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THEMED SECTION: VECTOR DESIGN AND DRUG DELIVERY

EDITORIAL

Delivery of magic bullets: on the still rocky road to gene therapy

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In this issue, the promises, problems and current progress towards gene therapy are examined in a themed set of six reviews. These cover the major methodologies deployed over the last twenty to thirty years to deliver a gene or other potentially therapeutic molecules into an organism. Initial enthusiasm and optimism concerning the prospects for gene therapy and more generally, the delivery of magic bullets, arose after the pioneering discoveries of monoclonal antibodies and retroviral infection during the 1970's and were fuelled by strategies to make synthetic viruses and the advent of chemical vectors over the succeeding twenty years. However, despite significant advances, to date, the early hopes of widespread gene therapy still remain largely unfulfilled.
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The first attempts over 30 years ago at transferring a particular gene into cells deficient in that gene were based on strategies using viral vectors. This is because viruses are extremely efficient in infecting cells and because the physical properties of DNA make penetration into cells difficult. However, as reviewed by Bouard *et al.* (2009), viral-mediated gene transfer is associated with a large number of now well-characterized constraints. Overcoming critical issues concerning for example the long-term efficiency and bio-safety of gene transfer using viral vectors is prerequisite to large-scale clinical application. In spite of the relatively few clear successes and some fatalities observed with most viral vectors, the impressive numbers of clinical trials using this technology attest to the continuing interest in the development of such vectors for gene therapy.

In order to circumvent the inherent problems associated with vectors made from viral material, much effort has been devoted to making synthetic viruses. As reviewed by Midoux *et al.* (2009), such compounds would be able to mimic the key steps that viruses naturally use during cell infection. As a consequence, it was rapidly recognized that DNA needs to be stabilized and protected. This lead to the development of transfection strategies using cationic lipids to encapsulate plasmidic DNA (Felgner & Ringold, 1989; Behr, 1994). Such lipopolyplexes generally enter cells by endocytosis and are internalized in endocytic vesicles. Survival of DNA in acidic endosomes and rapid escape of DNA from endosomes into the cytosol, allowing import into the nucleus, are crucial considerations for transfection efficiency. *In vivo* toxicity of such agents, due to uptake in the liver, is another critical issue. Current developments of next generation lipid-based transfection agents address these problems, mostly *in vitro*, indicating that clinical application is still in the distant future.

It has also been recognized that anti-sense oligonucleotides (ODN) and small interfering RNA (siRNA), which inhibit gene expression by interfering with transcription and/or translation, are potentially therapeutic agents of great interest. However, like DNA, these compounds suffer from their inherent instability in biological fluids and their lack of penetration into cells. Consequently, as reviewed by Fattal & Barratt (2009), ongoing work has focussed on the development of more stable compounds and encapsulated drug delivery systems using recent micro- and nanotechnologies. Even using the lessons learned from lipid-based DNA transfection strategies, many difficulties still need to be overcome before clinical application of ODN or siRNA can be envisaged.

An alternative strategy for the delivery of nucleic acids or other biomolecules that are drug candidates into cells is based

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on the use of cell-penetrating peptides (CPP). As reviewed by Heitz *et al*. (2009), the observation twenty years ago that some proteins shuttle within the cell or from one cell to another, lead to the identification of short peptide sequences (generally, <30 amino acids) endowed with the capacity to enter cells. CPP, which are either polycationic or amphipathic, are stable and rapidly penetrate cells. While the exact nature of the cellular uptake mechanism is a matter of debate, much subsequent work demonstrates that CPP are also capable of transporting cargo (including DNA, ODN, siRNA, peptides and proteins) into cells. Clinical trials with CPP are ongoing, and their outcomes will be crucial for future application.

Direct delivery of naked DNA into cells has been achieved using a number of physical methods, including gene gun, jet injection, and electro-transfer. As reviewed by Villemejane & Mir (2009), such physical methods have some clear advantages compared to viral or non-viral (chemical) vectors. In particular, these approaches may well be safer and have lower toxicity than viral or chemical vectors. On the other hand, increasing transfection efficiencies remains a major challenge for physical methods of direct gene transfer. Other constraints include the accessibility of DNA to various tissues and the tissue accessibility of the equipment needed to provoke DNA entry into cells. To date, only a very few clinical trials using such biophysical approaches for gene transfer are underway.

Clearly, adequate delivery methods for gene therapy are determinant for future success. Stability, bioavailability, cell targeting, transfection efficiency and duration, and especially safety are all clear gold standard criteria that need to be satisfactorily met before large-scale clinical application of gene therapy will become a reality.

On the other hand, therapeutic antibodies are presently the closest we have to the magic bullet concept, with over 20 monoclonal antibodies presently being commercialized. Chames *et al*. (2009) review the discovery of monoclonal antibodies and the subsequent major developments in antibody engineering that gave rise to the successful, second generation recombinant antibodies actually on the market as therapeutics. Current limitations and challenges are discussed as well as future directions. Of note is the development of intrabodies, but similar to the problems described above for gene therapy, a major bottleneck that needs to be resolved is how to deliver intrabodies into cells.

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