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Commentary

Future of adenoviruses in the gene therapy of arthritis

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Abstract

Recombinant adenoviruses are straightforward to produce at high titres, have a promiscuous host-range, and, because of their ability to infect nondividing cells, lend themselves to *in vivo* gene delivery. Such advantages have led to their widespread and successful use in preclinical studies of arthritis gene therapy. While adenoviral vectors are well suited to 'proof of principle' experiments in laboratory animals, there are several barriers to their use in human studies at this time. Transient transgene expression limits their application to strategies, such as synovial ablation, which do not require extended periods of gene expression. Moreover, there are strong immunological barriers to repeat dosing. In addition, safety concerns predicate local, rather than systemic, delivery of the virus. Continued engineering of the adenoviral genome is producing vectors with improved properties, which may eventually overcome these issues. Promising avenues include the development of 'gutted' vectors encoding no endogenous viral genes and of adenovirus–AAV chimeras. Whether these will offer advantages over existing vectors, which may already provide safe, long-term gene expression following *in vivo* delivery, remains to be seen.

Keywords: gene expression, gene therapy, immunity, vector, virus

Introduction

Adenoviruses possess many obvious advantages as gene-therapy vectors, particularly with regard to the ease of generating high-titre recombinant virus and the wide range of cell types that are susceptible to efficient transduction by such viruses [1]. Because susceptible cell types include nondividing cells, adenoviruses are well suited to gene delivery *in vivo*, which is otherwise difficult to achieve. Moreover, adenoviral vectors can accept large amounts of additional DNA, 30 kb or more in the case of the latest gutted vectors (see below). Such advantages have led to their widespread application both in preclinical and clinical studies. So far, 86 clinical trials, over 20% of all human gene-therapy protocols, have made use of adenoviruses for gene delivery. Until recently, adenoviruses had also been considered safe, a reputation now tarnished by the death in 1999 of a patient who had received a large dose of recombinant adenovirus.

Adenovirus has been used in a number of preclinical studies pertaining to the gene therapy of both rheumatoid arthritis and osteoarthritis. By the use of this vector, genes have been successfully delivered to intra-articular tissues, including the synovium [2–4], cartilage [5,6–9], menisci [10,11], and ligaments [12]. Systemic delivery has been achieved by intramuscular and intravenous injection. Moreover, there are several reports of the successful genetic treatment of animal models of arthritis using adenovirus as the delivery system [4,13–19].

MAP = mitogen activated protein; NF = nuclear factor; PEG = polyethylene glycol.

Success in preclinical studies raises the obvious question of clinical applicability, discussion of which forms the basis of this commentary.

Adenoviral vectors

Wild-type adenovirus contains a single, 36-kb, double-stranded DNA genome flanked by inverted terminal repeats. There are over 50 serotypes, from which serotypes 2 and 5 have been most developed for use as gene-therapy vectors. This virus infects the upper respiratory tract, producing symptoms similar to those associated with colds and influenza, but as far as is known, it does not normally cause more serious disorders.

After infection, the viral DNA escapes from the lysosome and is transported to the nucleus of the cell, where it persists as an episome; multiple genomes can coexist within the nucleus of an infected cell. The adenoviral genome has eight transcriptional units, expressed in temporal sequence as early (E), intermediate (I), and late (L) genes. There are four early genes (E1–E4), encoding proteins necessary for the replication of the viral genome. E1A is the first viral gene expressed, and its product transactivates the other promoters of early genes [20].

The first-generation vectors were constructed by deleting the E1 and E3 regions of the adenoviral genome. This strategy was intended to prevent expression of the late genes, upon which viral replication depends, and provide loci into which transgenes could be cloned, usually under the transcriptional control of a heterologous promoter. Recombinant adenoviruses of this type proved to be very useful vectors, infecting a wide variety of cell types very efficiently with minimal toxicity. The utility of these vectors, however, is limited continued synthesis of viral proteins by infected cells, despite the genetic deletions. These proteins render infected cells antigenic and thus liable to elimination by the immune system, a problem exacerbated by the subsequent discovery that the E3 domain of the virus encodes immunosuppressive proteins.

Second-generation vectors have deletions in the E2 or E4 regions of the genome [21]. These second-generation vectors are clearly improved with respect to immunogenicity and toxicity. It is unclear, however, whether these vectors' performance regarding gene expression is improved, as the inactivation of proteins encoded by E4 has been shown to impair seriously expression from heterologous promoters [22]. In the latest versions of adenoviral vectors, all viral coding sequences have been eliminated [23]. Production of these so-called 'gutted' vectors can be problematic and their ability to express transgenes in various tissues remains under investigation. Further improvements include the construction of adenovirus/adeno-associated virus chimeras that have the potential to provide both high transduction efficiencies and long-term transgene expression.

There are two reasons why it has proved difficult to obtain long-term gene expression with adenovirus. The first reflects the persistence of viral gene expression in cells infected with first-generation vectors. This renders the transduced cells immunogenic and thus liable to elimination by cytotoxic T lymphocytes [24]. Moreover, it appears that adenoviruses infect antigen-presenting cells, including dendritic cells, very effectively after delivery *in vivo* [25], contributing to the anti-adenoviral immune response. Of interest is our observation that transgene expression can persist for over a year in cells of the intervertebral discs of immunocompetent rabbits when first-generation adenovirus is used [26]. This finding suggests that long-term gene expression is possible in cells that are nondividing and protected from immune surveillance.

The episomal nature of genes delivered by adenoviruses is a second factor limiting the duration of gene expression. Episomal DNA is rapidly lost from dividing cells, but may be retained by nonmitotic cells. There are reports that genes delivered by gutted viruses are expressed for extended periods of time in organs such as liver and muscle, where cell division is rare.

Regardless of whether or not viral genes are expressed in transduced cells, all recombinant adenoviruses, like their wild-type parent strains, are highly antigenic. Most of us already carry antibodies to type 5 adenovirus. Furthermore, there is substantial experimental evidence that a single administration of a therapeutically useful dose of adenovirus generates a sufficient immune response to prevent successful readministration of the same vector [27]. Strategies to overcome this include switching of serotype, transient immunosuppression, 'tolerisation' (the induction of tolerance), and attaching polyethylene glycol (PEG) moities to the virus ('PEGylation'). Gene delivery *ex vivo* using a later-generation virus would also overcome problems associated with the immunogenicity of the adenovirus, but this would deprive the vector of one of its major advantages, efficient gene transfer *in vivo*.

The antigenicity of adenoviruses not only interferes with gene delivery, but also causes pathology, usually inflammation. This has been seen after the intra-articular injection of adenovirus in mice [28], rats [15], and rabbits [3], although not all authors have noted it [2]. Some of the variation may be due to batch differences. Depending upon the preparation, only 1–10% of recombinant virions may be infectious. Although noninfectious, the other 90–99% of the viral particles are antigenic and can contribute to inflammation. The purity of the viral suspension also affects its properties, and incomplete removal of cellular debris or chemicals used in the preparation of the virus will affect performance. In addition, adenovirus may be intrinsically inflammatory as a result of its ability to activate MAP kinases and NFκB by binding to integrins on the cell surface [29,30].

Before evaluating the future utility of adenoviral vectors in the gene therapy of arthritis, it is worth reviewing briefly the anti-arthritic strategies to which these vectors might be applied.

Strategies for the gene therapy of arthritis

Three major issues influence the present analysis [31]:

- Will the gene therapy be local or systemic?
- Will prolonged gene expression be necessary?
- Will redosing be necessary?

As its name suggests, local gene therapy implies that the gene will be delivered to a discrete anatomical location. For arthritis gene therapy, this is normally the joint, particularly the synovial lining of the joint [32], although it could also involve the use of cells with the ability to home in on joints [33]. The aim is to expose diseased tissues to the gene treatment without involving nontarget organs. Systemic delivery, in contrast, implies that the effects of the gene treatment are disseminated; this is achieved by, for instance, delivering genes encoding secreted, circulating products to tissues such as muscle, liver, and skin. The advantages of local, intra-articular gene delivery include a stronger therapeutic effect on the tissues of the joints with lower undesirable side effects. Systemic therapy, however, should provide greater benefit towards extra-articular manifestations of disease and provide a more expeditious route for treating polyarticular arthritis [31].

Strategies for the gene therapy of rheumatoid arthritis can be loosely divided into those that require prolonged periods of gene expression and those that do not. Among those not requiring long-term gene expression are local therapies intended to ablate the rheumatoid synovium by the delivery of, for example, apoptosis genes or the herpes thymidine kinase gene in conjunction with ganciclovir [34,35]; this drug is at present the subject of a human clinical trial. Success in such 'hit-and-run' protocols may require the transgene to be expressed for as little as one or two days, a duration that is easily achieved by existing adenoviral vectors. Experience with more conventional methods of synovial ablation suggests that remissions as long as several years may be obtained by this route. Subsequent recurrence of symptoms may be dealt with by repeating the gene therapy.

It may also prove possible to achieve prolonged tolerance or anergy in rheumatoid arthritis if immunocompetent cells are exposed for short periods of time to the products of the appropriate genes. Candidates include molecules that interfere with the costimulation of T lymphocytes and certain immunomodulatory cytokines [36]. Exactly how short a time these genes would need to be expressed for is impossible to predict for lack of experimental data, but timescales of a week or two, which are readily achievable with adenovirus vectors, are not unreasonable. The ability of the viruses to infect antigen-presenting cells *in vivo* [25] makes them suitable for applications to induce tolerance through expression of immunosuppressive gene products. Redosing will also be necessary if such therapies have a temporary effect. However, so little is known about the induction of tolerance by gene transfer that it is impossible to predict the frequency with which a successful tolerising gene treatment might need to be readministered.

In contrast to the above, most strategies for the treatment of both rheumatoid arthritis and osteoarthritis require extended periods of gene expression – possibly for the patient's lifetime. Such strategies include the expression of IL-1 and TNF antagonists, as well as type 2 cytokines, cartilage growth factors, and so forth [31,37]. The expression of genes encoding such proteins may also need to be regulated, but, as this problem is not unique to adenoviral delivery, we do not discuss it further here except to note that these vectors probably provide sufficient space for the necessary regulatory elements.

Suitability of adenoviruses in arthritis gene therapy

It is questionable whether adenoviruses are suited to arthritis gene therapies requiring long-term gene expression, regardless of whether these are systemic or local. The use of later-generation recombinant adenoviruses obviates the immunological problems engendered by the residual expression of viral proteins but not the more fundamental problem of episomal gene delivery. Continued engineering of the virus may improve the persistence of gene expression, but most investigators seeking prolonged gene expression are turning to alternative existing vectors, such as adeno-associated viruses ('AAVs') [38–40]. A related question for therapies directed towards synovium is the turnover kinetics of synoviocytes in health and disease. Clearly, prolonged transgene expression cannot be achieved by transducing cells that die, regardless of the vector system employed.

Problems associated with transient gene expression can be obviated by repeat dosing, but frequent redosing is not always practical and is impaired by the strong antigenicity of adenoviruses. Of the various strategies mentioned above for dealing with this issue, immunosuppression may be acceptable in RA, where immunosuppressive agents are already used therapeutically. The use of PEGylated virus and vectors developed from different serotypes is also of interest.

Safety concerns promise to curtail the application of adenoviral vectors to the systemic gene therapy of nonlethal diseases such as arthritis. The death of a patient with mild ornithine transcarbamylase deficiency after infusion of adenoviral vectors highlights this concern. Our own experimental data are consistent with the notion that

therapeutic doses of adenovirus run the risk of side effects when delivered systemically. As noted by Whalen *et al* [19], the amounts of adenovirus carrying the viral IL-10 gene (ad-vIL-10) needed to treat collagen-induced arthritis in mice by intravenous injection cause hepatitis. Other investigators have not reported this, but we do not know if they examined liver pathology. Significantly, ad-vIL-10 delivered locally has a stronger anti-arthritic effect at a much lower dose and without observable side effects [19].

There may, however, be a niche for adenovirus in the local treatment of arthritis under conditions where neither long-term gene expression nor frequent readministration is necessary. The most immediate of such applications is synovial ablation, as mentioned earlier. Indeed, we understand that a human clinical study in which adenovirus will be used to deliver the herpes thymidine kinase gene intra-articularly, in conjunction with ganciclovir, has been given regulatory approval in the Netherlands. If genetic synovectomy has a similar clinical effect as synovectomy by other means, the procedure may need to be repeated only rarely.

Conclusions

Adenoviral delivery systems are extremely useful in preclinical studies, where their ease of manufacture and application allows the rapid screening of candidate anti-arthritic genes, testing of hypotheses, and evaluation of feasibility. Nevertheless, at their present state of development, they would appear to have limited clinical application to the gene therapy of human arthritis. Their major problems include limited duration of gene expression, difficulties in repeat dosing, inflammatory responses, and questionable safety. The use of present vectors seems restricted to local, acute therapies such as those involving a genetic synovectomy. Such restrictions may ease as continued engineering of the virus produces vectors with improved properties, but impatient investigators may wish to make use of alternative existing vectors for which these problems already do not exist.

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