Editorial

Arthritis Gene Therapy Trials Reach Phase II

Arthritis has been on the gene therapy agenda for about 20 years[1]. Despite its impressive preclinical track record of efficacy and safety in animal models (as reviewed[5,6]), progress in carrying out clinical trials has been painfully slow[4]. The literature contains only 2 small Phase I studies[3,4] and a report of 2 subjects who experienced symptomatic relief following gene transfer[7]. The Phase I/II trial described by Mease, *et al* in this issue of *The Journal*[8] is thus very much to be welcomed. Not only does it greatly increase the number of subjects who have received gene therapy for arthritis, but it is also the first trial to address efficacy in a substantial fashion.

Although there are several different strategies for using genes as therapeutic agents in arthritis, by far the most progress has been made with the approach of delivering genes locally to individual diseased joints[2,3]. There are a number of reasons for this. Not only was it the first arthritis gene therapy strategy to be proposed[1], but also, by enabling sustained, endogenous, intraarticular synthesis of therapeutic gene products in selected joints, local delivery achieves something that no other technology can accomplish. Moreover, expressing the gene product intraarticularly minimizes exposure of non-target sites, thereby reducing the potential for unwanted side effects. The smaller requirements of local, rather than systemic, treatment also lower costs, especially as a successful gene therapy will require infrequent redosing. The burden of treating multiple joints individually in patients with rheumatoid arthritis (RA) may be less than first thought, following the discovery that the genetic treatment of just one joint in animals with polyarticular disease secures improvement in additional joints on the same individual[9]. The degree to which suppression of intraarticular disease mitigates extraarticular manifestations of RA remains to be determined.

Which gene to transfer and which vector to employ for this purpose are central questions for any gene therapy. Mease, *et al* used a cDNA encoding a human tumor necro-sis factor receptor-immunoglobulin Fc fusion protein (TNFR:Fc) equivalent to etanercept, whose efficacy in RA has been well established. The choice of adeno-associated virus, serotype 2 (AAV2) as the vector reflects the growing popularity of this parvovirus for human gene therapy[10]. It is injectable and has been used safely in over 50 clinical trials, including the recent successful genetic treatment of Leber's congenital amaurosis[11,12]. Moreover, AAV-based gene therapy has been granted orphan drug status for the treatment of familial lipoprotein lipase deficiency and X-linked juvenile retinoschisis. Drawbacks of AAV include the high cost of making clinical grade, recombinant virus, its small packaging capacity, and, because its genome comprises single-stranded DNA, the need for second-strand synthesis in the host cell before transgenes can be expressed. Depending on the cell type, second-strand synthesis can be very inefficient. AAV was also thought to avoid the problematic immune reactions provoked by certain other vectors, but data from a trial targeting hemophilia suggest otherwise[13,14].

The AAV-etanercept vector, known as rAAV2-TNFR:Fc, or tgAAC94, has shown efficacy when injected intraarticularly in rat streptococcal cell wall-induced arthritis[15]. Its safety in humans was previously evaluated in a small phase I study involving 14 subjects with RA and one with anklyosing spondylitis (AS)[6]. Subjects were given a single intraarticular injection of a suspension of 10^{10} or 10^{11} per ml DNase-resistant particles (DRP; the equivalent to viral particles). The volume injected depended upon the joint, ranging from 5 ml for knee joints to 0.5 ml for metacarpophalangeal joints. No severe adverse events were noted.

This led to the present Phase I/II study, which has a number of important differences from the Phase I trial. The dose range was expanded to include 10^{12} and 10^{13} DRP per ml, redosing was permitted, and, most controversially, concomitant treatment with conventional TNF blockers was allowed. This was based upon the assumption that sympto-

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matic joints in patients who are otherwise responding well to systemic TNF blockade will benefit from the additional etanercept provided locally by gene transfer. Entry criteria included adults with a diagnosis of RA, psoriatic arthritis, or AS with persistent swelling in at least one knee, ankle, elbow, wrist, or metacarpophalangeal joint. Subjects with RA were required to have had an inadequate response to at least one disease-modifying antirheumatic drug (DMARD), which could include a biologic DMARD. Exclusion criteria included discontinuation of etanercept because of safety concerns.

The trial was designed to treat 6 cohorts of 20 individuals, 15 of whom received rAAV2-TNFR:Fc and 5 of whom received placebo. The first 3 cohorts received 10^{11}, 10^{12}, or 10^{13} DRP per ml and cohorts 4–6 constituted a Phase II expansion of these dosages. Based upon the response of the target joint, a second open-label dose of vector was administered 12 or 30 weeks later. Subjects initially receiving placebo were administered vector as their second injection. Safety was evaluated by serial medical history and physical examination, complete blood count with white cell differential, blood chemistries, and urinalysis. Peripheral blood was screened for the presence of viral genomes by polymerase chain reaction (PCR); when available, synovial fluid and solid tissues were also analyzed. Transgene expression was assessed at the RNA level by reverse transcriptase-PCR and at the functional protein level by a TNF-binding activity radioimmunoassay. Humoral and cell-mediated immune responses to AAV2 were also assessed.

One problem for a study of this type is the lack of robust measures of efficacy when treating single joints in patients with RA. Various metrics were used to assess clinical outcomes: target joint swelling and tenderness were each evaluated on a scale of 0–3; subjects reported global symptoms, function, and satisfaction using a visual analog scale; the Disabilities of Arm, Shoulder, and Hand Questionnaire (DASH) and Rheumatoid Arthritis Outcome Score scales were used to assess joints within the upper and lower extremities, respectively. Various measures of systemic efficacy were also used.

Twelve serious adverse events were noted during this study, including 2 deaths. One of these, resulting from pulmonary emboli 30 days after a second injection of vector, was considered unlikely to be related to the study agent. The second fatality, however, aroused considerable press coverage, partly because it involved a young mother who fell seriously ill shortly after receiving her second injection of rAAV2-TNFR:Fc and who died 22 days later with disseminated histoplasmosis accompanied by a massive retroperitoneal hematoma[16]. The clinical trial was placed on hold while the US Food and Drug Administration and the Recombinant DNA Advisory Committee of the US National Institutes of Health launched investigations (see Evans, *et al*[17] for a detailed discussion). Eventually the study was

allowed to proceed in a slightly modified fashion and was completed uneventfully. Ultimately, the only severe adverse event ascribed to the trial was a case of septic arthritis of a knee joint. The only other adverse events of note involved several subjects who experienced dose-dependent administration site reactions; in 3 cases these were severe enough to require treatment with steroids.

Viral genomes were not detected in the peripheral blood of subjects given placebo or the lowest dose of vector. However, viral DNA was present in the blood of 46% of those receiving the middle dose and 61% of those receiving the highest dose, suggesting leakage from the joint of vector, or cells infected with vector. However, the copy number fell rapidly, and viral DNA was no longer detectable after 12–18 weeks. Joint aspiration, total joint replacement, and synovectomy, provided opportunities to analyze articular tissues for the presence of vector genomes. Data from different synovial fluid aspirates suggest that high copy numbers are present soon after injection of vector, but fall rapidly to very low levels. Viral DNA appeared to persist longer in synovium, but the small number of samples and their heterogeneity preclude strong conclusions. Analysis of autopsy material provided additional information. Intriguingly, large amounts of viral DNA were detected in scrapings from the surface of the articular cartilage of the injected knee, a finding of possible relevance to the question of whether AAV can penetrate the matrix of cartilage and transduce chondrocytes. Smaller amounts of viral DNA were recovered from synovium and ligament of the same knee, but virtually no viral DNA was detected in a wide range of additional tissues and organs, including liver, brain, heart, and the contralateral knee[16].

The PCR method used for detecting viral genomes did not distinguish between DNA within viral particles and DNA within transduced cells. This issue may be relevant to the lack of evidence for transgene expression at either the RNA or the functional protein level. Because rAAV2-TNF:Fc is a single-stranded virus, lack of gene expression could reflect problems with second-strand DNA synthesis. Nevertheless, the authors report a modest clinical response to the gene therapy. Reduced swelling, but not tenderness, was noted in the Phase I subjects, although this was not noted in the Phase II cohort, and trends toward improvements in patient-reported outcomes were observed.

Neutralizing antibodies to AAV2 were generated by the intraarticular injection of rAAV2-TNFR:Fc, possibly compromising the effectiveness of redosing. However, the lack of a detectable cell-mediated response to the vector is encouraging.

Overall, the study suggests that intraarticular gene therapy using AAV can probably be accomplished safely, with little sustained spread of vector genomes beyond the joint. However, fundamental issues of transgene expression need to be addressed before robust clinical responses can be

expected. It is now possible to produce recombinant AAV vectors with double-stranded DNA genomes that transduce synovial cells much more effectively and generate much higher levels of transgene expression than their single-stranded equivalents[18]. TNFR:Fc cDNA is too large to be packaged in such vectors, but there are other promising transgenes[2,3], and serotypes other than AAV2 may also be advantageous[19,20].

The sums of money needed to generate gene therapeutics and evaluate them in clinical trials are enormous, well beyond the typical resources of academic investigators[4]. However, researchers in this area can take heart from the fact that gene therapy is experiencing a nascent resurgence, with clinical successes reported recently for several diseases[21] and renewed confidence in its future[22]. Perhaps arthritis gene therapy is well positioned to catch this wave.

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