Immunotherapy Though Gene Transfer for RA

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Initially, gene therapy was proposed for monogenic diseases. The functional therapeutic gene restored the deficient activity. These diseases are uncommon, and 80% of the ongoing clinical trials of gene therapy concern acquired diseases, such as AIDS, cancer, and RA. The therapeutic proteins active in articular disease have a short half-life and low biodistribution. Gene transfer is an appropriate technique for delivery of biotherapies directly in the joints and allows production of the therapeutic protein locally at high concentrations. The feasibility of ex vivo gene transfer in human synovial cells has been demonstrated in two ongoing clinical trials in the USA (Pittsburgh) and in Europe. In these studies, the cells infected by retroviral vectors before implantation in the joints expressed IL-1 Ra for 1 to 4 weeks. It should be noted, however, that this protocol was not designed to evaluate the clinical efficiency as the joints into which the genetically modified cells were injected were in the final stage of disease. Rather, the trial was designed to demonstrate the feasibility of gene transfer and transgene expression in vivo as well as the safety of the procedure.

Immunotherapy for RA. The mediators that can be effectively targeted by immunotherapy in RA have been identified. The main target for biotherapies today are the proinflammatory cytokines, IL-1 and TNF-a, through the use of natural inhibitors or monoclonal antibodies (IL-1ra, IL-1 type I receptor, soluble TNF-a receptor, and monoclonal antibodies against TNF-a). These drugs have shown a dramatic effect on joint inflammation but the long-term effect on cartilage remains unknown. Moreover, the side effects related to systemic cytokine neutralisation remains unknown. TNF-a appears to play a pivotal role in the pro-inflammatory cytokine cascade and its inhibition has multiple beneficial effects through reduction in the production of pathogenic cytokine, chemokine, and adhesion molecules. The effective use of neutralising TNFa for the treatment of RA has been corroborated by several clinical trials.

Interleukin 10 (IL-10) is a Th2 immunosuppressive cytokine produced by T lymphocytes and monocytes. IL-10 inhibits secretion of proinflammatory cytokines in the synovium, including IL-1, TNF-a, IL-6, and GM-CSF. Moreover, IL-10 reduces antigen presentation and expression of MHC class II and adhesion molecules. T cells also are also affected by IL-10, which induces long-lasting anergy. A paradoxical immunological stimulation is associated with murine IL-10, which is related to its chemoattractive effects on CD8-positive lymphocytes. In contrast to cellular IL10, viral IL-10, originating from the EBV genome, shares 84% homology with human IL-10, but is exclusively immunosuppressive. Daily injection of recombinant murine IL-10 has been shown to inhibit experimental arthritis in DBA1 mice. Recombinant hIL-10 has been tested in human RA and clinical efficiency was demonstrated in a clinical trial that included seventy-two patients. In contrast to TNF neutralisation, IL-10 inhibits secretion of metalloproteases as well as inducing the secretion of the metalloproteases inhibitor, TIMP-1, by monocytes. The immunologic effects of IL-10 and TNF inhibition seem to be complementary. However, immunotherapy by targeting only one cytokine seems not to be sufficient to totally suppress RA and long term joint destruction. Combining dTNF-R and vIL10 gene transfer may be eficient to control definitively the disease.

rAAV technology. rAAV are non immunogenic vectors wich allows long term gene expression in synovial cells. Vector particles can be produced in the absence of wild type AAV, if the rep and cap gene products are provided in trans. For this, the recombinant AAV genome and a rep-cap expression cassette are transfected into cells

on two separate plasmids. The rep-cap construct has no sequence overlap with the recombinant genome, and the AAV ITRs have been replaced by the Adenovirus ITR. Human 293 cells, which are highly permissive to both AAV and adenovirus replication, can be efficiently transfected, and are of general use for vector production. Three significant improvements have now been made to the initial method. First, the level of Rep and Cap proteins produced after transfection has been shown to be critical for obtaining high titer rAAV. Surprisingly, low amounts of *rep* expression result in higher rAAV production, probably because of the negative influence of Rep on *cap* expression. Helper (*rep-cap*) plasmids designed to express low levels of Rep allow for the production of 5- to 10-fold more rAAV. Second, Adenovirus infection can now be replaced by the cotransfection of a deleted Adenovirus genome. The minimal helper construct only contains the E4, E2A, and VA genes, E1A and E1B being expressed by the 293 cells. Under these conditions, rAAV is being produced in the absence of adenovirus structural proteins or particles, and the purification step is facilitated. Third, the level of illegitimate recombination can be reduced to a minimum (less than 1 rep positive particle in 1 x 10^9), without affecting the production yield if the Adenovirus ITR are removed from the rep-cap helper construct.

One obvious goal is to obtain stable packaging systems for the production of rAAV. This would not only simplify the preparation method, but also facilitate the definition of specifications for the production of vectors to be used in pre-clinical and clinical experiments. The development of a universal packaging line is underway.

Of relevance to the present project, pharmacologic regulation of transgene expression has been obtained *in vivo* following rAAV-mediated transduction. Vectors carrying inducible promoters and transcription factors activated by doxycycline have been used successfully, and the construct described in this project will allow the control of the the therapeutic genes *in vivo*. A pilot feasability study will be possible within the forseen scale.