

Gut

Leading article – Molecular Biology series

Gene therapy

All at once gene therapy has burst upon the scene as a potential new treatment for genetic disorders, multifactorial conditions, and even for the provision of polypeptide hormones or enzymes to treat acquired disease. Why? Is this new found optimism justified or is it simply 'flavour of the month' – a mixture of 'hype' by the practitioners seeking large grants, the biotechnology companies striving in the corporate stakes, and journalists (whose favourite words are 'cure' and 'breakthrough')?

I declare a bias immediately; I am a practitioner, and I do have large grants in this field. But I also think that the optimism is justified, because of several new developments.¹ Pilot studies which replace the mutated gene for adenosine deaminase by a normal one in lymphocytes of several children affected by severe combined immunodeficiency have shown a clear clinical improvement. New studies show that targetting normal genes using viral and other systems to enter cells (from marrow, the lung and gut, liver and skin) may be easier than at first thought, and that DNA will function more or less normally once it gets into the cell. Further data show that the risk of causing harm by inactivating an essential gene in the host cell, or by recombining to form a pathogen, seems low (though we cannot yet say how low). Finally, there is a growing consensus (epitomised by the Clothier committee report)² that it is ethical to use gene therapy in a variety of somatic ways, but not in gametes or embryos, and indeed that gene therapy may provide a new general enabling technology for protein and drug delivery as well as for gene therapy through the entry of normal genes.

Ellen Solomon and Walter Bodmer first pointed out that the variation in human gene sequences represented a powerful new mapping tool³ which laid the basis for studies that led to the human genome project (in which the entire genome will be mapped, characterised functionally, and eventually sequenced). When we first cloned the human globin genes⁴ we realised that every cloned gene is person-specific, and therefore contains the particular sequences that instruct most of the features which we inherit and that determine our individuality. This combination of the person-specificity of cloning and the generality of mapping led to reverse genetics, or positional cloning: the position of a mutation (such as those causing muscular dystrophy or cystic fibrosis) is first defined by genetic linkage, and then physical maps of the DNA sequence from the chromosomal location are made from which the mutation is plucked by a mixture of intuition and hard sequence data.⁵ The most dramatic feature about the genes mutated so as to cause muscular dystrophy and cystic fibrosis, however, is that they were discovered by

reverse genetics – neither dystrophin nor cystic fibrosis transmembrane receptor were known to exist in the body before they were discovered by Kunkel and Tsui respectively.

It is necessary to know the gene (and the mutation) before even thinking of gene therapy. If a disease is caused by a mutation in a gene, it may be 'dominant' or 'recessive' – that is, it may occur when only one of the two copies is mutated in spite of the presence of a normal gene, or only when both are defective. Clearly it will be easier to treat a recessive disease using gene therapy than a dominant one, since it is only necessary to introduce the normal gene, not to remove the mutated copy. However, even this breaks down in some cases – for instance, in the form of hypercholesterolaemia due to a low density lipoprotein receptor defect, the pathology is quantitative, and introducing a normal gene may improve the prognosis, even in adults in whom the disease is dominant.

Cloning a normal human gene is no longer a problem; about 10 000 of the coding sequences (and the corresponding and larger genomic signals) have been cloned in bacterial viruses or as yeast artificial chromosomes. Getting these into human cells, en masse, in a functional form is more difficult. Several approaches have been proposed. The most advanced technology involves the use of viruses which have been engineered for safety and to accept human genes.

The RNA tumour viruses have been studied extensively, and have been used to carry the human adenosine deaminase gene successfully into children with severe combined immunodeficiency. They are small and can only carry a small amount of human DNA (which substitutes for some of the viral genome, providing additional safety as well as more space), and only infect dividing cells. They also always deliver their package of genes into the host chromosome, integrating more or less at random. Another favoured delivery system, or vector, is adenovirus: this is a bit bigger, and enters the cell via specific receptors that seem to provide some protection from breakdown of the nucleic acid once inside the human cell. However, adenovirus rarely integrates and cannot divide in the cell, so it would only be effective as long as the human cell into which it enters survives.

Are these viruses, which in their unmodified form can cause anything from colds (adenovirus) to cancer (mammary tumour virus), safe? Probably yes, but no one can be certain of safety in this context; the field is too new, and since treatment may have to be repeated many times, even an unlikely event may eventually happen. The viruses are inactivated by removing most of their pathogenic sequences, but they can recombine with wild type viruses in the

environment. It is not clear whether anything worse than the natural virus might result. Clearly, until these questions are answered, gene therapy will be reserved for life threatening diseases.

Other approaches, such as the use of human minichromosomes and the combination of the best features of the different viral and biochemical approaches, are under study, and may offer the best long term prospects. And it must be remembered that access to a tissue is often the least well thought out part of a proposal; there are many neurological conditions where gene therapy would be attractive if parts of the central nervous system were selectively accessible.

Cystic fibrosis is a clear target for gene therapy. In spite of improvements in treatment, average survival still hovers at around 20 years of age, and many of those affected have a severe clinical course requiring continuous therapy. The epithelial cells lining the lung and gut have a failure of ion transport due to a mutation of the gene coding for a cell membrane protein, the cystic fibrosis transmembrane regulator.⁶ Both the lung and gut are accessible to treatment, especially the lung, for which the aerosol technology for periodic introduction of vectors carrying a gene is well developed. In cystic fibrosis there are people who are severely ill for whom it would be justified to try treatments even at a very experimental stage. The major question, which cannot be answered until treatment has been attempted, is whether success would only result in preserving what function remains or whether the epithelial cells could reconstitute and restore function to damaged areas of the lung and gut.

Targetting gut epithelium has long term potential. For instance, it might be possible to introduce genes coding for specific receptors so as to alter the stomach or intestinal lining and provide treatment for ulcers. The possibility of introducing anti-oncogenes into those with a predisposition to colon cancer is undoubtedly one of the most exciting prospects.

Many single gene inherited diseases are caused by mutations which affect hepatic function, including haemophilia, phenylketonuria, and many of the disorders of cholesterol metabolism. It may be possible to target the liver by interaction with (for instance) transferrin receptors, which are abundant in this organ. It has even been suggested that a lobe of liver might be removed, disaggregated, treated with the gene in vitro, and then 're-seeded' into the hepatic vessels from whence it will repopulate the regenerating liver. This would, or course, only be justified for serious disease, but if more benign targetting techniques could be devised, it might be possible to use the liver as a target tissue for any interaction with serum metabolites, such as cholesterol.

Skin cells from any individual can be grown in vitro. Great advances have been made in this technique because of the interest in skin grafting, and fibroblasts do not transform to cancer cells easily in culture. A human gene can be inserted into fibroblasts using a retroviral vector, and the skin then grafted back to the donor. This has been attempted with some success using the gene for human factor IX, though synthesis continues for only a short time, and immune response in the mouse recipients proved a problem. The long term significance of using fibroblasts and skin grafting will emerge if it becomes possible to use very large inserts of genes coding for hormones, with associated sequences which allow response to physiological controls: for instance, inserting the entire normal insulin gene into fibroblasts and hoping that there will be a physiological insulin response. This is speculative but not entirely fictional; if it can be made to work by a combination of genetic and cellular engineering, it has amazing potential in gastroenterology.

Gene therapy is coming, and soon, for serious disease. It is here already for severe combined immunodeficiency, where the gene is cloned, the affected cell is easy to access, and the biochemistry is well understood. I suspect that other single gene disorders, such as cystic fibrosis and haemophilia B, will follow relatively quickly, certainly by the end of the decade. But the true excitement will be in the novel use of the technology to enable treatment of the common multifactorial diseases, even those not primarily due to a genetic defect, by genetic means at the somatic level. The obvious examples are hypercholesterolaemia, cancer of the colon and autoimmune disease; the time to begin thinking of these approaches is now. And while the ethical concerns are real enough, they are not novel in medicine, and are worth confronting for the benefits that will come to our patients and the community.

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Accepted 12 June 1992

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