Edging towards human gene therapy

False starts in identifying genes associated with psychiatric disease (see overleaf) and excessive regulatory hurdles facing human gene therapy could hamper research on gene manipulation. But the mood was optimistic at *Nature's* conference on Gene Manipulation in Biology and Human Disease.

Boston

INTRODUCING a session on the prospects for gene therapy at *Nature's* conference in Boston last week, Leon Rosenberg (Yale University) wisely refrained from predicting when the first trials of human gene therapy would commence. Progress towards them, he said, has been perhaps slower than some had hoped but faster than others had feared. The fact of the matter is that potential gene therapists have been beaver away to produce appropriate reagents and procedures in the full knowledge that, for no good reason, they will face far more barriers than are usual for experimental clinical trials.

Among the more genuine reasons why any trial of gene therapy is still some years off, is that many of the technical problems have been hard to crack. Another is the continuing memory of a premature attempt in the early 1980s to correct the faulty β -globin gene in two cases of thalassaemia; the attempts were mounted in Israel and Italy, but were led by a US researcher, whose research efforts have since been redirected.

It is ill wind, however, that blows no good. Those interested in globin gene therapy were forced to take stock. First, it became clear that the control of globin gene expression is more complex than had been thought. The frustrating result was that whereas it was possible to insert globin genes into cells, insufficient globin was produced to make the cells of any potential use in therapy. But a solution to that problem now seems to be within reach: DNA sequences that confer high expression on globin genes, despite being located far from them, have been identified and shown still to function after being engineered to be much closer to the genes.

In the meantime, ironically, the need for gene therapy in thalassaemia has receded, as bone marrow transplantation has increasingly found a place in the treatment of the most serious forms of the disease. Used in that way, transplantation is a type of gene therapy, albeit one that cocks a snook at the sophistication of genetic engineering. Whereas its use is limited by the availability of compatible bone marrow and the problems of graft rejection, both problems could, in principle, be solved were it possible to transplant the stem cells that are the progenitors for all

blood cells rather than whole bone marrow.

Such a prospect was held out by Irving Weissman (Stanford University). As the much-needed assay for human stem cells, he hopes to test their ability to reconstitute the haematopoietic system of the genetically immunodeficient SCID mouse. But, to judge by his experience with mouse stem cells, there would still remain the pressing problem of finding a practical way to stimulate the proliferation of isolated human stem cells if they are ever to be available in sufficient quantities for transplantation.

In principle, many genetic deficiencies could be treated by transplantation of the appropriate normal tissue, but because of the practical difficulties surrogate methods are being explored. One example that excited interest at the meeting was the 'neo-organs' or 'organoid neovascular structures' approach described by W. French Anderson (National Institutes of Health). Neo-organs are the end result of implanting rats with fibres of polytetrafluoroethylene (Gortex) coated with collagen, heparin binding growth factor 1 (also known as acidic fibroblast growth factor) and hepatocytes. After several months, the implanted structures have acquired a continuous blood supply from the liver, contain structures that resemble nerves and have grown to as much as half the size of the liver. That hepatocytes in neo-organs are functional has been shown by the apparent ability of neo-organs seeded with Lewis rat hepatocytes to restore to normal, for at least six months, the elevated levels of serum bilirubin in Gunn rats (Gunn rats have a genetic deficiency of the enzyme UDP-glucuronosyltransferase).

Even if cell or organ transplants may limit the need for specific gene therapy in some cases, the ingenuity of gene manipulators ensures that they will continue to thrive. Not all will be lost, for example, should there be no need to use β -globin gene therapy to treat thalassaemia, because knowledge of how to obtain adequate globin gene expression is already being used to develop a mouse model of sickle-cell anaemia in which to test new forms of therapy. The first step, as described by Richard Palmiter (University of Washington, Seattle), is to produce transgenic mice carrying the mouse equivalent of the mutant α -globin gene

that is the cause of the human disease. When these mice are crossed with a strain that lacks one copy of the β -globin gene, the resulting offspring have red blood cells that will sickle.

Mice carrying foreign genes, whether introduced into fertilized eggs (transgenic technology) or embryonic stem cells, are proving to be rich sources of animal models of human diseases. For example, neuroblastoma can be modelled by mice carrying a polyoma virus gene (Erwin Wagner, Institute of Molecular Pathology, Vienna), and the liver damage progressing to malignancy associated with hepatitis B virus infection can be modelled by a hybrid transgene composed of a hepatitis viral gene and an albumin gene promoter (Palmiter). A variation on this theme is to try and interfere with the function of a gene in order to produce a model of a genetic deficiency. Thus the cleft palates produced in transgenic mice carrying a hybrid viral *erbA*/retinoic acid receptor gene, provide a model in which to explore the possible role of retinoic acid in morphological development (Ronald Evans, Salk Institute).

The transgene in that case is a so-called dominant negative. It is the possibility of expressing in the blood cells of AIDS patients dominant negative genes for proteins of the human immunodeficiency virus (David Baltimore, Whitehead Institute) in order to interfere with human immunodeficiency virus function, that might yet drive the first successful attack on the growing battery of regulations that face anyone wishing to pioneer the use of gene therapy.

For now, the only tentative step towards such therapy that has been taken is the inclusion of a genetic marker in some of the 'tumour infiltrating lymphocytes' that are being used in an experimental procedure for cancer treatment. The marker is enabling the fate of the cells to be followed (Anderson) but plans are already afoot to include not just a marker gene but also a gene for interleukin-2 that may lengthen the life and efficacy of the cells. As their use will be tested only in patients for whom conventional forms of treatment have failed, there should be no more reason for excessive regulatory hurdles than is usual in such circumstances. Unfortunately, given that gene therapy is involved, even that is too much to hope for.

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