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Gene Therapy Challenges in Arthritis

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1. Introduction

1.1 From systemic to intra-articular gene therapy

The first experiments of gene therapy (GT) in arthritis were done in the mid- nineties.

Arthritis is a chronic, inflammatory disease responsible for joint inflammation and destruction resulting in an imbalance between pro-inflammatory and anti-inflammatory cytokines production. Moreover, we know that bone destruction is more active than bone regeneration. Therefore, there are different ways of targeting the disease. At the moment, there are three classes of drugs commonly used:

1. Non-steroidal Anti-inflammatory Agents (NSAIDs),
2. corticoids,
3. Disease Modifying Anti-rheumatic Drugs (DMARDS).

Biotherapies have been used for a decade. These treatments restore the balance of many functions such as the immune system and the bone metabolism. Agents used in biotherapy include recombinant proteins, monoclonal antibodies, growth factors, and vaccines. The efficiency of these treatments depends on the stage of the disease. Serious side effects and infrequent persistent remission are known as drawbacks. Furthermore, the systemic administration of immune regulator can affect several immune responses and trigger infections.

The objectives using gene therapy in arthritis are mainly 1) to obtain a therapeutic quantity of therapeutic molecule without side effects, 2) to get a long expression of the therapeutic molecule without multi injections, 3) to target the joint specifically resistant to the usual treatment (Fiocco & Punzi, 2011).

Arthritis models are still being used to improve gene therapy strategies: for instance we use induced arthritis models such as collagen induced arthritis (CIA) in mice developing sub-chronic poly arthritis or adjuvant induced arthritis or spontaneous models such as Tumour Necrosis Factor (TNF) transgenic mice.

Our team who had the first results in 1996 has seen many changes over the years in its approach due to scientific progress. Our first gene therapy experiments used the *ex vivo* strategy (using autolog transfected cells (immortalized fibroblast from DBA/1 mice) or heterolog transfected cells (xenogenic fibroblasts from Chinese hamster ovary (CHO) with plasmid encoding murine IL-4 or IL-13); then the viral vectors experiments were used until the risk of using this methods arose (Bessis et al., 1996). We also used a non viral vector as plasmid (Bloquel et al., 2004, 2007; Saidenberg-Kermanach et al., 2003). Several teams tried

to optimize the transfer of plasmid whereas others tried to improve the safety of viral vectors.
In order to decrease the risk of a systemic diffusion of the transgene and to target the joint which might not respond to the usual treatment we have developed a local intra-articular (i.a) gene delivery strategy.
It is important to note that i.a strategy has been the only choice for the two existing clinical trials using GT in patients with rheumatoid arthritis.
One of the two trials has been done using an adeno- associated virus (AAV) 2 encoding the human soluble Tumour necrosis factor receptor type I (hTNFR-Is) fused to the Fc fragment of human IgG1. The TNF-α binds the soluble TNFR-Is instead of binding the cell membrane receptor. The second clinical trial used cells transfected with a retroviral vector encoding IL-1Ra which binds the membrane receptor of IL-1 and prevents the binding of IL-1.
In this chapter, we will show the methods evaluated in arthritis models for the gene therapy challenges in rheumatoid arthritis.

1.2 Intra-articular gene therapy

Knees are formed of four bones (Femur, Tibia, fibula, patella) surrounded by a membrane called synovium. It produces the synovial fluid which lubricates and nourishes the bones and the cartilage. In rheumatoid arthritis, the synovium is overrun by white blood cells which produce inflammatory molecules as TNF-α, IL-1-β, IFN-γ. Intra-articular GT consists of a therapeutic gene injection in the articular space to allow a local production of the therapeutic molecule. In mice, the needle is placed in the middle of the patella (seen through the skin after having shaved the knee) as far as the femur, and gently withdraw the needle to allow the gene to mix with the synovial fluid. The uncertainty of this method is to find out which cells are being targeted. Synoviocytes, osteoclasts, osteoblasts, chondrocytes are interesting targets because synoviocytes are able to produce pro-inflammatory cytokines, osteoclasts are responsible for the bone degradation, the osteoblasts allow the bone regeneration and chondrocytes are responsible for formation, maintenance, and repair of articular cartilage.
Intra-articular treatments are currently used for patients with RA such as corticoids which are injected locally. The new approach by gene therapy is a challenge in rheumatology. Intra- articular GT allows to reduce the number of injections, the quantity of therapeutic molecules and to target the joint resisting the usual treatment (Bessis & Boissier, 2003).

2. From *ex vivo* to *in vivo* gene transfer

Two strategies have been assessed in order to introduce the gene into the joint: 1) the gene has been introduced into the cells before their injection into the joint (*ex vivo* gene therapy) or 2) the gene has been injected directly into the joint (*in vivo* gene therapy).

2.1 *Ex vivo* gene therapy

The choice of the *ex vivo* gene strategy requires to choose the cell type which must be transfected. It seems clear that using autolog cells are more effective than using heterolog cells. To regulate immune response in inflammatory diseases such as rheumatoid arthritis, cells presenting antigen (CPA) (dendritic cells, macrophages or B cells) are good candidates. However, it is difficult to transfect these cells *in vivo* therefore using *ex vivo* gene therapy is more feasible. CPA are interesting in the *ex vivo* strategy because they are able to reach the

inflamed area such as an arthritic joint where they interact with T cells to regulate immune response (Burke, 2003; Kim et al., 2006; Stoop et al., 2010). They are also able to migrate in the spleen and the lymph node to regulate the immune response (Ahrens et al., 2005; Kim et al., 2001).

Dendritic cells (DC) can direct the differentiation of T cell from Th0 towards Th1, Th17 or Th2. The activity of Th1 cells is predominant in animal model of autoimmune diseases such as RA at the expense of Th2 cells. Genetically modified DC allow to direct cell differentiation towards Th2 cells which have a therapeutic effect in decreasing Th1 activity.

In collagen induced arthritis (CIA), intravenous (i.v) injection of DC transfected with anti inflammatory cytokines (IL-4) can decrease the inflammation. After the injection, cells have been found 6h later in the lymph node and the spleen but not in other tissues or organs such as muscle, heart, lung and kidney. Transfected DC migrate to the secondary lymphoid organ where they modulate the T cell activity. These T cells produced less IFNγ than T cells from splenocytes of untreated mice. This shows that the injection of DC –IL-4 has directed T cells from Th0 to Th2 rather than to Th1 type (Kim et al., 2001).

On the other hand, DC transfected with adenovirus encoding FasL are able to induce T cell apoptosis in a specific manner. According to the authors it seemed that it was more efficient to direct T cells towards apoptosis than to direct them towards Th2 differentiation (Okamoto et al., 1998).

More recently, the discovery of small interfering RNA (siRNA) has allowed a change of strategy (Fire et al., 1998). Instead of introducing a gene into DC to increase expression of the transgene, DC can be transfected with a siRNA which will block the production of a pathogenic molecule.

It is known that CD40, CD80 and CD86 are important in the regulation of T cell activity by DC. From these data, Zheng et al. have studied the efficiency of DC transfected with siRNA targeting CD40, CD80 and CD86 in CIA. The i.a injection of transfected DC in mice resulted in less bone destruction and a reduced number of immune cells (neutrophils and mononuclear cells) infiltrating the joint (Zheng et al., 2010).

As described, DC are good cell candidates for *ex vivo* GT. The knowledge of their differentiation and their capacity to modulate the immune response has allowed the improvement of the therapeutic effect of genetically modified DC.

The new challenge now is to use regulatory T cells (Treg). Their suppressive activity on T cells effectors and their implication in tolerance process could be used in GT. The first study using T reg has shown that it was difficult to transfect these cells. Recently, Hombach et al. have demonstrated that primary T reg cells stimulated via the complex TCR/CD3 and the co-stimulatory molecule CD28 improve the gene transfer in these cells. It is important to note that the stimulation and the transfection have no effect on the properties of these cells (Hombach et al., 2009). From these data, Wright et al. have assessed the therapeutic potential of Treg transfected with TCR specific of antigen in arthritis induced adjuvant (AIA) model. They showed that primary Treg cells transfected with specific TCR and T CD4+ transfected with specific TCR and Foxp3 are able to reduce the disease (cartilage destruction and inflammation) (Wright et al., 2009).

Apart from DC and Treg cells, others such as mesenchymal cells, autolog fibroblasts or human like fibroblasts extract from human joints have also been used in arthritis models. The choice of the cell type depends on the immune response targeted, the feasibility to transfect them and the way to administer them: subcutaneously (s.c), i.v or in the joint. Several teams now develop a local *ex vivo* gene therapy to increase the number of

therapeutic cells in the inflamed target and to regulate locally the imbalance of the immune system or the bone metabolism.
The i.a strategy using *ex vivo* gene therapy has been studied in a big animal eg a rhesus monkey in CIA. Skin autolog fibroblasts from the animal and xenogenic CHO were transfected with a plasmid encoding LacZ then grafted in the synovium. This study showed that labelling in the joint grafted with autolog fibroblasts was stronger compared with the labelling in the joint grafted with heterolog cells CHO. The accessibility of these autolog fibroblasts is a serious advantage (Bessis et al., 2007).
Fibroblast-Like Synoviocytes (FLS) are the largest type of cells in the synovium and therefore they are more interesting to treat inflammation in the joint. In RA, they are partly responsible for the inflammation in producing pro-inflammatory cytokines. Their pro-inflammatory activity depends on their environment. *Ex vivo* GT was discussed because FLS are not easy to transfect and their access is difficult. However several teams showed the efficiency to use these cells to treat AIA (Adriaansen et al., 2006; Yamamoto et al., 2003).
Synovial Mesenchymal stem cells which are also in the synovium are more attractive than FLS because they are more accessible and they expand easily. They are used more specifically in the case of cartilage repair but the advantages mentioned above could be interesting to treat the inflammation and the destruction of cartilage (Horie et al., 2009).
To conclude on the type of cells used in *ex vivo* i.a GT, it is important to note that cells have to be transfected easily, extracted easily and have a good adhesion with the local joint environment.
Despite *ex vivo* GT being more complex than direct gene injection one of the clinical trials used *ex vivo* gene therapy. In this trial, autolog fibroblasts have been infected with a retroviral vector encoding IL-1Ra (Evans et al., 2005). Then, these cells have been injected in the joint. Wehling et al have published the first result of this strategy in 2009 (Wehling et al., 2009). Due to the serious secondary effects which happened in another unrelated protocol at the time of the trial, the team only injected two patients instead of six. Furthermore, they used the same retroviral vector backbone (MFG) which was the problem. The transfected cells have been injected in 3 metacarpophalangial joints in the hand. An intra-patient control system has been done with injection of autologous non transfected cells. For the first patient, the joint receiving 3.10^6 cells was less painful than the joint receiving 10^6 cells. Surprisingly, the pain in the joint receiving untransfected cells was reduced too. This first patient noticed an improvement as early as the day after the surgery whereas the second patient was less responding. She only noticed an improvement two to three weeks after surgery. Moreover she felt a slight pain improvement in the untreated joint. So, the feasibility was proven, however two surgical procedures are needed: one to take the autologous synoviocytes out and the second one to transplant these transfected cells. Evans et al. noticed that synoviocytes are able to produce IL-1Ra after three passages. This result shows the stability of the transfection. It is an important property which keeps the production of the therapeutic transgene for a long time (Wehling et al., 2009).

2.2 *In vitro* gene transfer progresses

Pioneers of the gene therapy were convinced that they could use viruses to bring a therapeutic gene into the cells (Hill & Hillova, 1972; Sambrook et al., 1968). For a long time, researchers have been using the property of the viruses to get into the cells naturally. However it was the risk of using these viruses which urged the researchers to find a non viral strategy. But early studies have shown the difficulty to transfect cells with non viral vectors. New vectors and new vectorisation methods had to be developed.

Calcium phosphate was one of the first methods used to transfect mammalian cells with DNA. Calcium phosphate and plasmid are able to co precipitate which allows DNA uptake into the cells. Cationic lipid and polymer have the same property. *In vitro* transfection reagents using different carrier molecules are now commercially available and have the advantage of being reproducible to always give the same results. Furthermore, using these reagents, fewer procedures are needed than in classical transfection methods. Calcium phosphate, polymer were used to transfect cells with non viral vectors but later on, they were used to increase the transfection with viral vectors such as adenovirus (Lee & Welsh, 1999).

A second method using electricity was set up in the nineties: electroporation and nucleofection are two examples (Gresch et al., 2004). With these technical strategies, electroporation and nucleofection, many more cell types can be used in GT. As a summary, the pulsed electric fields using electroporators modify the membrane potential resulting in breakdown of the lipid bilayer to form pores. These modifications are reversible and temporary. First of all, the vector encoding gene of interest and cells are mixed with a specific transfection buffer in a specific plate. Then, electric fields are applied. The choice of the waveform is an important step. Generators used to apply current can deliver two types of waveforms: an exponential decay waveform and a square waveform. However, it is important to note that: 1) the square waveform increases the temperature which leads to the death of the cells whereas the exponential decay waveform avoids overheating. 2) Furthermore, with the square form, the medium of the culture can change the transfection efficiency whereas the medium culture has less effect using exponential decay waveform (Lambert et al., 1990). The first electroporators were made to transfect DC or macrophage. But the number of cells transfected and their viability were limited. To transfect cells using the electroporator, the cells are put in a chamber where they received high electrical field to create transient pores in the cell membrane. A high number of cells and a high quantity of plasmid must be used to obtain a significant quantity of cells transfected.

Despite the progress of the electroporation the high percentage of death cell remained consequent. Another method called nucleofector has been applied. This apparatus increases the efficiency of the transfection and survival rate of the transfected cells compared with the classical protocol using lipofectamine or electroporation. Nucleofection has a better reproducibility than using electroporation. A review reports the efficiency of the gene transfer in different primary cell types and cell lines by nucleofection (Gresch et al., 2004). The lower primary cell type transfected was the human B cells and the lower cell line transfected was PC12 cells. It seems that the variation is not due to the transfection method but it depends on the preparation of the cells (human B cells and PC12 cells) and the donor of cells in the case of primary cells. Despite using electroporator or nucleofectors it is still difficult to transfect cells with a large plasmid (14.7 kb). Further studies are needed to improve the transfection of the cells with a larger plasmid. The improvement of electroporators which can deliver different voltages during the transfection and the improvement of the electrodes could improve the transfection (Cepurniene et al., 2010; Rebersek et al., 2007).

Furthermore, plasmid mobility is not well known. Authors are studying the role of electrophoresis in plasmid traffic in the cells to increase the DNA transfer (Cepurniene et al., 2010; Mir, 2009).

Electroporation involves several parameters: the form of the wave as seen above, the voltage (low or high) and the duration of each pulse applied. According to the parameters used,

both following effects can be observed: a thermal effect and electrochemical effects. Whereas pulse duration using microseconds or milliseconds has been used for a long time in order to destabilize the membrane and to involve the penetration of chemical agents (bleomycin) or DNA, the efficacy of this method using nanosecond is now being studied. The overheating created when electric pulses are applied is a drawback of the electroporation. Therefore duration could be limited to avoid this increase of heat and using nanoseconds could avoid the rise of temperature. Silve et al. explained the advantages and the limits in using nanoseconds in the book called Advanced Electroporation Techniques in Biology and Medicine (Silve et al., 2010).

With technical improvements primary cells such as synoviocytes can be transfected easily. Electroporation provides transfection efficiency, minimizes cell death in decreasing the quantity of the vector injected.

Despite the technical progress, the success of the *ex vivo* GT in arthritis models and the success of the first clinical trial in rheumatoid arthritis, the strategy remains difficult for patients who have to undergo two surgery procedures. This approach is still being used although other teams have focused their research on the *in vivo* GT.

2.3 *In vivo* gene therapy

In RA models, two methods can be used according to expected results: 1) to obtain a systemic effect on systemic immune disorders, 2) to obtain a local effect targeting the inflamed joint.

Systemic effects of a transgene are obtained after an intravenous (i.v), a subcutaneous (s.c) or an intra-peritoneal injection. However, drawbacks of the systemic injection have been reported:

1. there are side effects due to the lack of control of the diffusion of the transgene,
2. the immunogenicity or pro-inflammatory effects of vectors (Cottard et al., 2004; Ji et al., 1999; Zaiss & Muruve, 2005)
3. the need to inject a high dose of a vector in order to get an effective dose of therapeutic molecule.

In vivo gene therapy, via i.m injection of the vectors in arthritis models has been studied thoroughly (Bloquel et al., 2007; Chen S.Y. et al., 2009; Denys et al., 2010). The muscular cells have the advantage of being non-dividing cells, easy to access and they produce the protein encoded by the transgene for a long time (more than 6 months) (Bloquel et al., 2004). The efficiency of the transfection depends on the vector and the maturation of the muscle (Moisset & Tremblay, 2001). Indeed, viral vectors use viral membrane receptors to infect the cells. But as the cells get older they are less able to produce these receptors (Feero et al., 1997). In the case of non viral vectors we will see in section 2-5 that different methods have been studied to improve the transfection.

Initially, i.m injection was used to treat muscular disorders and to have a systemic secretion of a therapeutic molecule. In RA, the i.m injection is not totally adapted because the target is the joint. Besides, molecules injected in the muscle have less possibility to reach the joint than to diffuse in the body. Intra articular GT has also been suggested for the first time in 1992 (Bandara et al., 1993). The advantage of this injection was very quickly identified: 1) easy access to the joint 1) less quantity of vectors, 2) less risk of side effects because no or less vector and transgene diffusion, 3) high number of cells so high potential of cells to be transfected, 4) the FLS have a low mitotic rate ensuring a fairly long expression of the transgene. The efficiency of gene transfer using a systemic or a local injection has been

compared. Kim et al. demonstrated that the adenoviral vector encoding IL-4 injected intravenously or in the joint decreased the inflammation and the destruction of the bone and the cartilage in established CIA. The quantity of the vector injected was respectively 10^9 and 5.10^8 (Kim et al., 2000). Adenoviruses and Adeno-Associated-viruses (AAV) have been frequently used to work locally. However, as mentioned before, it seems important to develop a non viral strategy safer than a viral strategy. We studied the efficiency of the i.a gene transfer in CIA using a plasmid encoding mTNFR-Is. The knees of mice treated with this plasmid showed a decrease of both inflammation and destruction and there was no systemic effect (Denys et al., 2010). Examples of joint inflammation and bone destruction are illustrated in Fig. 1.

Although a "comparative study on intra-articular versus systemic gene electrotransfer in experimental arthritis" showed that i.m injection of plasmid (pVAX2) encoding IL-10 decreased the development of the disease, the i.a injection had no effect. In this study, contrary to all expectations, 1) the quantity of the plasmid injected in the joint (100µg) was higher than the quantity injected in the muscle (25µg), 2) i.a injection had no effect (Khoury et al., 2006) . The difference between the two could come from the transgene: the TNF-α could play a role locally whereas IL10 does not show the importance of the choice of the target. In our study, the expression of the transgene after i.a gene transfer was shorter than after i.m injection which is an important drawback. The higher production is about 7-8 days after the i.a injection. It decreased rapidly and was undetectable 14 days later (Bloquel et al., 2007; Denys et al., 2010; Khoury et al., 2006). We have shown that a second injection of a plasmid encoding msTNFR can allow a new expression of the transgene. However, the level of msTNFR obtained after the second injection was lower than the level obtained after the first injection may be because of a limited capacity of the cells to integrate the plasmid (Bloquel et al., 2007).

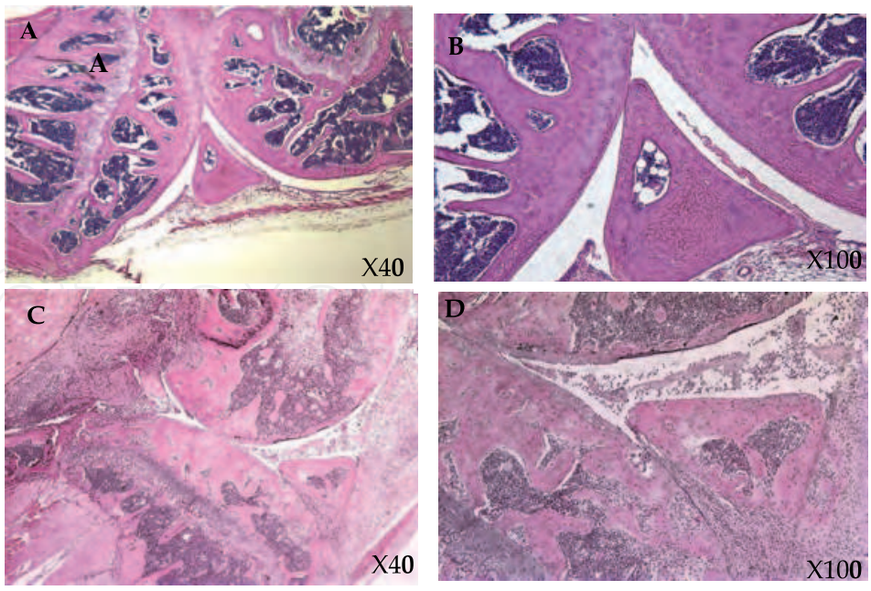


Fig. 1. Examples illustrating histological analysis of knee's inflammation and destruction. Haemalun/Eosin staining. (A-B) msTNFR-Is treated mice; aspect of normal synovial and normal cartilage. (C-D) Saline-treated mouse: inflammation and cartilage destruction of the knees.

A recent clinical trial using *in vivo* i.a strategy has been performed. An adeno-associated virus type 2 expressing TNF-α antagonist was injected in the knee of arthritic patients. These patients received a second injection either 12 or 30 weeks when necessary. Twelve adverse events and 2 deaths were reported after the second injection (Evans, 2010). The involvement of the vector in the pathogenecity has been rejected because this vector has been identified as a "good" vector and has been used in others studies (Boissier et al., 2007; Mueller & Flotte, 2008; Wu et al., 2006). In this study, viral genome was detectable in the blood as well as in the synovium where it remained longer. Therefore, this study has shown not only the feasibility but also the risk of i.a gene therapy.
This accident reminds us of the Jesse Gelsinger's case which shows the difficulty to select patients. This accident encourages researchers to improve the non viral gene transfer to be used safely in future treatments for humans and to improve the choice of patients' exclusion criteria.

2.4 Contralateral effect of *ex vivo* and *in vivo* gene therapy

It is important to underline that i.a injections could have a therapeutic effect in treated and untreated joints. The contralateral effect doesn't depend on :

1. the strategy using *ex vivo* or *in vivo* (Kim et al., 2002; Kim et al., 2005; Whalen et al., 1999)
2. the vector injected *in vivo* because it was observed using viral vector, adenovirus, adeno-associated virus and non viral vector (Chan et al., 2002; Ghivizzani et al., 1998; Van De Loo & Van Den Berg, 2002),
3. the model of arthritis because it is observed in CIA, AIA and Streptococcal Cell wall (SCW)-induced arthritis (Chan et al., 2002; Kim et al., 2005; Lechman et al., 2003),
4. species as observed in rabbits , in rats , in mice and also in human clinical trials (Chan et al., 2002; Ghivizzani et al., 1998; Van De Loo & Van Den Berg, 2002; Wehling et al., 2009).

The reason of the contralateral effect with *in vivo* gene transfer is not well known. However, authors have suggested that migration of APC in the case of *ex vivo* strategy might be responsible for that particular effect (Kim et al., 2002). Indeed, studies have showed before that these cells are able to travel to other sites of inflammation or secondary lymphoid tissues such as lymph nodes (Kim et al., 2000; Lechman et al., 1999; Morita et al., 2001).
In the case of *in vivo* gene strategy, local dendritic cells might be infected by the vector and they migrate to regulate the inflammation in the front paws or in the contralateral paw. We cannot control this distant effect at the moment therefore this property could be interesting to develop. It would be a serious advantage to be able to treat all the joints with the diffusion of the protein encoded by the transgene without the diffusion of the vector.

2.5 Technical strategies to improve *in vivo* gene therapy in rheumatoid arthritis

The first *in vivo* non viral gene delivery consisted in a single injection of the vector. However, the size of the DNA and anionic charges surrounding the DNA block the efficacy of the endocytosis. Mechanical, chemical or electrical approaches are being developed to improve gene delivery. We focused our description only on the methods used in arthritis models.

Liposomes

Liposomes are molecules which have been used for the last three decades and have become more and more performing. They were first used *in vitro* and the improvement in their

safety allowed them to be used for *in vivo* gene therapy to carry nucleic acids (DNA or RNAi).

They are microbubbles of lipids which are anionic, cationic or zwitterion. The composition of the liposomes can vary and their interaction with cells depends on this composition (Ropert, 1999). Cationic Liposomal carrier has been considered as a good potential to facilitate molecule internalisation in cells because they have similarities with the cell plasma membrane. The advantages of liposomes in GT have first been described in the *ex vivo* gene transfer: liposomes involve the adhesion of packaged nucleic acids to the cell surface, then the internalisation of them by endocytosis. They also confer a protection against DNase. Studies have shown that Cationic liposomes increase the transfection by 3 fold in the skeletal muscle (Felgner, 1996). However, *in vivo*, some drawbacks were reported following liposomes nucleic acids complex injection in the serum. Liposomes DNA complex also called lipoplexes could be responsible for a toxicity action and/or an inflammatory action. Therefore, they could limit the biodistribution. Advantages and drawbacks of Cationic liposomes used *in vivo* gene transfer have been described in the human gene therapy journal (Felgner, 1996).

In arthritis models, lipoplexes are used to carry siRNA. Khoury et al. also used siRNA targeting pro-inflammatory cytokines as TNF-α, IL-1β, IL6 and IL-18 (Khoury et al., 2006; Khoury et al., 2008). They have noted that the i.v simultaneous injection of the three lipoplexes blocking IL-1β, IL-6 and IL-18 has curative and preventive effects (Khoury et al., 2008).

Other teams have targeted the immune cells rather than the pro-inflammatory cytokines in CIA. Indeed, the development of rheumatoid arthritis depends on the interaction between T cells and CPA. Adreakos et al. used lipoplexes able to block CD40/CD40L interaction. Authors have developed a liposome compatible with the *in vivo* GT called amphotenic liposome which facilitates the encapsulation, the blood circulation and the cell release from an endosome (Andreakos et al., 2009). This liposome has been injected intravenously at the onset of the disease. It releases CD40 anti sense which prevents the CD40 expression on T cells. The improvement of the clinical signs of the disease was noticed only 24h after the treatment. Furthermore, the efficacy on all paws was noted 10 days after the first injection of liposomes.

Electrotransfer

Electric fields were first used *in vitro* as seen in section 2-2. Since then, they have been used in cancerology. The strategy called electrochemotherapy helps the endocytosis of drugs in tumour cells. The first application was conducted in the 1990's on subcutaneous and cutaneous tumours (Gilbert et al., 1997; Mir et al., 1991; Titomirov et al., 1991).

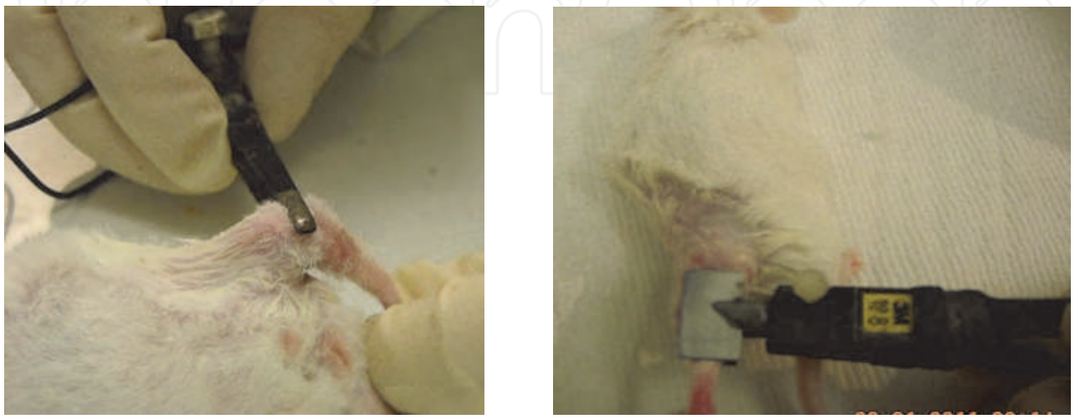
Besides, electric fields have been used to transfer genes into cells. This strategy called electrotransfer (ET) has also been used to kill tumour cells or to force the liver and then later on the muscles to produce therapeutic molecules (Aihara & Miyazaki, 1998; Heller et al., 1996; Rols et al., 1998).

The action of electric fields *in vivo* is the same as the *ex vivo* electroporation:

1. pore formation in the cell plasma membrane,
2. penetration of the vector into the cells.

The development of the i.m gene therapy using ET has been a major advance in genes transfer (Miyazaki & Miyazaki, 2008). The stability of the transgene expression between individual subjects was a very major advance of this method.

In 1998, Mir et al. have published the parameters which give an efficient and a reproducible muscle cells transfection. Furthermore, they obtained a temporary permeabilization of the cells and of many survival cells (Mir et al., 1998).
The generator used for the ET delivers square waves. The current is transmitted to the target via electrodes which are two parallel stainless steel plates, their size depending on the target. Fig.2. shows the position of the electrodes for the knee's elecrotransfer (A) or for muscle's electrotransfer (B).



A B

Fig. 2. Electrotransfer to mouse knee or muscle. DNA electrotransfer was performed with a square wave electroporation system. The current is transmitted to the target via electrodes which are two parallel stainless steel plates, their size depending on the target. A. Electrotransfer to mouse knee: the pair of electrodes was placed on either side of the knees and then 12 electric pulses were applied during 20ms. B. Electrotransfer to mouse muscle : the pair of electrodes was placed on either side of the muscle and then 8 electric pulses were applied during 20ms.

In arthritis models, conventional parameters are used to deliver genes in the muscles: 8 pulses for 20ms at low voltage (100-200V) for 1cm between the electrodes with a frequency of 1Hz. The parameters are changed to deliver genes into the joint: 12 pulses for 20ms at 250V/cm with a frequency of 2Hz (Denys et al., 2010; Khoury et al., 2006). It is interesting to note that the parameters of the ET can be better controlled in using mathematical modelling or *in vivo* test (Miklavcic et al., 2000). The parameters programmed in the electroporator are set for a unique position of the electrodes. However, during the ET, it is known that the muscle contracts and electrodes move. As a result there is a decrease of the permeabilization responsible for a decrease of the efficiency of the ET. Cukjati et al have described a method controlled by a computer which adapts the parameters according to the changes of the movement of the electrodes to achieve the permeabilization at the end of the pulses (Cukjati et al., 2007).
Despite the results with the application of low voltage (100-200V) and long duration (20ms), it seems that high and then low voltage must be applied: 1) to permeabilize the cells and 2) to promote the DNA transfer. These two steps have been described in several studies (Andre & Mir, 2010; Hojman, 2010; Satkauskas et al., 2005) . The following elements play a role in the transfer of DNA in the cells: 1) the electric pulses, 2) the structure of the DNA, 3) the composition of the buffer in which the DNA is diluted, 4) the physiology of the muscle.

The damages and the physiological modifications on muscular fibres have been studied after electrotransfer. The parameters used can alter the muscular fibres temporarily (Gissel, 2010; Hojman, 2010; Tevz et al., 2008). When we used a high voltage for 20ms, we did not notice any fibrosis (Bloquel et al., 2004).
Mir et al have studied the ionic flux, apoptosis marker expression and muscular function after ET using different combinations of pulses. As explained by the authors, the effects depend on the combinations (Hojman et al., 2008).
On the other hand, Peng et al. noted that the vector could be integrated not only by mature muscle cells but also by satellite cells after electrotransfer. These satellite cells replace the damaged muscular cells. Their transfection seems important in order to have a long expression of the transgene (Peng et al., 2005).
Besides the role of the pulse on the efficiency, let's not forget the electrodes configuration and composition.
Two types of electrodes can be used: invasive or non invasive electrodes. In arthritis models we used non invasive electrodes as described before. Other electrodes have been constructed according to the target. Gothelf and Gelth have described different types of electrodes used for skin electrotransfer (Gothelf & Gehl, 2010). Invasive electrodes have been a great evolution for transfected skin and tumours as they release the pulses directly into the tissue. The voltage used with these electrodes is less than the voltage used with non invasive electrodes because the current must go through several barriers before reaching its target (Liu & Huang, 2002). However, a huge step in invasive electrode is the set up of the syringe electrodes. They are formed with two electrodes, one of them being used to inject the DNA.
Syringe electrodes have originally been developed to treat neuronal diseases such as Alzheimer disease but it also opens a potential application in i.a gene therapy (Li et al., 2010). Intra articular ET showed therapeutic effects in arthritis models (Bloquel et al., 2007; Denys et al., 2010). However the application of non invasive electrodes on both sides of the joint is not always controlled which could explain some individual differences in the results.
Therefore, *in vivo* ET depends on the chosen electrodes, on the electric parameters applied and on the tissue composition. It can be difficult to determine the optimal electric parameters for a type of electrodes in a specific tissue. Numerical modelling described by Zupanic et al. can determine the optimum parameters" for ET in muscular tissue". (Zupanic et al., 2010). This approach can be used for different tissues.

Nanoparticles vectors

Chitosan is a nanoparticle charged positively capable of binding anionic nucleic acids. Furthermore, it is biocompatible, and remains small after nucleic acids binding (Takka & Gurel, 2010). In CIA, Howard et al. demonstrated that i.p injection of chitosan containing anti TNF-α siRNA delayed the arthritis development by targeting peritoneal activated macrophages (Howard et al., 2009).
In the rat AIA, the IL1-Ra-chitosan-folate complex (nanoparticles complex) decreased the inflammation and the destruction of the joints (Fernandes et al., 2008). Shi et al. have performed this strategy using hydrodynamic injections of the nanoparticles-complex in the vein of the hind paws. They became aware of the smaller size of the circumference of the ankles in rats treated than in non treated rats (Shi et al., 2011). A study shows the efficiency of i.a injection of other nanoparticles (atecollagen) associated to a double-stranded miRNA-15 in the arthritis development. As a result, authors noted a successful transfection of the

synovium cells and the downregulation of Bcl2 which is the target of the miRNA-15. Despite an incomplete action on the disease, the i.a injection of miRNA carried by atecollagen opens new perspectives to simultaneously administer several miRNA (Nagata et al., 2009).

3. Progress and prospects on the structure of naked nucleic acids and viruses to treat rheumatoid arthritis

This section focuses on the most important technological modifications concerning naked nucleic acids and viruses in order to increase their efficiency in arthritis models.

3.1 Plasmids

Plasmid DNA expression has been hampered by a low level of transfection and the short time of the expression of the transgene. Several changes have been made to improve its safety, its internalisation in the cells, its translocation in the nucleus and its integration (Conese et al., 2004; Gill et al., 2009; Lam & Dean, 2010). Plasmids are also: 1) reduced (minicircle DNA) 2) deprived of the bacteria resistant gene 3) deprived of its CpG motifs.
Studies showed that minicircles produce the transgene for a longer time than when it is produced from the conventional plasmid (Mayrhofer et al., 2009). Different elements explain this advantage:

1. minicircles have a supercoiled form that will protect the DNA from degradation by enzymes
2. minicircles have less CpG sequence motifs which are usually responsible for an immune response destroying the plasmids (Bloquel et al., 2004; Mayrhofer et al., 2009)
3. minicircles are small facilitating their penetration in the cells.

Despite these advantages, a few studies have demonstrated their therapeutic efficiency in RA(Chen Z.Y. et al., 2003; Stenler et al., 2009) . Some teams are focusing mainly on the improvement of their purification; others are trying to improve the efficiency of the plasmid. Researchers replaced CpG by other sequence motifs or by removing the resistance gene to antibiotics (Kay et al., 2010; Mayrhofer et al., 2008).
For a long time, the genes making cells or bacteria resistant to specific antibiotics have been the only way to select and isolate the cells / bacteria containing them. However, once the genes spread into the germs, these germs become resistant to antibiotics and are impossible to eradicate. Therefore, the scientists tried to construct plasmids without these genes and with a conditional replication origin (Marie et al., 2010; Soubrier et al., 1999).
Besides, further improvements are expected in order to have more plasmids translocated in the nucleus. Lan et al have described these approaches as well as the inclusion of transcription factor-binding sites for a cell-specific delivery (Lam & Dean, 2010).

3.2 siRNA

RA is characterized by an uncontrolled increase of the production of inflammatory cytokines (TNF-α, IL-1β, IL-6), an uncontrolled production of enzymes destroying cartilages and bones (metalloproteases). First of all, treatments and studies have targeted these pathogenic molecules. Then, the emergence of siRNA and small hairpin RNA offer the possibility to prevent the production of the pathogenic molecules. However these naked nucleic acids are unstable and are rapidly damaged by enzymes. Different approaches have been used to enhance their stability, to protect them against nuclease degradation, to perform their ability to penetrate in the cells (Khoury et al., 2006; Shim & Kwon, 2010). The

therapeutic effect in arthritis models has been demonstrated in blocking TNF-α by an i.a injection of siRNA and by an i.p injection (Howard et al., 2009; Schiffelers et al., 2005). The systemic injection of siRNA has been possible through the development of nanoparticles lipoplexes (Courties et al., 2011; Khoury et al., 2006). Another delivery approach has been developed using a cream containing siRNA (Takanashi et al., 2009). It targeted the osteopontin produced by macrophages and activated T cells. After application, osteopontin has been found reduced in the serum and siRNA ameliorate symptoms of the disease. Using a cream to deliver siRNA is an interesting new technology because this method acts locally without an invasive injection.

3.3 Viral gene therapy development in rheumatoid arthritis

The most common vectors used in RA are adenoviruses (AdV), retroviruses (oncoviruses and lentivirus) and recombinant adeno-associated viruses (rAAV). They have attractive characteristics for human GT (Bessis & Boissier, 2003; Cavazzana-Calvo et al., 2010; Herzog et al., 2010).

In 1985, for the first time, Roeschler et al. have injected an AdV encoding LacZ in the knees of rabbits. Authors showed that AdV are able to transfect both types A and B synoviocytes throughout the articular and periarticular knees (Roessler et al., 1993). Their use as a vector for gene delivery is limited due to the inflammatory effect and/or because they can be quickly lost when they transfect dividing cells. Researchers tried to construct AdV without viral sequences. The first result showed that the yield was weaker than for the first generation. Furthermore the process is difficult (Kochanek et al., 2001). Other modifications have been made to decrease the immunogenicity and to increase the stability and the specificity (Nayak & Herzog, 2010; Tang et al., 2010). Indeed, AdV under control of inducible promoter (human telomerase reverse transcriptase) can lyse synoviocytes which contain active telomerase in a rat CIA model. Furthermore authors noticed a replication of the AdV in the arthritic joints but not in the non- arthritic ones (Chen S.Y. et al., 2009). The ability of the AdV to target specifically pathogenic cells (without reaction against normal cells) is an important property to be developed for gene therapy in patients.

Retroviruses (RV) are divided into two classes. The first class called oncoretrovirus, used commonly, is derived from Monkey Murine Leukaemia oncoretrovirus. They are less adapted for i.a *in vivo* GT than AdV but the feasibility and safety of the *ex vivo* gene strategy has been demonstrated in patients RA (Wehling et al., 2009). The second class called lentivirus is able to easily transfect cells and then to produce the transgene for a long time. Several studies have shown that an i.a injection of lentivirus (encoding therapeutic molecules) decreases the development of arthritis in animal models (Gouze et al., 2003; Wang et al., 2010; Zondervan et al., 2008). Furthermore, lentivirus [encoding both a therapeutic molecule (galectin 1) and the shRNA of (galectin 3)] has been injected in the joint of arthritic mice (Wang et al., 2010). The joints injected with the lentivirus are less inflamed and show less bone degradation. Furthermore the angiogenesis seems lower in treated mice than in untreated mice. However, authors noted a lower vessel density.

RA is a disease developed from imbalance between pro and anti inflammatory mechanisms. This approach is also interesting because it shows the feasibility to target molecules playing an opposite role in the development of the pathology.

Recombinant adeno-associated viruses (rAAV) are non pathogenic and are able to produce the transgene for a long time. They exist under different serotypes, including eight characterized ones which differ by their ability to transfect specific tissues. The serotype

rAAV2 has been used in several clinical trials including the local treatment of RA (Mueller & Flotte, 2008). In the last few years, serotype 5 has been defined as a "good" vector to target the synovial tissue (Apparailly et al., 2005; Boissier et al., 2007). However, autoantibodies production directed against AAV are responsible for neutralization of rAAV (Cottard et al., 2000) . Boutin et al. have published the titer of Immunoglobulin G subclasses produced against each serotype in humans (Boutin et al., 2010). These results and the knowledge of the efficiency of Serotypes 5 to transfect synovium demonstrate that rAAV5 may be an ideal candidate for i.a gene therapy in RA. Furthermore, the integration of an inducible promoter is encouraging in order to target a specific inflamed tissue (Apparailly et al., 2002; Yang et al., 2010).

Using rAAV is limited because the production strategy is long, difficult and only gives a poor performance. Different processes have been followed to increase the yield and answer the requirements for clinical trials (Negrete & Kotin, 2008; Wright, 2008). For a long time rAAV have been produced from mammalian adherent cells restricting the production of large doses which prevents in turn limits a higher number of clinical trials and a commercial production. Another system avoiding the usual limit for producing rAAV has been developed. This system uses insect cells and baculoviruses vector. This experiment carries on in order to increase the yield whilst reducing the complexity of the system (Mena et al., 2010; Negrete & Kotin, 2008). Aslandi et al. have also established a stable insect cell line containing helper genes necessary for the production of vectors. These genes will only be active when the cells are transfected with baculovirus. The system has improved the yield and the protocol to produce rAAV (Aslanidi et al., 2009).

4. Development of specific regulated promoters

There are two classes of promoters: constitutive (viral and eukariotic) and inducible. Among constitutive viral vectors, Cytomegalovirus (CMV) is the most used promoter in mammalian expression systems because it allows a high level of gene expression. However, its expression is regulated by a large variety of stimuli which can cause an uncontrolled production of the transgene.

Different inducible promoters under the control of molecules such as antibiotics have been developed to target tumours or specific tissues (Robson & Hirst, 2003). For the first time Bakker et al. showed the feasibility to use an inducible adenovirus vector made up of 2-component promoters. The promoters have sites for Nuclear factor kappa B binding and are activated by pro-inflammatory cytokines. Authors have injected this vector in the joint of mice immunized with collagen. They noticed an improvement in the treated and untreated paws. They have obtained the same result in using the adenovirus encoding IL-1Ra under the control of CMV (Bakker et al., 2002). In order to get a more specific inducible therapeutic action of vectors, it is possible to use bioinformatic. Geurts et al. have elaborated a system to design proximal-promoters aiming at to RA (Geurts et al., 2009) . Therefore, inflammation responsive promoters provide a huge progress in rheumatoid arthritis gene therapy because this disease is characterized by temporary acute flares.

5. Conclusion

The studies described in this chapter show the advances in gene transfer and its applications in RA. The clinical trials applied on RA patients demonstrate:

1. that gene therapy is not only appropriate for monogenic diseases but also for multigenic diseases
2. the feasibility of the strategy
3. that progress must carry on in the years to come to become more efficient.

Each strategy (*ex vivo* and *in vivo*), each vector and each target have their advantages and drawbacks. Experimental protocols have improved the efficiency, the safety, of Gene therapy involving the development of clinical trials in RA patients. Three startegies of GT have been been tried : 1) RA-FLS genetically modified (by RV encoding IL-1Ra *ex vivo*) before i.a injection , 2) i.a injection of plasmid encoding the Herpes simples virus thymidine kinase , 3) i.a injection of AAV2 encoding hTNFRs (table 1).

Trial Country	Vector	Transgene	Date approved	Status
Canada	Adeno-associated virus	TNFR-Fc Ig fusion gene	2004	Closed
Germany	retrovirus	IL-1Ra		open
USA	retrovirus	IL-1Ra	1995	closed
	plasmid	HSV-TK	1998	closed
	Adeno-associated virus	TNFR-Fc Ig fusion gene	2003	closed
	Adeno-associated virus	TNFR-Fc Ig fusion gene	2005	open

Table 1. Gene therapy clinical trial in rheumatoid arthritis in the world. Three countries only have attempted RA gene therapy clinical trials (Edelstein, 2011).

Furthermore, the knowledge acquired on the mechanisms of the disease increases the number of possible targets. The recent studies on signalisation pathway offer the opportunity for new therapeutic strategies.

Other promising therapeutic systems must be developed to combine researches in biology, chemistry and engineering.

6. References

Adriaansen, J., Vervoordeldonk, M. J., Vanderbyl, S., de Jong, G. & Tak, P. P. (2006). A novel approach for gene therapy: engraftment of fibroblasts containing the artificial chromosome expression system at the site of inflammation. *J Gene Med*, Vol. 8, No. 1, pp. 63-71.

Ahrens, E. T., Flores, R., Xu, H. & Morel, P. A. (2005). *In vivo* imaging platform for tracking immunotherapeutic cells. *Nat Biotechnol*, Vol. 23, No. 8, pp. 983-987.

Aihara, H. & Miyazaki, J. (1998). Gene transfer into muscle by electroporation *in vivo*. *Nat Biotechnol*, Vol. 16, No. 9, pp. 867-870.

Andre, F. M. & Mir, L. M. (2010). Nucleic acids electrotransfer *in vivo*: mechanisms and practical aspects. *Curr Gene Ther*, Vol. 10, No. 4, pp. 267-280.

Andreakos, E., Rauchhaus, U., Stavropoulos, A., Endert, G., Wendisch, V., Benahmed, A. S., Giaglis, S., Karras, J., Lee, S., Gaus, H., Bennett, C. F., Williams, R. O., Sideras, P. & Panzner, S. (2009). Amphoteric liposomes enable systemic antigen-presenting cell-

directed delivery of CD40 antisense and are therapeutically effective in experimental arthritis. *Arthritis Rheum*, Vol. 60, No. 4, pp. 994-1005.

Apparailly, F., Millet, V., Noel, D., Jacquet, C., Sany, J. & Jorgensen, C. (2002). Tetracycline-inducible interleukin-10 gene transfer mediated by an adeno-associated virus: application to experimental arthritis. *Hum Gene Ther*, Vol. 13, No. 10, pp. 1179-1188.

Apparailly, F., Khoury, M., Vervoordeldonk, M. J., Adriaansen, J., Gicquel, E., Perez, N., Riviere, C., Louis-Plence, P., Noel, D., Danos, O., Douar, A. M., Tak, P. P. & Jorgensen, C. (2005). Adeno-associated virus pseudotype 5 vector improves gene transfer in arthritic joints. *Hum Gene Ther*, Vol. 16, No. 4, pp. 426-434.

Aslanidi, G., Lamb, K. & Zolotukhin, S. (2009). An inducible system for highly efficient production of recombinant adeno-associated virus (rAAV) vectors in insect Sf9 cells. *Proc Natl Acad Sci U S A*, Vol. 106, No. 13, pp. 5059-5064.

Bakker, A. C., van de Loo, F. A., Joosten, L. A., Arntz, O. J., Varley, A. W., Munford, R. S. & van den Berg, W. B. (2002). C3-Tat/HIV-regulated intraarticular human interleukin-1 receptor antagonist gene therapy results in efficient inhibition of collagen-induced arthritis superior to cytomegalovirus-regulated expression of the same transgene. *Arthritis Rheum*, Vol. 46, No. 6, pp. 1661-1670.

Bandara, G., Mueller, G. M., Galea-Lauri, J., Tindal, M. H., Georgescu, H. I., Suchanek, M. K., Hung, G. L., Glorioso, J. C., Robbins, P. D. & Evans, C. H. (1993). Intraarticular expression of biologically active interleukin 1-receptor-antagonist protein by *ex vivo* gene transfer. *Proc Natl Acad Sci U S A*, Vol. 90, No. 22, pp. 10764-10768.

Bessis, N., Boissier, M. C., Ferrara, P., Blankenstein, T., Fradelizi, D. & Fournier, C. (1996). Attenuation of collagen-induced arthritis in mice by treatment with vector cells engineered to secrete interleukin-13. *Eur J Immunol*, Vol. 26, No. 10, pp. 2399-2403.

Bessis, N. & Boissier, M. C. (2003). Vectors for gene therapy of joint disorders. *Joint Bone Spine*, Vol. 70, No. 6, pp. 471-473.

Bessis, N., Lemeiter, D., Laroche, L., Fournier, C., Huizinga, T., Brok, H., t Hart, B. & Boissier, M. C. (2007). Engraftment of cutaneous fibroblasts within synovial membrane in a nonhuman primate: short-term results. *Joint Bone Spine*, Vol. 74, No. 1, pp. 48-51.

Bloquel, C., Bessis, N., Boissier, M. C., Scherman, D. & Bigey, P. (2004). Gene therapy of collagen-induced arthritis by electrotransfer of human tumor necrosis factor-alpha soluble receptor I variants. *Hum Gene Ther*, Vol. 15, No. 2, pp. 189-201.

Bloquel, C., Fabre, E., Bureau, M. F. & Scherman, D. (2004). Plasmid DNA electrotransfer for intracellular and secreted proteins expression: new methodological developments and applications. *J Gene Med*, Vol. 6 Suppl 1, No. pp. S11-23.

Bloquel, C., Denys, A., Boissier, M. C., Apparailly, F., Bigey, P., Scherman, D. & Bessis, N. (2007). Intra-articular electrotransfer of plasmid encoding soluble TNF receptor variants in normal and arthritic mice. *J Gene Med*, Vol. 9, No. 11, pp. 986-993.

Boissier, M. C., Lemeiter, D., Clavel, C., Valvason, C., Laroche, L., Begue, T. & Bessis, N. (2007). Synoviocyte infection with adeno-associated virus (AAV) is neutralized by human synovial fluid from arthritis patients and depends on AAV serotype. *Hum Gene Ther*, Vol. 18, No. 6, pp. 525-535.

Boutin, S., Monteilhet, V., Veron, P., Leborgne, C., Benveniste, O., Montus, M. F. & Masurier, C. (2010). Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population:

implications for gene therapy using AAV vectors. *Hum Gene Ther*, Vol. 21, No. 6, pp. 704-712.

Burke, B. (2003). Macrophages as novel cellular vehicles for gene therapy. *Expert Opin Biol Ther*, Vol. 3, No. 6, pp. 919-924.

Cavazzana-Calvo, M., Hacein-Bey-Abina, S. & Fischer, A. (2010). [Ten years of gene therapy: thoughts and perspectives]. *Med Sci (Paris)*, Vol. 26, No. 2, pp. 115-118.

Cepurniene, K., Ruzgys, P., Treinys, R., Satkauskiene, I. & Satkauskas, S. (2010). Influence of plasmid concentration on DNA electrotransfer *in vitro* using high-voltage and low-voltage pulses. *J Membr Biol*, Vol. 236, No. 1, pp. 81-85.

Chan, J. M., Villarreal, G., Jin, W. W., Stepan, T., Burstein, H. & Wahl, S. M. (2002). Intraarticular gene transfer of TNFR:Fc suppresses experimental arthritis with reduced systemic distribution of the gene product. *Mol Ther*, Vol. 6, No. 6, pp. 727-736.

Chen, S. Y., Shiau, A. L., Shieh, G. S., Su, C. H., Lee, C. H., Lee, H. L., Wang, C. R. & Wu, C. L. (2009). Amelioration of experimental arthritis by a telomerase-dependent conditionally replicating adenovirus that targets synovial fibroblasts. *Arthritis Rheum*, Vol. 60, No. 11, pp. 3290-3302.

Chen, Z. Y., He, C. Y., Ehrhardt, A. & Kay, M. A. (2003). Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression *in vivo*. *Mol Ther*, Vol. 8, No. 3, pp. 495-500.

Conese, M., Auriche, C. & Ascenzioni, F. (2004). Gene therapy progress and prospects: episomally maintained self-replicating systems. *Gene Ther*, Vol. 11, No. 24, pp. 1735-1741.

Cottard, V., Mulleman, D., Bouille, P., Mezzina, M., Boissier, M. C. & Bessis, N. (2000). Adeno-associated virus-mediated delivery of IL-4 prevents collagen-induced arthritis. *Gene Ther*, Vol. 7, No. 22, pp. 1930-1939.

Cottard, V., Valvason, C., Falgarone, G., Lutomski, D., Boissier, M. C. & Bessis, N. (2004). Immune response against gene therapy vectors: influence of synovial fluid on adeno-associated virus mediated gene transfer to chondrocytes. *J Clin Immunol*, Vol. 24, No. 2, pp. 162-169.

Courties, G., Baron, M., Presumey, J., Escriou, V., van Lent, P., Scherman, D., Cantagrel, A., van den Berg, W. B., Jorgensen, C., Apparailly, F. & Davignon, J. L. (2011). Cytosolic phospholipase A2alpha gene silencing in the myeloid lineage alters development of Th1 responses and reduces disease severity in collagen-induced arthritis. *Arthritis Rheum*, Vol. 63, No. 3, pp. 681-690.

Cukjati, D., Batiuskaite, D., Andre, F., Miklavcic, D. & Mir, L. M. (2007). Real time electroporation control for accurate and safe *in vivo* non-viral gene therapy. *Bioelectrochemistry*, Vol. 70, No. 2, pp. 501-507.

Denys, A., Thiolat, A., Descamps, D., Lemeiter, D., Benihoud, K., Bessis, N. & Boissier, M. C. (2010). Intra-articular electrotransfer of mouse soluble tumour necrosis factor receptor in a murine model of rheumatoid arthritis. *J Gene Med*, Vol. 12, No. 8, pp. 659-668.

Edelstein, M. (2007). Gene therapy Clinical worldwide trial provided by Journal of Gene Medicine, In : Wyley InterScience, 2011, Available from: http://www.wiley.com/legacy/wileychi/genmed/clinical/

Evans, C. (2010). Arthritis gene therapy trials reach phase II. *J Rheumatol*, Vol. 37, No. 4, pp. 683-685.

Evans, C. H., Robbins, P. D., Ghivizzani, S. C., Wasko, M. C., Tomaino, M. M., Kang, R., Muzzonigro, T. A., Vogt, M., Elder, E. M., Whiteside, T. L., Watkins, S. C. & Herndon, J. H. (2005). Gene transfer to human joints: progress toward a gene therapy of arthritis. *Proc Natl Acad Sci U S A*, Vol. 102, No. 24, pp. 8698-8703.

Feero, W. G., Rosenblatt, J. D., Huard, J., Watkins, S. C., Epperly, M., Clemens, P. R., Kochanek, S., Glorioso, J. C., Partridge, T. A. & Hoffman, E. P. (1997). Viral gene delivery to skeletal muscle: insights on maturation-dependent loss of fiber infectivity for adenovirus and herpes simplex type 1 viral vectors. *Hum Gene Ther*, Vol. 8, No. 4, pp. 371-380.

Felgner, P. L. (1996). Improvements in cationic liposomes for *in vivo* gene transfer. *Hum Gene Ther*, Vol. 7, No. 15, pp. 1791-1793.

Fernandes, J. C., Wang, H., Jreyssaty, C., Benderdour, M., Lavigne, P., Qiu, X., Winnik, F. M., Zhang, X., Dai, K. & Shi, Q. (2008). Bone-protective effects of nonviral gene therapy with folate-chitosan DNA nanoparticle containing interleukin-1 receptor antagonist gene in rats with adjuvant-induced arthritis. *Mol Ther*, Vol. 16, No. 7, pp. 1243-1251.

Fiocco, U. & Punzi, L. (2011). "Are there any evidences for using the intra-articular TNF-alpha blockade in resistant arthritis?" *Joint Bone Spine*, Vol. No. pp.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature*, Vol. 391, No. 6669, pp. 806-811.

Geurts, J., Joosten, L. A., Takahashi, N., Arntz, O. J., Gluck, A., Bennink, M. B., van den Berg, W. B. & van de Loo, F. A. (2009). Computational design and application of endogenous promoters for transcriptionally targeted gene therapy for rheumatoid arthritis. *Mol Ther*, Vol. 17, No. 11, pp. 1877-1887.

Ghivizzani, S. C., Lechman, E. R., Kang, R., Tio, C., Kolls, J., Evans, C. H. & Robbins, P. D. (1998). Direct adenovirus-mediated gene transfer of interleukin-1 and tumor necrosis factor alpha soluble receptors to rabbit knees with experimental arthritis has local and distal anti-arthritic effects. *Proc. Natl. Acad. Sci. USA.*, Vol. 95, No. pp. 4613-4618.

Gilbert, R. A., Jaroszeski, M. J. & Heller, R. (1997). Novel electrode designs for electrochemotherapy. *Biochim Biophys Acta*, Vol. 1334, No. 1, pp. 9-14.

Gill, D. R., Pringle, I. A. & Hyde, S. C. (2009). Progress and prospects: the design and production of plasmid vectors. *Gene Ther*, Vol. 16, No. 2, pp. 165-171.

Gissel, H. (2010). Effects of varying pulse parameters on ion homeostasis, cellular integrity, and force following electroporation of rat muscle *in vivo*. *Am J Physiol Regul Integr Comp Physiol*, Vol. 298, No. 4, pp. R918-929.

Gothelf, A. & Gehl, J. (2010). Gene electrotransfer to skin; review of existing literature and clinical perspectives. *Curr Gene Ther*, Vol. 10, No. 4, pp. 287-299.

Gouze, E., Pawliuk, R., Gouze, J. N., Pilapil, C., Fleet, C., Palmer, G. D., Evans, C. H., Leboulch, P. & Ghivizzani, S. C. (2003). Lentiviral-mediated gene delivery to synovium: potent intra-articular expression with amplification by inflammation. *Mol Ther*, Vol. 7, No. 4, pp. 460-466.

Gresch, O., Engel, F. B., Nesic, D., Tran, T. T., England, H. M., Hickman, E. S., Korner, I., Gan, L., Chen, S., Castro-Obregon, S., Hammermann, R., Wolf, J., Muller-

Hartmann, H., Nix, M., Siebenkotten, G., Kraus, G. & Lun, K. (2004). New non-viral method for gene transfer into primary cells. *Methods*, Vol. 33, No. 2, pp. 151-163.

Heller, R., Jaroszeski, M., Atkin, A., Moradpour, D., Gilbert, R., Wands, J. & Nicolau, C. (1996). *In vivo* gene electroinjection and expression in rat liver. *FEBS Lett*, Vol. 389, No. 3, pp. 225-228.

Herzog, R. W., Cao, O. & Srivastava, A. (2010). Two decades of clinical gene therapy--success is finally mounting. *Discov Med*, Vol. 9, No. 45, pp. 105-111.

Hill, M. & Hillova, J. (1972). Virus recovery in chicken cells tested with Rous sarcoma cell DNA. *Nat New Biol*, Vol. 237, No. 71, pp. 35-39.

Hojman, P., Gissel, H., Andre, F. M., Cournil-Henrionnet, C., Eriksen, J., Gehl, J. & Mir, L. M. (2008). Physiological effects of high- and low-voltage pulse combinations for gene electrotransfer in muscle. *Hum Gene Ther*, Vol. 19, No. 11, pp. 1249-1260.

Hojman, P. (2010). Basic principles and clinical advancements of muscle electrotransfer. *Curr Gene Ther*, Vol. 10, No. 2, pp. 128-138.

Hombach, A. A., Kofler, D., Rappl, G. & Abken, H. (2009). Redirecting human CD4+CD25+ regulatory T cells from the peripheral blood with pre-defined target specificity. *Gene Ther*, Vol. 16, No. 9, pp. 1088-1096.

Horie, M., Sekiya, I., Muneta, T., Ichinose, S., Matsumoto, K., Saito, H., Murakami, T. & Kobayashi, E. (2009). Intra-articular Injected synovial stem cells differentiate into meniscal cells directly and promote meniscal regeneration without mobilization to distant organs in rat massive meniscal defect. *Stem Cells*, Vol. 27, No. 4, pp. 878-887.

Howard, K. A., Paludan, S. R., Behlke, M. A., Besenbacher, F., Deleuran, B. & Kjems, J. (2009). Chitosan/siRNA nanoparticle-mediated TNF-alpha knockdown in peritoneal macrophages for anti-inflammatory treatment in a murine arthritis model. *Mol Ther*, Vol. 17, No. 1, pp. 162-168.

Ji, L., Bouvet, M., Price, R. E., Roth, J. A. & Fang, B. (1999). Reduced toxicity, attenuated immunogenicity and efficient mediation of human p53 gene expression *in vivo* by an adenovirus vector with deleted E1-E3 and inactivated E4 by GAL4-TATA promoter replacement. *Gene Ther*, Vol. 6, No. 3, pp. 393-402.

Kay, M. A., He, C. Y. & Chen, Z. Y. (2010). A robust system for production of minicircle DNA vectors. *Nat Biotechnol*, Vol. 28, No. 12, pp. 1287-1289.

Khoury, M., Bigey, P., Louis-Plence, P., Noel, D., Rhinn, H., Scherman, D., Jorgensen, C. & Apparailly, F. (2006). A comparative study on intra-articular versus systemic gene electrotransfer in experimental arthritis. *J Gene Med*, Vol. 8, No. 8, pp. 1027-1036.

Khoury, M., Louis-Plence, P., Escriou, V., Noel, D., Largeau, C., Cantos, C., Scherman, D., Jorgensen, C. & Apparailly, F. (2006). Efficient new cationic liposome formulation for systemic delivery of small interfering RNA silencing tumor necrosis factor alpha in experimental arthritis. *Arthritis Rheum*, Vol. 54, No. 6, pp. 1867-1877.

Khoury, M., Escriou, V., Courties, G., Galy, A., Yao, R., Largeau, C., Scherman, D., Jorgensen, C. & Apparailly, F. (2008). Efficient suppression of murine arthritis by combined anticytokine small interfering RNA lipoplexes. *Arthritis Rheum*, Vol. 58, No. 8, pp. 2356-2367.

Kim, S. H., Evans, C. H., Kim, S., Oligino, T., Ghivizzani, S. C. & Robbins, P. D. (2000). Gene therapy for established murine collagen-induced arthritis by local and systemic adenovirus-mediated delivery of interleukin-4. *Arthritis Res*, Vol. 2, No. 4, pp. 293-302.

Kim, S. H., Kim, S., Evans, C. H., Ghivizzani, S. C., Oligino, T. & Robbins, P. D. (2001). Effective treatment of established murine collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express IL-4. *J Immunol*, Vol. 166, No. 5, pp. 3499-3505.

Kim, S. H., Lechman, E. R., Kim, S., Nash, J., Oligino, T. J. & Robbins, P. D. (2002). *Ex vivo* gene delivery of IL-1Ra and soluble TNF receptor confers a distal synergistic therapeutic effect in antigen-induced arthritis. *Mol Ther*, Vol. 6, No. 5, pp. 591-600.

Kim, S. H., Lechman, E. R., Bianco, N., Menon, R., Keravala, A., Nash, J., Mi, Z., Watkins, S. C., Gambotto, A. & Robbins, P. D. (2005). Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. *J Immunol*, Vol. 174, No. 10, pp. 6440-6448.

Kim, S. H., Bianco, N., Menon, R., Lechman, E. R., Shufesky, W. J., Morelli, A. E. & Robbins, P. D. (2006). Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. *Mol Ther*, Vol. 13, No. 2, pp. 289-300.

Kochanek, S., Schiedner, G. & Volpers, C. (2001). High-capacity 'gutless' adenoviral vectors. *Curr Opin Mol Ther*, Vol. 3, No. 5, pp. 454-463.

Lam, A. P. & Dean, D. A. (2010). Progress and prospects: nuclear import of nonviral vectors. *Gene Ther*, Vol. 17, No. 4, pp. 439-447.

Lambert, H., Pankov, R., Gauthier, J. & Hancock, R. (1990). Electroporation-mediated uptake of proteins into mammalian cells. *Biochem Cell Biol*, Vol. 68, No. 4, pp. 729-734.

Lechman, E. R., Jaffurs, D., Ghivizzani, S. C., Gambotto, A., Kovesdi, I., Mi, Z., Evans, C. H. & Robbins, P. D. (1999). Direct adenoviral gene transfer of viral IL-10 to rabbit knees with experimental arthritis ameliorates disease in both injected and contralateral control knees. *J Immunol*, Vol. 163, No. 4, pp. 2202-2208.

Lechman, E. R., Keravala, A., Nash, J., Kim, S. H., Mi, Z. & Robbins, P. D. (2003). The contralateral effect conferred by intra-articular adenovirus-mediated gene transfer of viral IL-10 is specific to the immunizing antigen. *Gene Ther*, Vol. 10, No. 24, pp. 2029-2035.

Lee, J. H. & Welsh, M. J. (1999). Enhancement of calcium phosphate-mediated transfection by inclusion of adenovirus in coprecipitates. *Gene Ther*, Vol. 6, No. 4, pp. 676-682.

Li, Y., Wang, J., Liu, J. & Liu, F. (2010). A novel system for *in vivo* neprilysin gene delivery using a syringe electrode. *J Neurosci Methods*, Vol. 193, No. 2, pp. 226-231.

Liu, F. & Huang, L. (2002). A syringe electrode device for simultaneous injection of DNA and electrotransfer. *Mol Ther*, Vol. 5, No. 3, pp. 323-328.

Marie, C., Vandermeulen, G., Quiviger, M., Richard, M., Preat, V. & Scherman, D. (2010). pFARs, plasmids free of antibiotic resistance markers, display high-level transgene expression in muscle, skin and tumour cells. *J Gene Med*, Vol. 12, No. 4, pp. 323-332.

Mayrhofer, P., Blaesen, M., Schleef, M. & Jechlinger, W. (2008). Minicircle-DNA production by site specific recombination and protein-DNA interaction chromatography. *J Gene Med*, Vol. 10, No. 11, pp. 1253-1269.

Mayrhofer, P., Schleef, M. & Jechlinger, W. (2009). Use of minicircle plasmids for gene therapy. *Methods Mol Biol*, Vol. 542, No. pp. 87-104.

Mena, J. A., Aucoin, M. G., Montes, J., Chahal, P. S. & Kamen, A. A. (2010). Improving adeno-associated vector yield in high density insect cell cultures. *J Gene Med*, Vol. 12, No. 2, pp. 157-167.

Miklavcic, D., Semrov, D., Mekid, H. & Mir, L. M. (2000). A validated model of *in vivo* electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy. *Biochim Biophys Acta*, Vol. 1523, No. 1, pp. 73-83.

Mir, L. M., Belehradek, M., Domenge, C., Orlowski, S., Poddevin, B., Belehradek, J., Jr., Schwaab, G., Luboinski, B. & Paoletti, C. (1991). [Electrochemotherapy, a new antitumor treatment: first clinical trial]. *C R Acad Sci III*, Vol. 313, No. 13, pp. 613-618.

Mir, L. M., Bureau, M. F., Rangara, R., Schwartz, B. & Scherman, D. (1998). Long-term, high level *in vivo* gene expression after electric pulse-mediated gene transfer into skeletal muscle. *C R Acad Sci III*, Vol. 321, No. 11, pp. 893-899.

Mir, L. M. (2009). Nucleic acids electrotransfer-based gene therapy (electrogenetherapy): past, current, and future. *Mol Biotechnol*, Vol. 43, No. 2, pp. 167-176.

Miyazaki, S. & Miyazaki, J. (2008). *In vivo* DNA electrotransfer into muscle. *Dev Growth Differ*, Vol. 50, No. 6, pp. 479-483.

Moisset, P. A. & Tremblay, J. P. (2001). Gene therapy: a strategy for the treatment of inherited muscle diseases? *Curr Opin Pharmacol*, Vol. 1, No. 3, pp. 294-299.

Morita, Y., Yang, J., Gupta, R., Shimizu, K., Shelden, E. A., Endres, J., Mule, J. J., McDonagh, K. T. & Fox, D. A. (2001). Dendritic cells genetically engineered to express IL-4 inhibit murine collagen-induced arthritis. *J Clin Invest*, Vol. 107, No. 10, pp. 1275-1284.

Mueller, C. & Flotte, T. R. (2008). Clinical gene therapy using recombinant adeno-associated virus vectors. *Gene Ther*, Vol. 15, No. 11, pp. 858-863.

Nagata, Y., Nakasa, T., Mochizuki, Y., Ishikawa, M., Miyaki, S., Shibuya, H., Yamasaki, K., Adachi, N., Asahara, H. & Ochi, M. (2009). Induction of apoptosis in the synovium of mice with autoantibody-mediated arthritis by the intraarticular injection of double-stranded MicroRNA-15a. *Arthritis Rheum*, Vol. 60, No. 9, pp. 2677-2683.

Nayak, S. & Herzog, R. W. (2010). Progress and prospects: immune responses to viral vectors. *Gene Ther*, Vol. 17, No. 3, pp. 295-304.

Negrete, A. & Kotin, R. M. (2008). Strategies for manufacturing recombinant adeno-associated virus vectors for gene therapy applications exploiting baculovirus technology. *Brief Funct Genomic Proteomic*, Vol. 7, No. 4, pp. 303-311.

Okamoto, K., Asahara, H., Kobayashi, T., Matsuno, H., Hasunuma, T., Kobata, T., Sumida, T. & Nishioka, K. (1998). Induction of apoptosis in the rheumatoid synovium by Fas ligand gene transfer. *Gene Ther*, Vol. 5, No. 3, pp. 331-338.

Peng, B., Zhao, Y., Lu, H., Pang, W. & Xu, Y. (2005). *In vivo* plasmid DNA electroporation resulted in transfection of satellite cells and lasting transgene expression in regenerated muscle fibers. *Biochem Biophys Res Commun*, Vol. 338, No. 3, pp. 1490-1498.

Rebersek, M., Faurie, C., Kanduser, M., Corovic, S., Teissie, J., Rols, M. P. & Miklavcic, D. (2007). Electroporator with automatic change of electric field direction improves gene electrotransfer in-vitro. *Biomed Eng Online*, Vol. 6, No. pp. 25.

Robson, T. & Hirst, D. G. (2003). Transcriptional Targeting in Cancer Gene Therapy. *J Biomed Biotechnol*, Vol. 2003, No. 2, pp. 110-137.

Roessler, B. J., Allen, E. D., Wilson, J. M., Hartman, J. W. & Davidson, B. L. (1993). Adenoviral-mediated gene transfer to rabbit synovium *in vivo*. *J Clin Invest*, Vol. 92, No. 2, pp. 1085-1092.

Rols, M. P., Delteil, C., Golzio, M., Dumond, P., Cros, S. & Teissie, J. (1998). *In vivo* electrically mediated protein and gene transfer in murine melanoma. *Nat Biotechnol*, Vol. 16, No. 2, pp. 168-171.

Ropert, C. (1999). Liposomes as a gene delivery system. *Braz J Med Biol Res*, Vol. 32, No. 2, pp. 163-169.

Saidenberg-Kermanach, N., Bessis, N., Deleuze, V., Bureau, M., Scherman, D. & Boissier, M.-C. (2003). Efficacy of interleukin-10 gene electrotransfer into skeletal muscle in mice with collagen-induced arthritis. *J Gene Med*, Vol. 5, No. 2, pp. 164-171.

Sambrook, J., Westphal, H., Srinivasan, P. R. & Dulbecco, R. (1968). The integrated state of viral DNA in SV40-transformed cells. *Proc Natl Acad Sci U S A*, Vol. 60, No. 4, pp. 1288-1295.

Satkauskas, S., Andre, F., Bureau, M. F., Scherman, D., Miklavcic, D. & Mir, L. M. (2005). Electrophoretic component of electric pulses determines the efficacy of *in vivo* DNA electrotransfer. *Hum Gene Ther*, Vol. 16, No. 10, pp. 1194-1201.

Schiffelers, R. M., Xu, J., Storm, G., Woodle, M. C. & Scaria, P. V. (2005). Effects of treatment with small interfering RNA on joint inflammation in mice with collagen-induced arthritis. *Arthritis Rheum*, Vol. 52, No. 4, pp. 1314-1318.

Shi, Q., Wang, H., Tran, C., Qiu, X., Winnik, F. M., Zhang, X., Dai, K., Benderdour, M. & Fernandes, J. C. (2011). Hydrodynamic delivery of chitosan-folate-DNA nanoparticles in rats with adjuvant-induced arthritis. *J Biomed Biotechnol*, Vol. 2011, No. pp. 148763.

Shim, M. S. & Kwon, Y. J. (2010). Efficient and targeted delivery of siRNA *in vivo*. *Febs J*, Vol. 277, No. 23, pp. 4814-4827.

Silve, A., Villemejane, J., Joubert, V., Ivorra, A. & Mir, L. M. (2010). Advanced Electroporation Techniques in Biology and Medicine,In:*Nanosecond Pulsed Electric Field Delivery to Biological Samples: Difficulties and Potential Solutions*.James C. Lin, pp CRC press, 9781439819067

Soubrier, F., Cameron, B., Manse, B., Somarriba, S., Dubertret, C., Jaslin, G., Jung, G., Caer, C. L., Dang, D., Mouvault, J. M., Scherman, D., Mayaux, J. F. & Crouzet, J. (1999). pCOR: a new design of plasmid vectors for nonviral gene therapy. *Gene Ther*, Vol. 6, No. 8, pp. 1482-1488.

Stenler, S., Andersson, A., Simonson, O. E., Lundin, K. E., Chen, Z. Y., Kay, M. A., Smith, C. I., Sylven, C. & Blomberg, P. (2009). Gene transfer to mouse heart and skeletal muscles using a minicircle expressing human vascular endothelial growth factor. *J Cardiovasc Pharmacol*, Vol. 53, No. 1, pp. 18-23.

Stoop, J. N., Harry, R. A., von Delwig, A., Isaacs, J. D., Robinson, J. H. & Hilkens, C. M. (2010). Therapeutic effect of tolerogenic dendritic cells in established collagen-induced arthritis is associated with a reduction in Th17 responses. *Arthritis Rheum*, Vol. 62, No. 12, pp. 3656-3665.

Takanashi, M., Oikawa, K., Sudo, K., Tanaka, M., Fujita, K., Ishikawa, A., Nakae, S., Kaspar, R. L., Matsuzaki, M., Kudo, M. & Kuroda, M. (2009). Therapeutic silencing of an endogenous gene by siRNA cream in an arthritis model mouse. *Gene Ther*, Vol. 16, No. 8, pp. 982-989.

Takka, S. & Gurel, A. (2010). Evaluation of chitosan/alginate beads using experimental design: formulation and *in vitro* characterization. *AAPS PharmSciTech*, Vol. 11, No. 1, pp. 460-466.

Tang, B., Cullins, D. L., Zhou, J., Zawaski, J. A., Park, H., Brand, D. D., Hasty, K. A., Gaber, M. W., Stuart, J. M., Kang, A. H. & Myers, L. K. (2010). Modulation of collagen-induced arthritis by adenovirus-mediated intra-articular expression of modified collagen type II. *Arthritis Res Ther*, Vol. 12, No. 4, pp. R136.

Tevz, G., Pavlin, D., Kamensek, U., Kranjc, S., Mesojednik, S., Coer, A., Sersa, G. & Cemazar, M. (2008). Gene electrotransfer into murine skeletal muscle: a systematic analysis of parameters for long-term gene expression. *Technol Cancer Res Treat*, Vol. 7, No. 2, pp. 91-101.

Titomirov, A. V., Sukharev, S. & Kistanova, E. (1991). *In vivo* electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. *Biochim Biophys Acta*, Vol. 1088, No. 1, pp. 131-134.

van de Loo, F. A. & van den Berg, W. B. (2002). Gene therapy for rheumatoid arthritis. Lessons from animal models, including studies on interleukin-4, interleukin-10, and interleukin-1 receptor antagonist as potential disease modulators. *Rheum Dis Clin North Am*, Vol. 28, No. 1, pp. 127-149.

Wang, C. R., Shiau, A. L., Chen, S. Y., Cheng, Z. S., Li, Y. T., Lee, C. H., Yo, Y. T., Lo, C. W., Lin, Y. S., Juan, H. Y., Chen, Y. L. & Wu, C. L. (2010). Intra-articular lentivirus-mediated delivery of galectin-3 shRNA and galectin-1 gene ameliorates collagen-induced arthritis. *Gene Ther*, Vol. 17, No. 10, pp. 1225-1233.

Wehling, P., Reinecke, J., Baltzer, A. W., Granrath, M., Schulitz, K. P., Schultz, C., Krauspe, R., Whiteside, T. W., Elder, E., Ghivizzani, S. C., Robbins, P. D. & Evans, C. H. (2009). Clinical responses to gene therapy in joints of two subjects with rheumatoid arthritis. *Hum Gene Ther*, Vol. 20, No. 2, pp. 97-101.

Whalen, J. D., Lechman, E. L., Carlos, C. A., Weiss, K., Kovesdi, I., Glorioso, J. C., Robbins, P. D. & Evans, C. H. (1999). Adenoviral transfer of the viral IL-10 gene periarticularly to mouse paws suppresses development of collagen-induced arthritis in both injected and uninjected paws. *J Immunol*, Vol. 162, No. 6, pp. 3625-3632.

Wright, G. P., Notley, C. A., Xue, S. A., Bendle, G. M., Holler, A., Schumacher, T. N., Ehrenstein, M. R. & Stauss, H. J. (2009). Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proc Natl Acad Sci U S A*, Vol. 106, No. 45, pp. 19078-19083.

Wright, J. F. (2008). Manufacturing and characterizing AAV-based vectors for use in clinical studies. *Gene Ther*, Vol. 15, No. 11, pp. 840-848.

Wu, Z., Asokan, A. & Samulski, R. J. (2006). Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol Ther*, Vol. 14, No. 3, pp. 316-327.

Yamamoto, A., Fukuda, A., Seto, H., Miyazaki, T., Kadono, Y., Sawada, Y., Nakamura, I., Katagiri, H., Asano, T., Tanaka, Y., Oda, H., Nakamura, K. & Tanaka, S. (2003). Suppression of arthritic bone destruction by adenovirus-mediated dominant-negative Ras gene transfer to synoviocytes and osteoclasts. *Arthritis Rheum*, Vol. 48, No. 9, pp. 2682-2692.

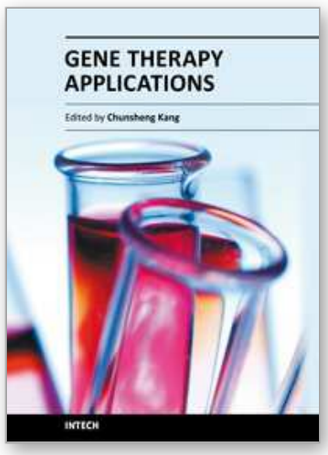
Yang, T., Duan, R., Cao, H., Lee, B. H., Xia, C., Chang, Z., Keith Tanswell, A. & Hu, J. (2010). Development of an inflammation-inducible gene expression system using helper-dependent adenoviral vectors. *J Gene Med*, Vol. 12, No. 10, pp. 832-839.

Zaiss, A. K. & Muruve, D. A. (2005). Immune responses to adeno-associated virus vectors. *Curr Gene Ther*, Vol. 5, No. 3, pp. 323-331.

Zheng, X., Suzuki, M., Ichim, T. E., Zhang, X., Sun, H., Zhu, F., Shunnar, A., Garcia, B., Inman, R. D. & Min, W. (2010). Treatment of autoimmune arthritis using RNA interference-modulated dendritic cells. *J Immunol*, Vol. 184, No. 11, pp. 6457-6464.

Zondervan, R., Xia, T., van der Meer, H., Storm, C., Kulzer, F., van Saarloos, W. & Orrit, M. (2008). Soft glassy rheology of supercooled molecular liquids. *Proc Natl Acad Sci U S A*, Vol. 105, No. 13, pp. 4993-4998.

Zupanic, A., Corovic, S., Miklavcic, D. & Pavlin, M. (2010). Numerical optimization of gene electrotransfer into muscle tissue. *Biomed Eng Online*, Vol. 9, No. pp. 66.



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