Gene therapy in rheumatic diseases
BACKGROUND

In Western countries the population is ageing associated with an increasing incidence of chronic diseases. Among these, diseases of bones and joints, e.g. osteoporosis, rheumatoid arthritis (RA) and osteoarthritis (OA), as well as systemic diseases, e.g. Sjögren's syndrome (SS) and Systemic lupus erythematosus (SLE), reduce the quality of life and impose a huge drain on healthcare funds. A better understanding of the pathophysiology of RA has led to important and innovative approaches to its treatment. In the case of confirmed diagnosis the recommended gold standard is to initiate methotrexate, possibly in combination with other conventional disease-modifying anti-rheumatic drugs (DMARDs) and/or corticosteroids. If disease activity cannot be controlled by conventional treatment, biological treatment is considered. During the last decade, development of TNF blockade, together with an improved timing and dosing of conventional therapy, has significantly improved the outcome of RA in many patients, but not all. Other biological treatments that have become available include CTLA4-Ig (abatacept), anti-CD20 (rituximab), and anti-IL-6 receptor (tocilizumab) treatment. Despite all these treatment options, remission is achieved in only a minority of the patients, leaving most patients with at least monoarticular or oligoarticular disease activity. Furthermore, one major problem of the treatment with biologicals is the need for ongoing systemic therapy. Gene therapy can offer in theory lifelong treatment selectively at the site of inflammation, resulting in remission without the need for frequent re-administration of the gene construct. For RA, and to a lesser extent for other immune-related inflammatory disorders, the proof of concept has been extensively demonstrated in a variety of pre-clinical studies. However, only few clinical trials have been conducted in the field of rheumatologic diseases. Gene therapy approaches for the treatment of non-lethal diseases are still experimental, but recently a breakthrough was reached; the European Commission granted marketing authorization for the first gene therapy application namely Glybera®. Glybera® is approved under exceptional circumstances as a treatment for adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) confirmed by genetic testing, and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions.

This section of the educational course will summarize the current status of the developments in the field of gene therapy for RA and related diseases. Gene transfer technologies will be discussed including route of administration, viral and non-viral delivery systems, therapeutic targets and recent clinical trials.

The course will focus on the developments with the greatest potential in the near future, especially gene therapy in RA, since most of the pre-clinical and clinical gene therapy studies have been performed in this field. Other rheumatologic diseases, e.g. OA, SS and SLE, are mentioned briefly. Cell therapies are addressed in a separate section.

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From:
I. GENE THERAPY: ROUTES OF ADMINISTRATION AND VECTORS

I.1 Introduction
In gene therapy studies a “correct copy” or “wild type” gene is provided to the target cell that is missing this gene or has an inactive gene. Generally, it is not an exact replacement of the “abnormal,” disease-causing gene, but rather extra, correct copies of genes are provided to complement the loss of function. In the treatment of rheumatic diseases the purpose of the gene therapy is different, since no specific defect in the gene is known to cause the disease. In the area of autoimmune disease the focus is more directed towards modulation of the inflammatory process either with or without decreasing bone and cartilage destruction. The objective is to insert a gene coding for a therapeutic protein, usually in combination with a promoter sequence, into a target cell resulting in the constitutive production of the therapeutic protein in the body. Promoters are DNA sequences that are used to regulate gene expression. The promoter is placed upstream of the therapeutic gene (see Figure 1 for a schematic representation). Without a promoter the gene will not be expressed.

Since the DNA by itself does not have the machinery to enter the cell, a vector, either viral or non-viral, needs to be used in order to facilitate cell entry of the therapeutic gene. Thereafter, the vector unloads its genetic material containing the therapeutic gene into the target cell and the cell can start to produce the therapeutic protein using its own machinery (see Figure 2 for a schematic representation). Currently, the most efficient type of vectors are viruses that have been genetically modified to carry therapeutic genes. Of those viruses most of the viral genes are removed to prevent replication of the virus and to reduce its immunogenicity. In the first section the routes of administration of the vectors are discussed, the second section will be focused on the vectors. An overview of gene therapy approaches tested in different animal models of arthritis is given in Table 1.

Figure 1. Schematic representation of a therapeutic gene. The gene consists of a promoter, a therapeutic gene and a poly(A) tail. There are different promoters that have specific functions to control gene expression. The poly(A) tail is a long stretch of adenosine nucleotides at the tail or 3'end of the gene. It is of importance for transcription termination, nuclear export, translation and stability of mRNA.
Table 1. Targets and vectors used in experimental arthritis models for RA

<table>
<thead>
<tr>
<th>Transgene</th>
<th>Method</th>
<th>Administration</th>
<th>Vector</th>
<th>Animal model</th>
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<tr>
<td>IL-1Ra</td>
<td>Ex vivo</td>
<td>Synovial fibroblasts</td>
<td>Retrovirus</td>
<td>Rabbit AIA, Rat SCW-induced arthritis, mouse CIA</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
<td>Mouse IL-1Ra KO</td>
</tr>
<tr>
<td>IL-1RaCp</td>
<td>Ex vivo</td>
<td>Local (ia)</td>
<td>Stable transfection</td>
<td>Rabbit AIA</td>
</tr>
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<td>sIL-1R</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
<td>Rabbit AIA</td>
</tr>
<tr>
<td>TNFR2:Fc</td>
<td>Ex vivo</td>
<td>Local (ia)</td>
<td>Retro</td>
<td>Rabbit AIA</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
<td>Rat SCW-induced arthritis,</td>
</tr>
<tr>
<td>TNFR1:Fc</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>AAV</td>
<td>Rat AA, mouse CIA</td>
</tr>
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<td>sTNFR</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>AAV</td>
<td>TNF-transgenic mouse</td>
</tr>
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<td>IL-4</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
<td>RAT AA, mouse CIA</td>
</tr>
<tr>
<td>vIL-10</td>
<td>In vivo</td>
<td>Systemic (iv)</td>
<td>Adeno</td>
<td>Mouse CIA</td>
</tr>
<tr>
<td>IL-13</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Plasmid</td>
<td>Mouse CIA</td>
</tr>
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<td>IFN-β</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Retro</td>
<td>Mouse CIA</td>
</tr>
<tr>
<td>VIP</td>
<td>In vivo</td>
<td>Systemic (ip)</td>
<td>Lenti</td>
<td>Mouse CIA</td>
</tr>
<tr>
<td>Immune cells</td>
<td>CD40-ASO</td>
<td>In vivo</td>
<td>Systemic (iv)</td>
<td>Liposome</td>
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<tr>
<td></td>
<td>BAFF</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Lenti</td>
</tr>
<tr>
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<td>IKKβ-dn</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
</tr>
<tr>
<td>signaling</td>
<td>BAFF</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>ODN/ liposome</td>
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<td>Adiponectin</td>
<td>In vivo</td>
<td>Systemic (iv)</td>
<td>Adeno</td>
</tr>
<tr>
<td></td>
<td>PTEN</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
</tr>
<tr>
<td>Induction of</td>
<td>FasL</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
</tr>
<tr>
<td>apoptosis</td>
<td>FADD</td>
<td>In vivo</td>
<td>Local</td>
<td>Adeno</td>
</tr>
<tr>
<td></td>
<td>HSV TK plus ganciclovir</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
</tr>
<tr>
<td>Other</td>
<td>AAT</td>
<td>In vivo</td>
<td>Systemic (ip)</td>
<td>AAV</td>
</tr>
<tr>
<td></td>
<td>IDO</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Adeno</td>
</tr>
<tr>
<td></td>
<td>GILZ</td>
<td>In vivo</td>
<td>Systemic (iv)</td>
<td>Liposome</td>
</tr>
<tr>
<td></td>
<td>GILZ</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>AAV</td>
</tr>
<tr>
<td></td>
<td>Galectin-1 and 3</td>
<td>In vivo</td>
<td>Local (ia)</td>
<td>Lenti</td>
</tr>
</tbody>
</table>

1. IL1-Ra = interleukin-1 receptor antagonist; IL-1RacP = interleukin-1 receptor accessory protein; sIL-1R = soluble interleukin-1 receptor; TNFR:FC = TNF receptor extracellular domains fused to the FC domain of IgG; sTNFR = soluble tumor necrosis factor receptor; IL-4 = interleukin-4; IL-10 = interleukin-10; VIL-10 = viral interleukin-10; IL-13 = interleukin-13; IFN-β = interferon-β; VIP = vasoactive intestinal peptide; CD40-ASO = CD40 antisense oligonucleotide; BAFF = B cell activating factor belonging to the TNF family; PTEN = phosphatase and tensin homolog deleted from chromosome 10; Fasl = Fas ligand; FADD = Fas-associated death domain protein; HSV TK plus ganciclovir = herpes simplex virus thymidine kinase plus ganciclovir; IKKβ-dn = IKKβ dominant negative; NF-κB = nuclear factor-kappa B; AAT = alpha-1 antitrypsin; IDO = indoleamine 2,3-dioxygenase; GILZ = Glucocorticoid-induced leucine zipper.

2. ia = intra-articular; im = intramuscular; iv = intravenous.

3. HSV = herpes simplex virus; AAV = adeno-associated virus; ODN = oligodeoxynucleotide.

4. SCW = streptococcal cell wall; CIA = collagen induced arthritis; LPS = lipopolysaccharide; AIA = antigen-induced arthritis; AA = adjuvant-induced arthritis.
I.2.1 Ex vivo gene therapy

An ex vivo approach involves the transduction of autologous cells in culture. The cells are exposed to a vector, most often a retrovirus (see section 1.2.3), that is carrying a therapeutic gene. The virus enters the cells and releases the gene into the cells. Thereafter, the transduced cells are expanded in the laboratory followed by the injection of these modified cells into the target tissue (see Figure 3). This can be done systemically (e.g. intravenous or intramuscular) or directly into the inflamed joint. This method permits the transduced cells to be screened for unwanted replication-competent virus before re-injection in the patient.

Different cell types can be used for this approach. Requirements for cells used for ex vivo gene therapy are i) accessibility of the cells and ii) the ability to survive for a longer period of time ex vivo without a significant change of phenotype. Cells used for ex vivo gene therapy approaches include modified T cells, dendritic cells, (skin) fibroblasts, and bone marrow-derived mesenchymal stem cells. This type of gene therapy is also known as “adoptive cellular transfer” since these cells have the ability to migrate to sites of inflammation and to express and deliver immunoregulatory products after ex vivo transduction.

The first phase I trial using gene therapy in RA patients was based on this ex vivo gene therapy approach. In this trial fibroblast-like synoviocytes (FLS) from the patients were ex vivo
transduced with a retroviral vector containing an interleukin-1 blocking agent before injection into the metacarpophalangeal (MCP) joints of the same patients (see also section “clinical trials”). FLS are very suitable cells for transduction, since they have a low mitotic rate ensuring long-term expression and they can relatively easily be obtained from the joint by miniarthroscopy. However, the process of acquiring autologous cells, ex vivo transduction, expansion of the cells, and transplantation into the joint is laborious and expensive. This hampers the development of this technique towards clinical application for the treatment of RA patients.

I.2.2 In vivo gene therapy

Using in vivo gene therapy, therapeutic genes are directly delivered to the target tissue using a viral or non-viral vector. In this way different cell types in the synovium can be transduced (see Figure 4). Compared to ex vivo gene therapy, in vivo gene therapy is less laborious, easier and much cheaper. Therefore, in vivo gene therapy is most promising for clinical development. The most recent clinical gene therapy program in RA has used this approach. Viral-mediated gene transfer is presently the most efficient delivery system, although non-viral delivery methods are also under development. Multiple viruses have been used to accomplish in vivo gene transfer (see also “vectors for gene therapy”). In vivo gene therapy can be applied using both systemic delivery of the vectors to extra-articular locations, resulting in the treatment of multiple joints, and local delivery, resulting in gene transfer to the synovial tissue in individual joints. One unexpected finding after local delivery has been the ‘contralateral effect’ in which the local gene delivery to one joint of an animal with
polyarticular disease leads to improvement of the contralateral, uninjected joint. This effect was first reported in an experimental arthritis model in rabbits and later in the adjuvant arthritis model of RA in rats. The beneficial effects do not seem to be related to the vector or the transgene. The exact mechanism remains unclear and cannot only be explained by trafficking of the modified cells to the contralateral joint. It is conceivable that antigen-specific mechanisms or neurological processes are involved. Clinical trials will need to elucidate if this phenomenon also occurs in human patients. Different gene transfer methods are summarized in Figure 5.

**I.3 Vectors for gene therapy**

There are several types of vectors available for the transduction of target cells with a gene of interest. Characteristics of a good vector are high transduction efficiency, high expression of the therapeutic gene, long-term expression, low immunogenicity and a good safety profile. Viral vectors are generally more potent than non-viral vectors. With the use of a viral vector the natural ability of viruses to infect cells and introduce their genetic material is preserved. Viruses have evolved to be highly efficient in stable transductions of various cell types and can in principle enable lifelong treatment. To allow safe gene therapy, these viruses are genetically modified to prevent them from replicating and cause disease, while preserving their infectious properties. Examples, among others, include recombinant herpes virus, retrovirus, lentivirus, adeno-associated virus (AAV), and adenovirus (Ad). Non-viral vectors are also under development. The most frequently used vectors are discussed in this section.

A summary of vectors used for gene therapy is given in Table 2. An overview of vectors used in experimental arthritis animal models for RA is provided in Table 1.

**I.3.1 Recombinant adeno-associated virus-mediated gene transfer**

Recombinant AAV vectors are based on a non-pathogenic, replication deficient member of the parvovirus family with a 4.7 kb single-stranded DNA genome. Recombinant AAV (rAAV) needs a helper virus to complete its life-cycle, such as an adeno- or herpes simplex virus. Currently more than 11 different serotypes have been identified, and there are over 100 variants. The different serotypes have unique epitopes, which are antigenic determinants that are recognized by the immune system. These epitopes make it possible for rAAV to interact with different receptors on target cells, resulting in different tropism. The most frequently used rAAV vector is based on serotype 2. The discovery of other AAV serotypes has resulted in new rAAV production techniques that allow packaging of AAV2-based genomes into capsids of different AAV serotypes (see Figure 6). This is called cross-packaging. The advantage of this technique is the possibility to transduce other tissues that are less permissive for rAAV2 and are more relevant for the different diseases, thereby broadening the application of rAAV vectors. For example, airway epithelial cells and nervous tissue are better transduced by rAAV5 and rAAV1, rAAV8 vectors are much better suited for liver transduction. Recombinant AAV4 is most efficient in infecting retinal cells. Two independent studies have shown that comparing serotypes 1 up to 5, rAAV serotype 5 was the most efficient rAAV vector for transducing synovial tissue in vivo, followed by rAAV2. Cross-packaging also allows re-administration of the vector genome, using a different serotype. This can be useful when neutralizing antibodies against the vector capsid arise, as these antibodies can decrease the gene transfer efficiency.
### Table 2. Vectors used for gene therapy

<table>
<thead>
<tr>
<th>Vector</th>
<th>Type</th>
<th>Insert size</th>
<th>T.E.</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>DNA</td>
<td>&lt; 4 kB</td>
<td>High</td>
<td>- Non-pathogenic, non-toxic</td>
<td>- Small insert size (cloning capacity)</td>
</tr>
<tr>
<td></td>
<td>(single stranded)</td>
<td></td>
<td>- Low-immunogenic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Different serotypes have different tropisms</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Stable infection</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Transduction of non-dividing cells</td>
<td></td>
</tr>
<tr>
<td>Adeno</td>
<td>DNA</td>
<td>&lt;7.5 kB</td>
<td>High</td>
<td>- Efficient transduction of dividing and non-dividing cells</td>
<td>- Transient expression due to immunogenicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Easy to produce</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Do not integrate in host genome</td>
<td></td>
</tr>
<tr>
<td>Retro</td>
<td>RNA</td>
<td>&lt; 8 kB</td>
<td>High</td>
<td>- Simple production of vector</td>
<td>- Transient expression due to transduction of dividing cells (MMLV)</td>
</tr>
<tr>
<td></td>
<td>Lenti</td>
<td></td>
<td></td>
<td>- Transduction of non-dividing cells (Lenti)</td>
<td>- Limited cloning capacity (MMLV)</td>
</tr>
<tr>
<td></td>
<td>MMLV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>DNA</td>
<td>&lt; 20 kB</td>
<td>Low</td>
<td>- Large insert size,</td>
<td>- Immunogenic</td>
</tr>
<tr>
<td></td>
<td>(double stranded)</td>
<td></td>
<td>- Transduction of non-dividing cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-viral</td>
<td>-</td>
<td>&gt; 20 kB</td>
<td>Low</td>
<td>- Good cloning capacity</td>
<td>- Low transduction efficiency</td>
</tr>
<tr>
<td></td>
<td>Liposome</td>
<td></td>
<td></td>
<td>- Relatively inexpensive</td>
<td>- Inflammatory response</td>
</tr>
<tr>
<td></td>
<td>Plasmid</td>
<td></td>
<td></td>
<td>- Stable and long-term expression (ACes)</td>
<td>- Transient expression (liposome, plasmid)</td>
</tr>
<tr>
<td></td>
<td>Aces</td>
<td></td>
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</table>

T.E. = transduction efficiency; AAV = adeno-associated virus; MMLV = moloney murine leukaemia virus; HSV = herpes simplex virus; ACes = artificial chromosome gene expression system.
Figure 4. Local in vivo gene therapy for RA. rAAV is injected into the arthritic joint by intra-articular injection. Cells present in the synovium (fibroblast-like synoviocytes, macrophages, dendritic cells, B cells and T cells) can be transduced and will produce therapeutic protein at the site of inflammation. Originally published in Rheumatology. Adriaansen J, Vervoordeldonk MJBM, Tak PP. Gene therapy as a therapeutic approach for the treatment of rheumatoid arthritis: innovative vectors and therapeutic genes. Rheumatology 2006; 45; 656-68.

Figure 5. A description of different gene transfer methods, the cells that are involved and the route of administration. DC = dendritic cell; MΦ = macrophage.
Techniques that increase the transduction efficiency are currently under development in order to decrease the viral load necessary to obtain sufficient production of the transgene. This is expected to help reducing the immunogenic load and to reduce the costs of this application.

A disadvantage of the rAAV vector is the low cloning capacity. If the gene encoding the therapeutic protein in combination with the promoter exceeds 4700 base pairs, the rAAV gene construct cannot be produced efficiently.

During the last decade, remarkable progress has been made in the development of rAAV as vector for human gene therapy because of its high safety profile. In addition, rAAV has been shown to confer long-term gene transfer in a variety of tissues, showing expression in muscle tissue in dogs for even more than 11 years. Recent developments in rAAV production and purification have revolutionized the AAV field. At least 100 clinical trials have been conducted or are in progress with rAAV vectors carrying different transgenes. Most of the preclinical and clinical experience to date has been with AAV2, the first serotype to be isolated and fully characterized. Other serotypes are also used in clinical trials like AAV1, AAV6, AAV8, and AAV9. The clinical studies have been performed in a variety of diseases, including hemophilia, cystic fibrosis, Duchenne muscular dystrophy, lipoprotein lipase deficiency, Leber’s hereditary optic neuropathy, and alpha 1-antitrypsin deficiency (Gene therapy trials worldwide, www.abedia.)
Overall, a high safety profile of all vectors was observed in the trials performed. Besides the recent approval of Glybera™ to the market, a breakthrough after more than a decade of clinical trials in hemophilia has been reached. A single treatment with an rAAV8 vector expressing clotting Factor IX (FIX) was enough to increase human factor IX (FIX) to sustained therapeutic levels and to show efficacy.

To-date only few clinical gene therapy trials have been performed in the field of rheumatologic diseases and only one group used AAV. Targeted Genetics Inc. (TG) performed two clinical trials in RA patients using an in vivo gene therapy approach with rAAV2. The phase I study investigated the safety of an intra-articular injection with an rAAV2 vector coding for a TNF receptor IgG1 fusion gene (tgAAC94). These studies are described in more detail in section II.2.

Although overall a high safety profile of the vector was observed in the trials performed, some effects in humans were never seen in the animal models used, underscoring the importance of performing clinical trials in order to develop innovative gene therapy approaches for treatment in patients and learning from these experiments in patients. Results in humans undergoing gene transfer indicate that capsid-specific T cell responses directed against transduced cells may limit the duration of transgene expression following AAV gene transfer. In several clinical studies, AAV administration resulted in capsid-specific T-cell responses. In these studies the vector was infused into the hepatic artery of hemophilia B subjects. The T-cell responses appeared to be vector dose dependent and were only seen in patients treated with the high vector dose ($12 \times 10^{10}$ viral genomes (vg)/kg). The formation of capsid-specific T-cells did not cause any harm to the patients. In the hemophilia B trial the T-cell responses resulted in a loss of transgene expression. In contrast, in the studies where alipogene tiparvovec (Glybera) was administered via multiple direct intra-muscular injections into the legs, AAV1 vector DNA was found in all injection site muscle biopsies 26 weeks after administration, including in the subjects who demonstrated T-cell cytotoxicity. Such a (cytotoxic) cellular immune response was also noted in clinical studies where $\alpha_1$-antitrypsin was expressed after local injection of a rAAV1 vector into the muscle. However, sustained expression of $\alpha_1$-antitrypsin was observed for at least 1 year after delivery, suggesting that in this case the cellular immune responses to the AAV capsid had not eliminated transgene expression.

Immune responses to gene therapy vectors are dependent on variables such as the serotype used, the injection site, the target tissue/organ and the transgene. As would be expected, administration of the gene therapy vector to immune-privileged sites, such as to the eye for retinal disorders, has negligible or no impact from the immune system. Whether this would be the case after injection of the therapeutic vector into the joints of RA patients who are on systemic immunosuppressive drugs can only be answered by performing clinical trials addressing this issue.

### I.3.2 Adenoviral-mediated gene transfer

The adenoviral (Ad) vector is a non-enveloped virus with a 36 kb double-stranded linear DNA genome. The virus can cause disease in humans, but serotypes 2, 5 and 35 are not associated with severe human pathology.

Ads have many characteristics useful for gene therapy purposes. In addition to be able to transduce both dividing and non-dividing cells, Ad is very efficient in introducing DNA into the host cell, relatively easy to produce high titers and Ad has high cloning capacity. Ads do
not integrate into the host genome. To prevent the possibility of replication of the vector, a part of the viral genome is deleted (early region 1). Although part of the viral genome is deleted, infected cells will still synthesize viral proteins. The host immune response against adenoviral particles and the transduced cells is a major problem. Animal experiments have shown transient expression of Ad vectors, indicating that the currently used Ads are too immunogenic for long-term expression of a therapeutic gene necessary in a chronic disease like RA. Many RA patients also have neutralizing anti-Ad5-antibodies in the synovial fluid. For other applications, especially in the oncology field, Ad vectors containing genes that can induce cells death of tumor cells are developed to reduce the tumor size of inoperable tumors. Ad vectors also remain a powerful tool for screening new therapeutic genes for their application in animal models of rheumatic diseases.

The newest generation Ad vectors relates to the so-called gutted vectors. In these vectors all viral genes have been deleted. To transduce target cells, these gutted vectors need a helper virus, and therefore they are also called helper-dependent Ad vectors. Currently, there are still technical problems with these vectors, preventing clinical application. Deletion of the E4 region has deprived the vector of one of its major advantages, the efficient gene transfer in vivo.

1.3.3 Retroviral vectors

Retroviral vectors have been used extensively in the laboratory and in the majority of gene therapy clinical trials. Most retroviral vectors are based on the Moloney murine leukaemia virus (MMLV) and have a major limitation because of their inability to infect non-dividing cells. The cloning capacity is 8-10 kb and these vectors do not retain any of the native viral coding sequences. MMLV-based vectors are usually employed for ex vivo gene therapy as described in section 1. Because this virus is capable of integrating its genome into that of the host cell, the gene of interest will be present in all daughter cells. A possible drawback of this virus is the random manner in which retroviruses integrate into the host genome, which can result in insertional mutagenesis leading to pathology, including malignancies.

This oncogenic potential became clear in a retroviral gene therapy trial in which five out of more than twenty treated patients with X-linked severe combined immunodeficiency developed leukaemia. The leukaemia most likely developed due to insertional mutagenesis of the vector at an oncogenic site.

Lentiviral vectors are based on complex retroviruses (lentiviruses) such as human immunodeficiency virus (HIV). There are several advantages with the use of a lentiviral vector. The vector has a relatively high cloning capacity (up to 8 kb), the production process of the vector is relatively simple and the host-inflammatory reactions are moderate. Moreover, in contrast to murine retroviral vectors, lentiviral vectors transduce a variety of quiescent cells very efficiently. Over the past years it has been shown that lentiviral vectors mediate efficient transduction of various cell types in vitro and in vivo. However, more development is required to employ these vectors for gene transfer directly into the joint.

Few studies have been done on the use of lentiviral vectors for gene therapy in animal models of RA, which showed favorable results. A first human gene therapy trial with this virus has been conducted in HIV patients, with no serious side-effects and positive outcomes, hereby paving the way for future trials with lentiviral vectors.
I.3.4 Herpes simplex virus

Like adenoviruses, herpes simplex virus (HSV) based vectors can effectively transduce a wide variety of dividing and non-dividing cells. There is room for the insertion of multiple genes and complex regulatory sequences, because 43 out of the 81 known sequences are non-essential for replication in vitro. Successful treatment of RA might involve the delivery of a combination of several types of proteins and require controlled coordinate expression. Like Ad vectors, HSV vectors also express low levels of viral proteins and are therefore immunogenic. Further research is necessary before administration into human joints can be tested.

I.3.5 Non-viral vectors

Non-viral vectors represent a synthetic delivery system, including a DNA molecule encoding a gene of interest combined with a carrier compound. Non-viral systems are essentially based on nucleic acid compaction by electrostatic interaction between nucleic acid phosphates and cationic polymers or cationic lipids that can compact DNA. They are routinely used with great success as transfection agents in tissue culture. Formulation of plasmid DNA with cationic lipids, often in combination with a neutral phospholipid, results in condensed particles, termed lipoplexes, formed by ionic interaction between the cationic lipid and the negatively charged DNA and subsequent hydrophobic interactions between the lipid moieties. The DNA in these formulations is protected from the environment and exhibits increased resistance to nucleases. The lipids in the vector can incorporate into the lipids of cell membranes, bringing the DNA contained within the vector into the cell.

The advantages of using non-viral expression systems are that no viral antigens are produced, there are no vector size limitations, there is no risk of recombination with or induction of vector replication by wild type viruses, and these vectors are usually inexpensive. While transfection of eukaryotic cells in vitro is fairly simple and effective, in vivo transfection of intact tissue proves to be an extremely difficult task. In general, levels of gene expression following non-viral gene transfer are at least a log order lower than when viral vectors are used and persist for only a short period of time. In addition, inflammatory responses following in vivo delivery of DNA by non-viral systems have been reported by several groups. The inflammatory mechanism appears to include reactions to both the liposomes themselves and to CpG motifs within the plasmids.

Although there is a lot of interest in the development of optimized non-immunogenic non-viral vectors because of their potential high safety profile, a lot of research still needs to be done before non-viral gene therapy methods will be ready for clinical application.

I.4 Immune responses to viral vectors: neutralizing antibody formation

Important for the translation of gene therapy from pre-clinical studies into a clinical application is to elucidate if possible immune responses or pre-existing immunity can have an effect on the gene therapy approach used. Since for RA at this moment in vivo viral gene therapy approaches have the best potential to go into the clinic, immune responses to viral vectors are discussed in more detail. Transduction of cells in vivo inevitably leads to the presentation of viral antigens to B cells, resulting in CD4+ T cell activation and subsequent differentiation of B cells into plasma cells, which may produce antibodies specific for the viral shell proteins
and which may have neutralizing effects. Neutralizing antibodies (NAB) are able to neutralize an infection in vivo by specific immune response leading to the clearance of these cells. As a result, in vivo transduction may be complicated by pre-existing immunity directed against wild-type adenovirus or AAV. For adenovirus type 5, most commonly used for gene therapy purposes, the incidence of pre-existing immunity is 50%. It has been reported that this can be circumvented by the use of alternative serotypes.

Capsids being derived from AAV, which are naturally occurring viruses, could elicit immune responses to some extent in treated patients. Therefore, a potential concern about the use of AAV-based vectors for gene therapy is the formation of AAV (neutralizing) antibodies to the capsid proteins. The formation of AAV antibodies after delivery of the first vector dose does not impact the transduction efficacy of this treatment but could reduce or prevent the transduction after a second injection with the same AAV vector. In addition, many individuals have pre-existing (neutralizing) antibodies to various AAV serotypes following natural exposure to wild-type AAV. Extensive surveys on the prevalence of anti-AAV antibodies in humans have been published. In adults, anti-AAV2 antibodies are the most prevalent (up to 70% of healthy humans), followed by serotypes like AAV5, AAV9, and AAV8, which are much less prevalent. AAV5 has a very low seroprevalence in humans in general and in particular in RA patients (about 30-40%) with only 3-5% demonstrating AAV5 neutralizing antibodies.

Pre-existing anti-AAV neutralizing antibodies can reduce or completely prevent transduction of a target tissue, resulting in lack of efficacy. The effect of pre-existing neutralizing antibodies on transduction is dependent on the route of administration, dose of vector and type of vector. Studies defining the optimal strategy or combination of strategies, to successfully treat subjects with pre-existing antibodies to AAV due to natural infection or to prior administration of AAV vectors, are ongoing.

Since immune responses against the vector are vector dose-dependent, great effort is put into optimizing the expression cassettes resulting in higher expression levels while using lower doses of vector. Another approach under investigation is the use of immune suppression strategies at the time the vector is injected until about 12 to 16 weeks after the injection in order to avoid the development of immune responses. In case antibodies are formed, switching serotype for a second administration would be possible. A more sophisticated approach is the development of AAV capsid mutants that can escape not only the induction of immune responses by avoiding uptake of the vector by antigen presenting cells, but also escaping the negative effects of pre-existing neutralizing antibodies to AAV in humans. The approach is still under development and has as yet not been tested in a clinical trial. More recently, the addition of defined quantities of empty capsids in the vector batches to reduce the effect of NAB during administration of the vector are under investigation. Currently, seropositive subjects (patients with pre-existing NAB) are excluded from the clinical studies.

Immune responses to the transgene occur mostly in diseases where gene therapy is used to replace a “missing” gene and when the transgene is relatively large.
II. TARGETS FOR GENE THERAPY FOR RHEUMATOID ARTHRITIS

II.1 Pre-clinical studies in animal models of arthritis

With the discovery of more cellular and molecular mechanisms implicated in the pathogenesis of RA, novel targets for treatment have been identified. Current strategies tested in in vivo pre-clinical models of arthritis involve the restoration of the cytokine balance by over-expressing immunomodulatory cytokines or inhibiting pro-inflammatory cytokines, targeting of signal transduction pathways, and the induction of apoptosis in synovial tissue. Some studies have used therapeutic genes mainly targeting cartilage and bone degradation, for instance by inhibiting the action of matrix metalloproteinases (MMPs). However, since inflammation is of utmost importance in RA and targeting the inflammatory pathways often also results in a decrease in cartilage and bone degradation, these approaches are more likely suited for clinical application in the near future and will therefore be discussed in more detail in this section. It is however possible that on the long term, when gene therapy for RA is proven feasible, these approaches will be developed in combination with conventional therapies or other therapeutic genes. Especially for the treatment of OA targets associated with bone and cartilage might be valuable.

The different transgenes are tested in several animal models of arthritis using both in vivo and ex vivo gene therapy strategies for either systemic or local delivery of the vector. A selected overview of the most frequently tested strategies is described in this section and shown in Table 1. Currently, a lot of research is ongoing in the field of arthritis gene therapy and more different therapeutic genes, or combinations of these, and approaches, including the use of small interfering RNAs, are under investigation, several of which cannot be discussed in this educational course.

II.1.1 Inhibition of pro-inflammatory cytokines

The inflammatory pathway activated in arthritis is currently the most studied target for biological therapy. Since it was shown that TNF plays a key role in the initiation and perpetuation of synovial inflammation and biologicals targeting TNF have shown to be successful in animal models and in the clinic, several gene therapeutic approaches targeting TNF have been tested in the past years.

As proof of principle, adenoviral vectors expressing soluble TNF receptor I coupled to the Fc part of IgG (TNFR1:Fc) have been used for local in vivo gene therapy in rabbits and mice and showed reduction in the severity of arthritis. However, as discussed in section 1.3.2 the adenoviral vector also stimulates a host immune response that may lead to elimination of the adenovirally transduced cells. Therefore, other groups used the rAAV vector that has more potential for clinical application, in animal studies. Intra-articular injection of rAAV2 encoding a soluble TNFRI in the joint in human TNF-α transgenic mice at the onset of disease decreased spontaneous synovial pathology. In a study using streptococcal cell wall (SCW)-induced arthritis in rats, administration of an rAAV2 vector containing cDNA encoding a rat p75 TNFR2:Fc fusion gene suppressed the arthritis as reflected by decreased inflammatory cell infiltration, pannus formation, cartilage and bone destruction, and mRNA expression of pro-inflammatory cytokines in the joints. These studies formed the basis for a clinical trial in which an rAAV2 vector expressing the human TNFR2:Fc protein was tested in patients with inflammatory disease (see also section “clinical trials”). Since in a comparative study rAAV5 was found to be the most
efficient AAV vector in transducing synovial tissue, more recently this AAV serotype was used in two animal models of arthritis, namely adjuvant-induced arthritis (AA) in rats and collagen-induced arthritis (CIA) in mice, to evaluate the therapeutic potential of a TNF-blocking agent in combination with this vector. The rAAVS vector contained a chimeric human TNFα soluble receptor 1 variant (TNFR1:Fc). In rats with AA treated with TNFR1:Fc under control of a disease-inducible promoter (meaning that the gene will only be expressed when the disease is active) a significant reduction of paw swelling was observed accompanied by a decrease in cartilage and bone destruction. In addition, a clear reduction of paw swelling was seen in the left, uninjected paw. The use of this vector in CIA in mice revealed comparable results. Together, these data provide evidence that local gene transfer of a TNFα-blocking agent using the most optimized rAAV vector may be a feasible interventional approach for the treatment of arthritis.

Non-viral approaches were also explored to block TNF in animal models of arthritis. Two groups used in vivo electrotransfer to deliver plasmids encoding TNF blocking agents intramuscularly in CIA in mice. The most potent effects were seen when a plasmid expressing TNFR1:Fc, the same as expressed by the rAAVS vector as mentioned in the previous paragraph, was used. Comparison of the electrotransfer approach with repeated recombinant protein (etanercept) injections highlighted the potential practical advantage of the gene therapy approach for CIA, which leads to a sustained therapeutic effect after single treatment. These results show that electrotransfer may be a useful method to deliver cytokine or anti-cytokine therapy in RA, but non-viral methods need, as discussed in section 1, further development before they can be translated into a clinical application.

Another approach to inhibit the inflammatory response is the use of the interleukin1 receptor antagonist (IL-1Ra). IL-1Ra is a naturally occurring inhibitor of the pro-inflammatory interleukin-1-mediated activation of the interleukin-1 receptor (IL-1R). IL-1Ra has been tested in clinical trials and anakinra, the recombinant form of IL-1Ra, has been approved for the treatment of RA.

Limitations of IL-1Ra as a pharmaceutical compound include its lack of oral availability and its short biological half-life. Therefore, gene therapeutic approaches, resulting in continuous expression of the protein, have been tested in animal models of arthritis. These studies revealed that the transfer of the IL-1Ra gene provided a far more potent biological effect than administration of the recombinant protein in animal models. An example is provided by a study in of antigen-induced arthritis in rabbits. Repeated injections of recombinant human IL-1Ra had no effect in this model of RA beyond inhibition of synovial fibrosis, occurring in the chronic stage of the disease. In contrast, a dramatic beneficial effect was observed on cartilage matrix metabolism as well as a moderate anti-inflammatory effect when IL-1Ra was administered locally to joints via ex vivo gene transfer. Similarly, human IL-1Ra gene transfer into synovial fibroblasts prevented cartilage destruction in human cartilage co-implanted with transduced synovial fibroblasts in SCID mice. From these and other experimental arthritis models, it has become clear that administration of IL-1Ra, both locally and systemically, has a protective effect on cartilage and bone turnover and reduces the inflammatory cell infiltration normally seen in experimental arthritis. These studies have resulted in the first ex vivo gene therapy clinical trial using a retroviral vector expressing IL-1Ra in RA patients. This phase I trial has demonstrated that human IL-1Ra cDNA can be safely transferred to and expressed within rheumatoid joints using ex vivo gene therapy (see also section ‘clinical trials’). Currently, research on IL-1Ra mutants
GENE THERAPY IN RHEUMATIC DISEASES

with enhanced antagonistic activity is ongoing. Since anakinra treatment in RA patients results mainly in the protection against joint destruction while it is only marginally anti-inflammatory, the IL-1Ra gene therapy approach is perhaps more effective in patients with osteoarthritis.

II.1.2 Anti-inflammatory and immunomodulatory cytokines

Anti-inflammatory cytokines are cytokines that inhibit the production of pro-inflammatory cytokines and counterbalance for instance Th1-driven responses. Th1-driven responses (“cellular immunity”) include the differentiation of Th1 cells, which subsequently activate macrophages and stimulate B-cells to produce IgG antibodies that are involved in opsonisation of extracellular pathogens, facilitating uptake by phagocytic cells. Anti-inflammatory cytokines showing a therapeutic effect in experimental arthritis include interferon-beta (IFN-β), IL-4, IL-10 and IL-13.

IFN-β reduces the secretion of mediators of inflammation and destruction like IL-6, TNF-α, MMPs and prostaglandin E2, which are key players in the pathogenesis of RA. IFN-β has in addition to anti-inflammatory properties an important role in bone homeostasis. Furthermore, IFN-β has anti-angiogenic properties, which could boost a therapeutic effect in RA. It has been shown that continuous IFN-β treatment, using daily IFN-β protein injections or ex vivo transduced cells secreting IFN-β, is very effective in CIA in both mice and Rhesus monkeys.

IFN-β has been approved as therapy for multiple sclerosis and has also been tested in a phase II clinical trial in RA patients. In this study 209 RA patients, stable on methotrexate treatment, received 2.2 µg IFN-β-1a, 44 µg IFN-β-1a, or placebo subcutaneously three times weekly for a period of 24 weeks.

This dosing regimen resulted in flu-like symptoms and injection site reactions and did not lead to clinical improvement or protection against joint destruction, perhaps because higher and more constant levels of IFN-β may be required at the site of inflammation to translate into clinical benefit. Local long-term treatment through gene therapy might be a promising approach to achieve this. Intra-articular IFN-β gene therapy has been tested in a number of pre-clinical studies in two different rat models of arthritis using either an Ad or an rAAV5 vector. Local delivery of Ad or rAAV5 vectors expressing rat IFN-β after the onset of disease reduced paw swelling in both the treated and untreated, contralateral joint. Moreover, IFN-β treatment protected against joint destruction, which is a hallmark of RA. These results provide a strong rationale for IFN-β gene therapy as a novel therapeutic approach for arthritis.

IL-10 and IL-4 are produced by Th2 cells that can inhibit the synthesis of TNF and IL-1 in synovial cells. A lot of gene therapeutic approaches using IL-10 as vector have been tested. Considerable evidence indicates that delivery of viral and non-viral vectors encoding either homologues of IL-10 or viral IL-10 (which lacks immunostimulatory properties and is predominantly immunosuppressive) in CIA in mice results in decreased T cell proliferation in response to type II collagen, delayed onset and reduced severity of disease, and significant amelioration of established disease. While these reports from studies in experimental animal models seemed very promising, the prolonged elevation of systemic IL-10 in a clinical trial was associated with toxic effects such as anaemia in RA patients. Therefore, a strategy targeting IL-10 expression to cells that modulate the ongoing autoimmune response and to achieve high local but low systemic concentrations of IL-10 was tested in CIA in mice. Intranasal gene delivery of a plasmid encoding IL-10 (pIL-10) significantly delayed arthritis onset and reduced disease
severity. Intranasally delivered pIL-10 targeted monocytes and macrophages and showed dissemination to inflamed joints and draining lymph nodes. Importantly, systemic levels of IL-10 were only transiently elevated and were undetectable after 4 days. The mechanism underlying the therapeutic effect still needs to be clarified in order to further optimize this approach for a possible application in patients with RA.

Reports on the anti-inflammatory effect of AdIL-4 are conflicting; however, all data available so far showed that IL-4 overexpression provides impressive protection against cartilage degradation and bone erosion. Therefore, it has been suggested that the combination of viral IL-10, mainly affecting inflammation, in combination with IL-4 gene therapy might be beneficial for the treatment of RA.

Interleukin-13 can, similar to IL-4, inhibit pro-inflammatory cytokine release by macrophages, but it also has an effect on blood vessel formation. In the rat AA model of RA intra-articular administration of an Ad.IL-13 vector showed reduced inflammation and diminished vascularity and bone destruction. In a more recent study, it was shown that administration of an Ad.IL-13 vector to mice with immune complex-induced arthritis actually increased the number of synovial joint inflammatory cells, but IL-13 gene transfer also abrogated chondrocyte apoptosis via down-regulation of FCγRI and MMP-mediated proteoglycan degradation induced by immune complexes. More studies are needed to determine whether IL-13 is a good target for the treatment of RA patients.

Another anti-inflammatory molecule that has been investigated for the treatment of arthritis is vasoactive intestinal peptide (VIP). Neuropeptide VIP potently inhibits Th1 responses and promotes immune tolerance through the induction of Treg cells. Pre-clinical studies in arthritis models using VIP gene therapy showed promising results. A single ip injection of the VIP gene expressed by a lentiviral vector was highly effective in mouse CIA, showing even when administrated after onset of arthritis complete regression of the disease.

II.1.3 Immune cells

Therapeutic approaches affecting immune cell activation hold great promise for the treatment of RA. Abatacept (CTLA-4Ig) blocks the interaction between T-cells and antigen presenting cells through CD28-CD80/86 interaction inhibition. Another way to block the interactions between the cells would be the administration of an anti-CD40 monoclonal antibody, but this was accompanied by significant adverse effects (unexpected thromboembolic events) which impeded further clinical development. A gene therapy approach that was used to block the CD40-CD40L interactions using an antisense oligonucleotide directed against CD40, systemically delivered through a liposomal carrier. A single iv injection of the antisense/liposome complex in CIA in mice resulted in improved disease symptoms and downregulation of proinflammatory cytokines in the joints.

In addition to blocking T-cell activation, B-cell depletion by use of the anti-CD20 antibody rituximab has proven to be successful. Another approach to interfere with the humoral response could be silencing the expression of TNF superfamily member B cell-activating factor (BAFF). In mouse CIA an intra-articular injection of a lentivirus expressing BAFF-short hairpin RNA that can down-regulate BAFF expression, resulted in long-term suppression of arthritis development, amelioration of joint pathology, suppressed generation of plasma cells and Th17 cells, followed by inhibition of proinflammatory cytokine expression. The success of targeted therapies, such as abatacept and rituximab, together with the results of gene therapeutic strategies targeting similar pathways in pre-clinical models, show the potential of specific targeting of immune mechanisms.
II.1.4 Intracellular signaling: transcription factors

Targeting of transcription factors involved in the regulation of inflammatory mediators might be an alternative approach to modulate arthritis activity. In contrast to the targets mentioned in the previous paragraph, transcription factors are located intracellularly. Targeting transcription factors by gene therapy implies the need for specific transduction of the cell of interest, whereas transducing cells to make them produce anti-inflammatory proteins may result in an indirect on neighboring, non-transduced cells.

The transcription factor NF-κB may be a good target to inhibit. NF-κB is highly activated in the synovium of RA patients, and among other effects it can induce the transcription of pro-inflammatory cytokines. NF-κB activation is also a pivotal factor protecting the synovial cells against apoptosis (programmed induced cell death). Suppression of NF-κB can markedly enhance apoptosis in the synovium. Phosphorylation of the inhibitor of NF-κB (IκB) proteins is an important step in NF-κB activation and is induced by IκB kinase (IKK). Interfering with the phosphorylation of IKK can result in decreased NF-κB activation.

Blocking NF-κB through local overexpression of either IκB or transduction with a dominant negative IκB-β (Ad.IKKβdn) significantly ameliorated arthritis activity in the rat AA model of RA, as shown by decreased paw swelling. Recently, it was shown that rAAV5-mediated IKKβdn gene transfer to the synovium also reduced the severity of arthritis in rats with AA when the treatment was started after the onset of disease. Another approach to suppress NF-κB signaling may be by overexpression of the hormone adiponectin using an adenoviral vector. In the mouse CIA model this approach resulted in reduced disease activity and inflammation, and decreased joint destruction.

Another intracellular pathway involved in RA is the phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway. PI3-kinase signaling and Akt activation can be blocked by a phosphatase and tensin homolog deleted from chromosome 10 (PTEN). In the rat CIA model ia AdPTEN administration resulted in an amelioration of arthritis symptoms.

Together, these data suggest that an NF-κB- or PI3-kinase/Akt directed approach might be a novel anti-rheumatic strategy using local gene therapy.

II.1.5 Induction of apoptosis

In RA some FLS proliferation is present but the most important pathogenic mechanism for pannus growth is probably impaired FLS apoptosis. Arthritis can be temporarily ameliorated by removal of inflammatory synovium, performed surgically, chemically or by radiation therapy. In addition to these techniques, gene therapeutic approaches to induce apoptosis in RA synovium have been tested in animal models. Beneficial effects have been shown in animal models, reducing the inflamed tissue mass and ameliorating the disease severity. The death factor Fas/Apo-1 and its ligand (FasL) play pivotal roles in the induction of apoptosis of immune cells and the maintenance of immune tolerance to self antigens. High levels of Fas are expressed on activated synoviocytes and infiltrating leukocytes within the inflamed joint. Unlike Fas, the levels of functional FasL in the RA joint are extremely low. Gene transfer of Fas ligand (FasL) has increased the frequency of apoptotic cells in the synovium of mice and rabbits with arthritis. Determination of the effects of FasL gene transfer on human inflammatory synovium in vivo would be an important next step towards development of this approach for the treatment of RA.
Fas-associated death domain protein (FADD) also plays a key role in Fas-mediated apoptosis of synovial cells in RA patients. Experiments using FADD gene transfer to human synovium in vitro and in vivo (human rheumatoid synovium engrafted in a SCID mouse) resulted in apoptosis of the synovial cells.

Alternatively, induction of apoptosis in synoviocytes may be achieved by delivery of the herpes thymidine kinase gene (tk) followed by administration of ganciclovir, resulting in apoptosis of the transduced cells. Intra-articular injection of an adenoviral vector encoding tk into arthritic knees of rhesus monkeys, followed by treatment with ganciclovir, showed increased apoptotic cell death in injected joints compared to non-treated joints. Although induction of apoptosis appears successful in experimental models, it is still a matter of debate whether this is the preferred way to remove synovial tissue over more conventional methods like synovectomy.

### II.1.6 Other approaches

More recently, several new gene therapeutic approaches have been evaluated for the treatment of RA. Overexpression of glucoprotein human alpha-1 antitrypsin (hAAT), indoleamine 2,3-dioxygenase (IDO) and the endogenous lectin galectin-1, gene expression silencing of glucocorticoid-induced leucine zipper (GILZ) and knock down of galectin-3 through different viral vectors all resulted in disease amelioration and an anti-inflammatory effect in CIA models. This indicates that there may still many new targets with therapeutic potential for the treatment of RA.

### II.2 Clinical studies in RA patients

Over the past 20 years, remarkable progress has been made in the development of effective gene therapy for the treatment of various diseases. However, only few clinical trials have been conducted in the field of rheumatologic diseases (see Table 3), and only three trials have been reported in detail and will be described briefly in this paragraph.

The first trial in RA patients involved ex vivo gene therapy using a retroviral vector expressing IL-1RA used to transduce autologous FLS. The main objective of this trial was to test whether this procedure was safe and feasible in humans; therefore, only patients waiting for joint replacement were included in this study. The study involved nine postmenopausal women with advanced RA who required unilateral sialastic implant arthroplasty of the 2nd-5th MCP joints. In a dose escalation, double-blinded fashion, two MCP joints were injected with transduced cells, and two MCP joints received control cells. One week later, injected joints were resected and examined for evidence of successful gene transfer. All subjects tolerated the procedure without adverse events. Unlike control joints, those receiving transduced cells showed mRNA transgene expression. In addition, clusters of cells expressing high levels of IL-1RA were present in the synovium of transduced joints. Thus, it is possible to transfer a potentially therapeutic gene safely to human rheumatoid joints and to obtain intra-articular transgene expression. A similar German study confirmed the ability of IL-1RA delivery to the synovium and intra-articular transgene expression. However, as discussed in section 1, ex vivo gene therapy is very expensive and time consuming and therefore this approach was not further developed for clinical application.

A recent clinical trial performed by Targeted Genetics Inc. (TGI) was one of the first trials using direct in vivo gene therapy for a non-genetic and non-lethal disease. This study is also
important in the translation of gene therapy for arthritic diseases from animal models to the patient. The phase I study was undertaken to evaluate safety of an intra-articular injection of the TNFR2-Immunoglobulin 1 (IgG1) fusion (TNFR2:Fc) gene (tgAAC94) using an rAAV2 vector. The data demonstrate that tgAAC94 was well-tolerated at doses up to \(1 \times 10^{11}\) particles per ml of joint volume. Although this study was not powered to evaluate efficacy, there was an indication of sustained improvement in signs and symptoms in nine out of eleven patients 24 weeks after treatment with tgAAC94. These results lead to the initiation of a second study.

In the Phase I/II study, 127 patients with arthritis were included; continuation of systemic TNF blockade was permitted in this trial. RA patients, but also patients with other inflammatory arthritic diseases, mainly psoriatic arthritis, were included. The patients received a single intra-articular injection of tgAAC94 (at 3 different dosages: \(1 \times 10^{11}\), \(1 \times 10^{12}\), or \(1 \times 10^{13}\) particles per ml joint volume) or placebo, followed by an open-label injection of tgAAC94 after 12 to 30 weeks, depending on whether the target joint met criteria for re-injection. The clinical response was assessed and the patient-reported outcomes showed a non-significant trend towards response, but there was no change in scores for arthritis upon physical examination. One patient, who was treated with systemic anti-TNF antibody treatment in combination with intra-articular injection with tgAAC94, developed fatal disseminated histoplasmosis. This tragic event was ultimately considered unrelated to the study agent (see also ‘in depth discussion’). In 12% of the patients injection site reactions were reported, but treatment was otherwise well tolerated.

Obviously, these trials are encouraging for the development of gene therapy for RA, as they showed feasibility and safety. However, no data are available on the expression of the TNF-blocking agent in the joints, molecular effects at the site of inflammation, the influence of NAB on the transduction efficacy, and on the efficacy of the second injection. Future trials need to elucidate these questions and need to determine whether long-term expression of the therapeutic gene can be obtained using an rAAV vector and if this may result in a therapeutic effect in the joint.

Currently a clinical trial administrating IFN-\(\beta\) locally in the joint of RA patients is under development and injection of the first patient is anticipated to take place in the first quarter of 2015. GLP toxicity and biodistribution studies have been initiated evaluating the safety profile of the clinical rAAV5 vector expressing human IFN-\(\beta\) (ART-I02). Preliminary results of these GLP studies indicated that local treatment with high doses of ART-I02 does not show any toxicity and is well tolerated in the animals.

An overview of current gene therapy clinical trials in patients with rheumatoid arthritis is shown in Table 3.

### III. GENE THERAPY FOR RHEUMATOLOGIC DISEASES OTHER THAN RHEUMATOID ARTHRITIS

#### III.1 Osteoarthritis

Morphological changes observed in OA include loss of cartilage, changes in the bone, and a variable degree of synovial inflammation. Experimental studies aimed at developing gene therapy for OA have not yet progressed as far as those for RA. Like in RA, many gene delivery studies have focused on transducing synovial cells. In OA, synovial tissue is not the primary
target tissue, but synovial cells could produce therapeutic proteins with a beneficial effect on cartilage and bone. Using a technology targeting both articular cartilage and synovial tissue may represent an ideal strategy for OA. There are several possible therapeutic strategies for gene therapy in OA, which include overexpression of growth factors, the inhibition of pro-inflammatory cytokines or angiogenesis, and the prevention of chondrocyte apoptosis.

To achieve cartilage repair, the synthesis of cartilage matrix should ideally be improved and cartilage degradation should be suppressed. Chondrocyte-stimulating factors like growth factors (TGF-β related molecules, insulin-like growth factor 1 (IGF-1), fibroblast-like growth factor 2 (FGF-2)) or BMPs (bone morphological proteins; BMP-2, BMP-4, BMP-7) could have therapeutic value, since they are involved in cartilage homeostasis. In a rat model based on mechanically induced lesions in the joint, AAV IGF-1 gene transfer did not show improvement of cartilage. It was concluded that AAV vectors were capable of inducing the expression of IGF-I in vitro, but the effects of treatment were not sufficient to protect the cartilage from serious damage in vivo. However, the virus concentration was almost 2 logs lower than normally used in experimental models of RA, as discussed in section 2.

A key pro-inflammatory cytokine involved in the degradation of cartilage is IL-1. In vitro studies have shown beneficial effects of IL-1Ra on the degradation of cartilage. In OA synovium a relative shortfall in the production of IL-1Ra has been demonstrated. This, coupled to up-regulation of the receptor, is an additional enhancer of the catabolic effect of IL-1 in OA. These findings strongly support the rationale for developing therapeutic strategies aimed at IL-1 blockade for the treatment of OA. An in vivo study has indeed shown that intra-articular injections of IL-1Ra can slow down the progression of experimental OA. In a rabbit OA model FGF combined with IGF-1 and IL1-Ra gene therapy through adenoviral transduction showed promising results.

Angiogenesis may accelerate inflammation and contribute to disease severity and angiogenesis; inhibition can result in improved cartilage regeneration and repair. Inhibition of angiogenesis, by overexpression of thrombospondin-1 (TSP-1), was tested in a rat model of OA. Overexpression of TSP-1 with an adenoviral vector resulted in reduced inflammation and suppressed progression of the disease.

Chondrocyte apoptosis is another possible mechanism that could be targeted in OA. Inhibition of NO (nitric oxide) synthase and treatment with hyaluronan reduced progression and suppressed apoptosis in a rabbit model of OA. Transduction of chondrocytes and

<table>
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<th>Vector</th>
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<th>Status</th>
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<td>HSV-tk</td>
<td>Plasmid</td>
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RA = rheumatoid arthritis; PsA = psoriatic arthritis; AS = ankylosing spondylitis; IL-1ra = interleukin 1 receptor antagonist; TGF-β = transforming growth factor beta; TNFR:Fc = tumor necrosis factor receptor Fc domain of immunoglobulin; HSV-tk = herpes simplex virus thymidine kinase (expression together with ganciclovir administration results in synovial cell death); n = number of subjects in study.
chondroprogenitor cells can be achieved using different vectors. Injections of adenoviral vectors into normal joints do not transduce chondrocytes, but transduction using neutral liposomes that contain Sendai virus has been reported, resulting in stable expression in rat joints.

Another experimental technique for OA treatment is based on cell therapy, using adult mesenchymal stem cells (MSCs). These stem cells are made to differentiate into cells of the chondrogenic lineage and used for the regeneration and maintenance of articular cartilage. MSCs can also be used for the production of cartilage implants. Currently 2 trials have been initiated in the United States of America and Korea, to investigate the efficacy and safety of allogeneic human chondrocytes expressing TGF-β1 after in vitro retroviral transduction in knee osteoarthritis, of which results still are awaited.

### III.2 Sjögren’s syndrome

Sjögren’s syndrome (SS) is an autoimmune disorder characterized by decreased lachrymal and salivary gland function, which can also affect multiple organs, including kidney and lung. The principal lesion in SS is lymphocytic infiltration in target tissues. Currently, there is no effective treatment for salivary gland dysfunction. Therapies are aimed at palliative care, including artificial saliva, dental prophylaxis and saliva stimulation by muscarinic agonists. Other options are the use of steroids and other immunomodulatory therapies. A potential target organ for gene therapy in SS is the salivary gland. Via the lumen of the gland, the salivary gland is easily accessible and allows local treatment. Moreover, slowly dividing salivary epithelial cells can ensure long term gene expression.

Similar to the treatment of RA, SS therapy is aimed at altering the immune response. Possible approaches include inhibition of pro-inflammatory molecules like TNF-α, IL-12, IL-17 and interferon-γ, reducing pro-inflammatory activity, and the use of anti-inflammatory molecules such as interferon-α and IL-10, antagonizing the pro-inflammatory response. Another possibility is targeting lymphocyte adhesion to the salivary gland by inhibiting adhesion molecules, such as intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. T cells and B cells could also be targeted by specific antibodies inhibiting cell activity or cell interaction.

There are also several potential non-immunological therapeutic approaches in SS. Dysregulation of apoptosis in the salivary gland could be targeted by altering pro- and anti-apoptotic factors. A different approach is restoring the fluid secretion in salivary glands by increasing the expression of aquaporins (AQP), membrane proteins that function as water channels and respond to osmotic gradients. SS patients have less expression of AQP-1, possibly related to the pathology in SS. A third option is directed at neuro-inflammatory pathways. The muscarinic type 3 receptor function has been shown to be impaired in SS and possibly stimulating this function could lead to improved secretory function of the gland.

At present gene therapy for SS is still in the experimental phase. Unfortunately, an animal model that corresponds to all the characteristics seen in SS patients is as yet not available. However, some animal models, like the non-obese diabetic (NOD) mouse, share some features with SS, such as mononuclear infiltration in the salivary glands and production of autoantibodies. However, this animal model lacks stability and the mice develop diabetes which makes it more difficult to evaluate the impact of the gene therapy approach. Still, local rAAV gene transfer of immunomodulatory proteins (e.g. IL-10 and VIP) into the salivary glands of NOD mice has shown promising results in the NOD model of SS. Local inhibition of TNF demonstrated an
opposite effect with decreased salivary gland activity. Gene therapy with soluble ICAM-1 only showed beneficial effects in mice when treatment was initiated early in the disease.

### III.3 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a complex autoimmune disease, characterized by multi-organ involvement. Many factors contribute to the disease pathology. A strong genetic predisposition exists in SLE. The genetic background of patients has been shown to play a role in the development of the disease, with a higher prevalence among women, a higher concordance rate in monozygotic twins and a predilection for certain racial and ethnic groups.

Current strategies for gene therapy in SLE are aimed at correcting the abnormal immunological response, for instance by interfering with cytokines, cell activation and migration, cell gene methylation status, or cell-cell interaction. Single gene targeting remains difficult due to the many genetic and other factors contributing to disease pathology.

Gene therapy aimed at changing the cytokine profile has shown promising results in a mouse model for lupus, the MRL\textit{Ipr/Ipr} mice. This animal model shows defective apoptosis of T cells, resulting in a phenotype similar to SLE with severe glomerulonephritis and production of autoantibodies. Possible approaches include blocking for example interferon-\(\alpha\) (IFN-\(\alpha\)), interferon-\(\gamma\) (IFN-\(\gamma\)) and B-lymphocyte stimulator (BlyS) or enhancing cytokines like transforming growth factor-\(\beta\) (TGF-\(\beta\)), interleukin-2, and interleukin-12. A plasmid encoding an IFN-\(\gamma\) receptor/ IgG1 Fc fusion protein injected in an MRL\textit{Ipr/Ipr} mouse model resulted in increased survival, lower levels of autoantibodies and milder renal disease. Administration of a plasmid coding for TGF-\(\beta\) and BlyS blockade through an adenovirus coding for TACI (BlyS receptor/ FC fusion protein) gave similar results. However, targeting TGF-\(\beta\), IL-2, or IL-12 has shown contradictory results, probably in part due to the use of different vectors (both viral and plasmids) and administration techniques.

Interference with cell-cell interaction is also an attractive option since SLE patients exhibit changes in T-cell function; generally SLE T cells show an over-excitable phenotype. Interference with co-stimulatory signals using an adenovirus encoding CTLA4-Ig gene has shown promising results in animal models of SLE: CTLA4-Ig gene therapy in dogs showed amelioration of experimentally induced lupus-like disease skin lesions and reduction of antinuclear antibodies (ANA). Using the same vector after intravenous administration in MRL\textit{Ipr/Ipr} mice resulted in almost complete amelioration of lupus nephritis. Treatment with AAV8-CTLA4-Ig in neonatal NZB/NSW mice before disease onset delayed autoantibody production and kidney disease and prolonged lifespan. Addition of AAV8-CD40-Ig resulted in a synergistic effect. In adult mice this combination resulted in a decrease in autoantibody levels, suppression of proteinuria and prolongation of lifespan.

B cell elimination through an adenoviral vector coding for anti-CD20 antibody in BWF1 mice resulted in reduced anti-dsDNA antibody levels, impeded proteinuria development and improved survival.

Finally, chemokines and intracellular molecules can also be targeted by gene therapy. A chemokine of interest is monocyte chemoattractant protein-1 (MCP-1/CCL2). Gene therapeutic inhibition of this chemokine resulted in decreased cellular proliferation in renal glomeruli and the lungs. Intracellular pathways might be targeted to correct IL-2 production. Overall, gene therapy for SLE remains at present in an experimental phase and the focus is on the identification of the best therapeutic genes.
REFERENCES


