Preface

The Successful Clinical use of Viral Vectors for Human Gene Therapy

The field of human gene therapy has had a long and contorted evolution but has now attained a stage of adolescence and even very early maturity. Coincident with the publication of this volume is the 40th anniversary of the publication of an early description of the potential and even the logical necessity of gene therapy for human disease; that is, the development of treatments aimed directly at the underlying genetic defects responsible for disease rather than at their biochemical and functional consequences (Friedmann and Roblin, 1972). From the outset, the development of techniques of gene therapy has centered largely on the use of disabled recombinant viruses to achieve efficient transfer of therapeutic genetic information into cells to correct genetic defects and reconstitute genetic deficiencies. That was largely because geneticists and molecular biologists realized that nature had long ago invented tool to carry out the central task of gene therapy, that is, the highly efficient introduction of foreign genetic information into mammalian cells in ways that permit stable and heritable expression of new genetic functions in the cells. Those agents are called viruses and their role in gene transfer in mammalian cells was established by the studies of Dulbecco and colleagues showing that the tumorigenic papova viruses produce neoplastic changes in cells by integrating part of their genome in stable and functional form into the genome of infected cells (Westphal and Dulbecco, 1968). Further, coincident with the emergence of the concepts of gene therapy and the discovery of viral gene transfer mechanisms came the tools for creating the kinds of recombinant DNA molecules that made possible the production of viral derivatives that retained the mechanisms that native viruses have for highly efficient cell entry of wild-type viruses but that had been disabled of their pathogenic functions by deletion of the viral replicative and cytotoxic functions (Shimotohno and Temin, 1981; Tabin *et al.*, 1982; Wei *et al.*, 1981). By providing viral replicating functions *in trans* and by ligating potentially therapeutic genes into the recombinant molecules, methods quickly became available to produce large amounts of fully infectious but disarmed viral particles that served as vectors for shuffling foreign genes into cells.

It was widely realized that eventual successful functional genetic correction would require a degree of efficient gene transfer much higher than could be achieved by nonviral techniques. Nevertheless, many nonviral gene transfer "transfection" methods continued to be developed and such chemical methods have indeed also demonstrated variable degrees of efficacy in correcting genetic defects in model studies (Friedmann and Rossi, 2006). Naked DNA, lipid-formulated complexes (liposomes), and other chemical and synthetic nucleic acid complexes, and more recently, even protein-based transduction methods, have all been shown to permit functional and even reasonably efficient gene transfer and even correction of genetic defects in human and other mammalian cells. However, to the present time, it is only the viral vector systems that have finally matured to the point of robust and clinically useful gene transfer and convincing disease therapy. Principal among these viral systems are those that utilize recombinant forms of the gamma retroviruses, lentiviruses, and adeno-associated viruses.

Retroviruses. From their inception, retrovirus vectors have been the most widely used viral vector system for transducing mammalian cells. They had the advantage of ease of construction, very high efficiency, and a capacity for carrying and delivering functional forms of most full-length cDNAs into mammalian cells. However, they were hampered by two major inadequacies, that is, their inability to transduce most quiescent and nonreplicating cells such as neurons and their genotoxic potential resulting from the relative promiscuous mechanisms of integration into the host cell genome leading to insertional mutations in the genome. The development of the lentivirus vector system has made possible the highly efficient transduction of quiescent cells and has thereby obviated the target cell deficiency of other classes of retrovirus vectors (Friedmann and Rossi, 2006).

The drawback represented by the insertional mutagenic potential of retrovirus vectors became harsh reality in the clinically successful studies using these vectors for the treatment of the human immunodeficiency disease X-SCID. Despite the remarkable therapeutic success of these studies, at least five children treated with this generation of oncoretrovirus vectors developed leukemia as a result of insertional oncogene activation (Paruzynski *et al.*, 2011). Although recent advances described in this volume have made major improvements in our understanding of the insertional properties of retrovirus vectors, much still needs to be learned about targeting these and other integrating vectors to appropriate "safe harbor" sites in the genome where integration would not cause proliferative aberrations and tumorigenesis. Valuable steps are being taken in this direction, as exemplified by the use of hybrid zinc finger-targeted endonucleases to specify the locations of double-stranded genomic breaks and subsequent repair (Porteus and Baltimore, 2003; Wilson, 2003).

Adeno-associated viruses. During the search for vectors that would avoid or correct the inadequacies of retroviruses, the parvovirus class of viruses

represented by the adeno-associated viruses (AAV) became a very attractive system for the development of transducing vectors for human and other mammalian cells because of their extreme genetic simplicity and because AAV was not known to be associated with human disease and generally were thought to be nonintegrating. AAV vectors were therefore expected to be safe in the context of human clinical use. On the other hand, AAV were hampered by their very small size and therefore by their low carrying capacity for therapeutic cDNAs. Nevertheless, recent clinical studies have borne out the advantages provided by AAV vectors in selected clinical applications and have given unequivocal evidence of therapeutic efficacy (Grieger and Samulski, 2011).

It has also become clear that genetic reconstitution and gene therapy, even when clinically effective, are likely to be beset by difficult associated adverse consequences, as in the case of the leukemias in the SCID studies. Gene therapy will, like most other forms of therapy, be complicated by the unwanted but intrinsic side effects of intervening in highly interactive genetic networks and processes, both normal and pathological. Currently available tools for gene therapy are largely based on augmentation of a defective genome by addition of a complementing but largely unregulated genetic function rather than by true genetic correction of the underlying defect. That mechanism would be expected to lead to persistent aberrations of complex interactive genetic networks in the modified cell. Only true reversion of a genetic defect to a normal genotype by sequence correction would probably obviate such effects. There is reason to be optimistic that site-specific genetic correction will be feasible in the not-too-distant future.

As has been made abundantly clear by recent clinical experience with human gene transfer studies, there is no ideal vector for all applications in human gene therapy. The choice of vector systems in human gene therapy obviously depends crucially on many factors, including the replicative and functional properties and location of the target cell, the nature of the genetic and physiological deficiencies to be corrected, and the need for fine developmental and temporal control of transgene expression, at least in some cases. And so the search continues for other approaches to viral and nonviral gene transfer, other classes of vectors, other and improved methods of gene delivery. Nevertheless, despite the attractiveness and promise of many of these alternative gene delivery systems, there is a medical imperative to use available tools, even if imperfect, for the relief of human disease. We restrict the discussion in this volume to the vectors that have most clearly and most definitively produced therapeutic benefit for patients, that is, the retrovirus, lentivirus, and AAV vectors. They are not perfect, but they have proven to be therapeutically effective in a growing number of clinical settings.

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