

Preface

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Ever since the discovery of the DNA double helix by Watson and Crick tremendous advances in our knowledge of molecular genetics and cell biology have made it one of the most exciting areas of science for more than 50 years. We have now progressed beyond the realm of basic research in genetics to the development of biotechnology, which has allowed us to produce human proteins in bacteria and to use these, as in the case of insulin, for therapy of human disease. In many cases the therapy derived from biotechnology is designed as replacement therapy to make up for defects at the DNA level, which result in either the wrong protein being made or no protein at all. A more sophisticated approach than replacement therapy would be to correct a deficit at the level of the gene by either regulating gene expression or replacing or substituting for a defective gene. Once the discovery and characterization of restriction enzymes made it possible to identify, isolate, and clone specific genes the way was open to begin to think about gene therapy. This book describes the state of the art of gene therapy, which is designed to either directly introduce a good copy of a defective gene or to have the cell express a new protein or regulatory RNA which will block the deleterious effects of a defective gene.

The requirements for executing gene therapy successfully are relatively few. First the molecular nature of the defect to be corrected needs to be understood. This has been achieved for a large number of diseases, although multifactorial diseases still pose a challenge. Second, the corrective DNA sequence (i.e., the gene) needs to be determined and sufficient amounts for

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use produced. Many genes have been cloned and are available. The main obstacle to successful gene therapy is the requirement for a vector to carry the corrective gene into the cell. A successful vector must be able to deliver the gene to the target cell, and get it through the cytoplasm and into the nucleus where the cell must be able to express the gene contained in the vector. A variety of approaches described in the introductory chapters of this book, have been tried, including naked DNA, DNA in lipid vesicles, and viruses, which have been adapted to carry "transgenes" in place of normal viral genes. To date the most effective, in terms of delivery and expression, have been vectors derived from viruses. Viruses are natural vectors which deliver their genomes into cells where the DNA or RNA is successfully expressed. Many viruses are also capable of establishing a persistent presence in infected cells. However, viruses have potential downsides as vectors. Most of the better-characterized viruses cause serious human disease and any vector derived from such viruses must be modified to minimize the likelihood of toxicity. Secondly the body recognizes viruses as foreign and mounts an immune response. Viruses have evolved to evade the host response, but it is clear from both animal models and clinical trials that the nature of the host response has to be taken into account.

Shortly after the concept of gene therapy had gained currency there was a push to attempt it in clinical trials. With one or two notable exceptions, there was little associated toxicity, but even less in the way of apparent efficacy. It was difficult to attain therapeutic levels of transgene expression and in most instances to maintain the levels of expression achieved initially after infection. What became evident was that a much better understanding of the process of viral infection at the levels of both the cell and the intact host was needed before gene therapy could become an accepted way to treat various diseases (similar considerations apply equally well to other types of vectors). Particular issues which needed elucidation included mechanisms by which the vector interacts with the host immune system, the process of cellular uptake and trafficking of the vector, regulation of transgene expression, and modification of the vector genome to prevent insertional mutagenesis (all DNAs will recombine into the genome, some slowly and others much more efficiently). Significant advances have been made in our knowledge of the basic processes and this has greatly increased the likelihood of successful gene therapy.

The notion of gene therapy was initially received with enthusiasm. Early clinical trials were closely followed by the media. However, the early optimism soon gave way to pessimism because of the lack of evident clinical success and because of adverse effects, notably the death of one trial participant. The response mirrors earlier reactions to research into various diseases. Early trials with polio vaccines gave more people polio than protective antibody. It took many years before two successful vaccines were developed, nearly simultaneously. Although we have had success with AIDS therapy, our efforts to develop a successful vaccine have still not borne fruit. As a people Americans are impatient.

We have now achieved clinical success in two diseases using gene therapy. In France and the UK, infants with a form of severe combined immunodeficiency disease (SCID-X1) that was untreatable by available therapies have been "cured" by treating cells of their bone marrow with a vector derived from Maloney Murine Leukemia virus. In France 11 of 12 infants treated were cured; however, 4 of those cured developed leukemia, directly attributable to the vector. Three of these had the leukemia successfully treated as well. This trial is clearly a success; despite the morbidity associated with the vector. Without treatment, all would likely have died. A somewhat happier result has been achieved in the past year. An adeno-associated virus vector has been used to treat patients with a rare form of retinitis pigmentosa. All of the patients had been legally blind for years and now almost all have achieved striking improvements in their eyesight. Most encouraging, were similar phase I clinical trials done by three groups nearly simultaneously, and all achieved similar positive results. Thus, although the trials were designed primarily to show the safety of the vector, efficacy seems to be pretty clear. Similarly, treatment of adenosine deaminase deficiency (ADA-SCID) continued to be successful using a protocol similar to that for SCID-X1 but without incidence of leukemia.

Thus, once again the future looks bright for gene therapy. The chapters in this book stand testament to this view and as you read on, you will probably have the same sense of optimism for what this exciting field has in store for us.