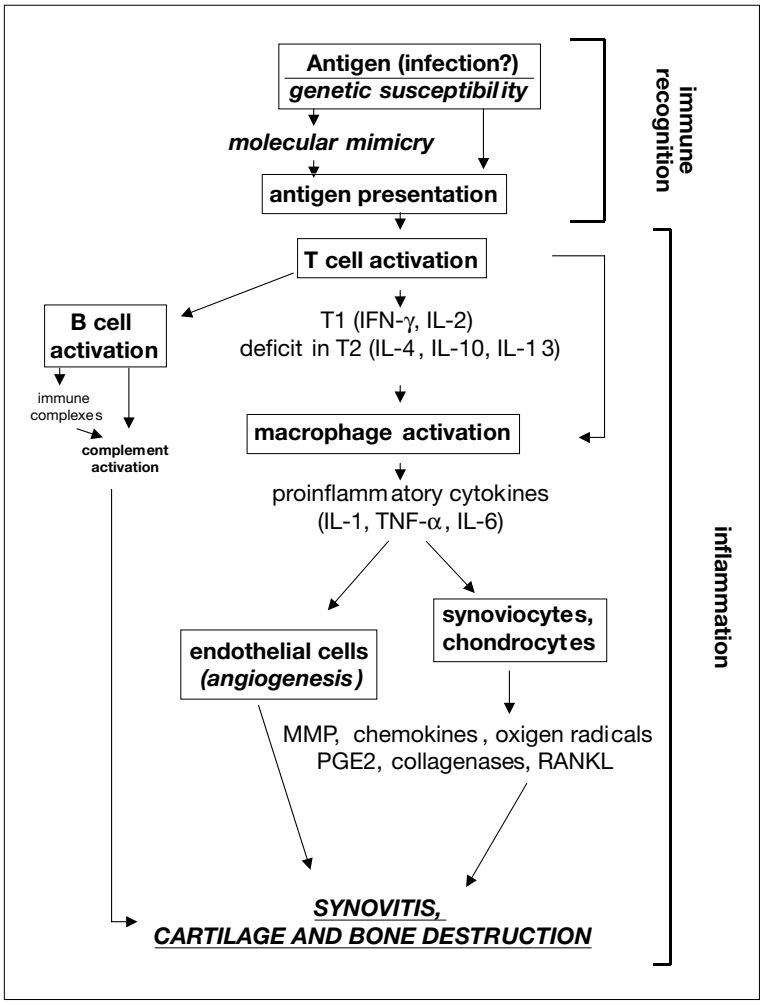

Figure 1 - Immunopathogenesis of rheumatoid arthritis (adapted from) (48).

RA should target the triggering autoantigen or the receptor specific for the relevant epitope of that antigen. Unfortunately, this phase of the pathogenesis of RA remains unelucidated. An alternative strategy is to interrupt the cascade set off by the specific antigen stimulus. Gene therapy can achieve this in several ways, for instance by increasing CTLA-4 or CD28 expression (69) to block T-cell activation in response to presentation of the antigen or by increasing soluble CD40 levels to inhibit B-cell differentiation and to block interactions between T cells and B cells (70, 71).

The inflammatory reaction itself is currently the most studied target for biological therapy. IL-1 and TNF-α act in synergy to orchestrate the entire inflammatory process (72). IL-1 can be effectively blocked by IL-1Ra, which binds to the IL-1 receptors, making them unavailable for IL-1 (73). The second IL-1 receptor (IL-1RII) is also an IL-1 inhibitor: when located on the cell membrane, it binds IL-1 but fails to transmit the signal, acting as a decoy receptor (74); and when soluble, it binds IL-1, decreasing the amount of soluble IL-1 available for binding to the IL-1RI receptor. Targeting IL-1RI would probably be of limited efficacy; in particular blocking this receptor characterized by high affinity for the natural IL-1 inhibitor IL-1Ra, may increase the availability of membrane IL-1RI, thereby increasing transmission of the signal that activates IL-1 (40, 75). TNF-α, the other major player in the inflammatory process, can be inhibited by overexpression of one of its receptors, either p55 (type I) or p75 (type II). Thus, an adenovirus containing the gene for the fusion protein sTNFRI/IgG1 inhibits collagen-induced arthritis (CIA) in mice (23, 26, 28) and antigen-induced arthritis in rabbits (AIA) (17). The gene encoding the monomeric form of sTNFRII, which is expressed after *ex vivo* splenocyte infection by a retrovirus, inhibits CIA (25). Another means of inhibiting inflammation is to increase levels of anti-inflammatory cytokines. IL-4, IL-13, and IL-10 can inhibit the release of pro-inflammatory cytokines and can decrease the production of Th1 cytokines such as interferon- γ (37-39). This effect is accompanied with increased production of IL-1Ra and with enhanced release of Th2 cytokines (self-amplification loop). Viral IL-10 (homologous to IL-10 and encoded by the Epstein-Barr virus), in contrast to mammalian IL-10, has anti-inflammatory effects but causes only minimal immunosuppression (8, 10, 11).

Further downstream along the cascade, the balance between tissue repair and tissue destruction can be altered by modifying metalloproteinase inhibition or growth factors. Growth factors such as BMP-2, IGF-1, FGF, or TGF-ß may be useful for repairing cartilage or bone lesions (76). A major difficulty with biological therapies focused on tissue repair

may be timing: the treatment would probably not be useful in the advanced disease, at a stage when the lesions are irreversible.
It is also possible to achieve synovectomy by gene therapy (gene therapy-mediated synovectomy): the local transfection of synoviocytes by the thymidine kinase gene of herpes simplex virus followed by administration of the prodrug ganciclovir causes lysis of the synoviocytes (46); the thymidine kinase converts ganciclovir to a nucleotide analogue that blocks the synthesis of DNA, thus destroying dividing cells. An alternative is transfection of the Fas ligand gene, which causes apoptosis of the synoviocytes. Fas gene expression is considerably increased in RA synoviocytes, whereas Fas ligand (FasL) levels are low, resulting in increased survival and in proliferation of these cells within the rheumatoid synovium. Fas-L concentrations can be upregulated by injecting the corresponding gene into the joint. This method has been shown to induce apoptosis of cultured synovial cells from human rheumatoid membrane (42) and to improve CIA (41). Fas-L stimulation can also be achieved by transferring the FADD gene (Fas-associated death domain) into the synovial cells (77).

LOCAL OR SYSTEMIC TREATMENT

Two different methods could be used in RA: one is local treatment, i.e., injection in or about the joints, and the other is systemic treatment by parenteral injection (intramuscular, intravenous, subcutaneous or, in animals, intraperitoneal). Although flares sometimes predominate in one or two joints, a far more common pattern is polyarticular disease and in some cases extra-articular involvement (49).
Local treatment seeks to achieve high concentrations of the therapeutic protein within the joint fluid and/or synovial membrane. Most studies have used genes encoding a secreted form of a protein, which is delivered to cells residing in tissues within the joint. A major advantage of this method is that high protein levels can be obtained at the arthritic site. However, systemic effects can occur, in particular as a result of trans-synovial diffusion. Soluble molecules easily cross the synovial membrane, which has no basement membrane, and consequently any local articular treatment has the potential to induce systemic effects. It explains why a vector injected into a joint can be found throughout the body. Furthermore, local vector injections may have contralateral effects (36).
These considerations have led to the development of systemic treatments, which may obviate the need for injecting multiple joints. The rationale for systemic treatment is that RA is a systemic disease whose joint manifestations depend, at least in part, on systemic immune disorders. A theoretical obstacle is that the far greater production of therapeutic protein needed for systemic therapy requires injection of a higher dose of vector, which can be difficult to produce. Furthermore, the higher concentrations in the bloodstream may cause side effects related to the vector and/or to the therapeutic protein. Consequently, development of this strategy is compatible only with models or applications in which efficacy is demonstrated after introduction into the body of a moderate amount of the vector with its therapeutic gene. From a long-term perspective, systemic treatments may prove easier to use.

IN VIVO OR EX VIVO STRATEGIES

The goal of gene therapy is to replace conventional biological methods by achieving continuous expression during a given period of time, production and, in most cases, release of a therapeutic protein. Cells capable of expressing the gene of interest are chosen. The gene is introduced into those cells, either *ex vivo* or *in vivo*. To this respect, gene therapy follows the same rules in RA as in other polyallelic diseases.
Ex vivo gene transfer was the first method used for gene therapy in arthritis models. Synovial cells are harvested and synovial fibroblasts (type B synovial cells) cultured and infected with a retroviral vector encoding the gene of interest. This gene was IL-1Ra in the earliest studies. After expansion and infection with the vector, the synovial cells are injected into the joints of the donor animals. Thus, this method is similar to autologous grafting. Experiments conducted with IL1-Ra have provided convincing evidence that the IL-1Ra gene is expressed within the synovium of the injected joint and that IL-1Ra is present in the joint fluid. Other cell types can be transfected *ex vivo* and reinjected into the animal, including myoblasts, skin fibroblasts, T cells, and dendritic cells. Reinjection can be performed at a site other than the joint to achieve systemic therapy of RA. Splenocytes transfected *ex vivo* by a retrovirus encoding sTNFR or TGF (6) inhibit CIA arthritis in mice. Furthermore, the *ex vivo* method can use nonretroviral vectors, such as adenoviruses (23). Plasmid vectors have al-

so been used successfully. Lines of xenogeneic fibroblasts (Chinese hamster ovary cells) have been transfected with plasmids encoding various anti-inflammatory cytokines; the transfected lines were grafted into the subcutaneous tissues of mice with CIA (39). Despite the short lifespan of the cells expressing the therapeutic gene, significant efficacy was found. The cells can be protected by encapsulation into hollow fibres permeable to therapeutic molecules, thus constituting an implantable bioreactor (37). However, after *ex vivo* plasmid transfection, autologous skin fibroblasts seem to be the most effective cell type for treating CIA (78). Finally, the transfected cells used in all these *ex vivo* methods can be viewed as supervectors that ensure delivery of the therapeutic gene at a selected site. All *ex vivo* transfection methods allow stringent quality control of gene introduction, good quantitation of transfection efficiency, and control of the site of gene expression prior to reinjection. The difficulty is greatest, however, when *ex vivo* gene therapy is coupled with a local strategy. The transfected cells injected into the joint are first harvested from a joint (synovial fibroblasts), and the patient must undergo two invasive procedures requiring a high level of accuracy and involving sites that can be hard to access.

In vivo gene transfer is obviously simpler, whether the systemic route or intra-articular injection is used. There is no need to harvest material from the patient or to perform complex manipulations of these cells in the laboratory. However, systemic therapy requires a high vector dose which can be difficult to obtain for some types of vector.

FROM PRECLINICAL EXPERIMENTS TO CLINICAL TRIALS

The evaluation of therapeutic strategies for RA faces a major obstacle, which is the absence of animal models replicating all the aspects of human RA. Available models each replicate one facet of the disease. Consequently, extrapolation of experimental findings to humans requires extreme caution. Overall, available models simulate a RA flare rather than RA itself, which is characterized by flares on a background of chronic disease. Conclusions drawn from experiments should be evaluated in the light of the limitations of a particular model and are not necessarily relevant to other models.

All available arthritis models are characterized by a phase of acute or subacute joint inflammation. The degree of joint destruction is variable. Extra-articular manifestations are inconspicuous or receive little attention. Taken in aggregate, animal experiments establish that gene therapy for arthritis is feasible. Table I summarizes the main findings. They confirm that delivery of an anti-inflammatory molecule related to overexpression of the corresponding gene induces the expected effect, whether the local or the systemic route is used.

The first successful experiments conducted with transfected fibroblasts or 3T3 cell lines in four different animal models of RA, (16, 21, 52) prompted a clinical trial in humans with RA. The overall strategy was the same as in the models: local treatment with reinjection into a joint of synovial cells infected with a retrovirus encoding IL-1Ra. This study, whose protocol is described in detail elsewhere (79) has been completed very recently. The results demonstrate that this gene therapy strategy is feasible in humans. The procedure was extremely cumbersome, however. The clinical study was conducted in patients scheduled for prosthetic replacement of the metacarpophalangeal joints (MCPs). The first step was collection of synovial tissue from a joint. Synovial cells were infected with the MFG-IRAP retrovirus containing cDNA for IL-1Ra, cultured for one week, and prepared for injection into joints other than the donor joint. One week after the injection, the joints were harvested during the MCP replacement procedure. Examination of the joints showed local expression of IL-1Ra. These results prompted similar trials in Europe and the United States.

FUTURE DIRECTIONS

The active research conducted to improve gene therapy is not specific to rheumatology. Clearly, the various components of gene therapy strategies will have to be noticeably improved before considering routine use in human patients. Intensive research efforts focusing on nonviral vectors have led to the development of electrotransfer, which substantially improves transfection efficiency. Studies on the efficiency of articular electrotransfer are ongoing. The development of synthetic vectors is also a major focus for research.

The selection of genes for transfection has benefited from improvements in our understanding of the biological mechanisms involved in RA. Gene

therapy provides access to intracellular molecules, particularly key enzymes or second messengers. In particular, cyclin-dependent kinase inhibitors administered into the joint via adenoviral vectors inhibit synovial proliferation, thus ensuring resolution of arthritis (44). Similarly, synovial-cell apoptosis can be stimulated by inhibitors of nuclear translocation of nuclear factor-kappaB (NF-kB) transferred via an adenoviral vector (66). The ability to regulate the release of the protein of interest is another advantage that, in theory, is specific of gene therapy. Most of the transgenes used to date are expressed under the control of viral promoters that are not amenable to regulation. In the treatment of inflammation related to joint destruction, the benefits of the anti-inflammatory effect should be weighed against the potential risks related to presence in the body of anti-inflammatory molecules in high levels (risks of immunosuppression, for instance). Gene expression can be regulated by exogenous molecules (tetracyclines for instance) acting on transgene promotors. Other more subtle strategies are conceivable. An example is self-regulation of the transgene by the inflammation itself. Very recent investigations have shown that intra-articular IL-1Ra gene delivery can be regulated by using the promotor naturally controlling the gene of C3 inflammation protein. Moreover, this strategy was found to prevent CIA in mice (80). The multiplicity of the factors involved in RA suggests that several molecules used in combination may be more effective than a single molecule. For instance, vIL-10 was shown to synergize with sTNFRI and sIL-1R also synergizes with sTNFR to inhibit arthritis (29).
Taken in aggregate, published studies have firmly established the scientific validity of gene therapy in RA models. Nevertheless, advances are needed to define the reference strategy. To this end, further experimental and preclinical studies must be conducted.

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RIASSUNTO

Rheumatoid arthritis (RA) is a severe autoimmune systemic disease. Chronic synovial inflammation results in destruction of the joints. No conventional treatment is efficient in RA. Gene therapy of RA targets mainly the players of inflammation or articular destruction: TNF-α or IL-1 blocking agents (such as anti-TNF-α monoclonal antibodies, soluble TNF-α receptor, type II soluble receptor of IL-1, IL-1 receptor antagonist), anti-inflammatory cytokines (such as IL-4, IL-10, IL-1), growth factors. In this polyarticular disease, the vector expressing the therapeutic protein can be administered as a local (intra articular injection) or a systemic treatment (extra articular injection). All the main vectors has been used in experimental models, including the more recent lentivirus and adeno-associated virus. Ex vivo gene transfer was done with synovial cells, fibroblasts, T cells, dendritic cells, and different cells from xenogenic origin. In vivo gene therapy is simpler, although less controlled method. Clinical trials in human RA has started with ex-vivo retrovirus expressing IL-1 receptor antagonist and have demonstrated the feasibility of the strategy of gene therapy. The best target remains to be determined and extensive researches have to be conducted in pre-clinical studies.

Key words - Rheumatoid arthritis, inflammation, cytokines, gene therapy
Parole chiave **-** *Artrite reumatoide, infiammazione, citochine, terapia genica*

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