**Conference reports**

**Genes and gene therapy—prospects and problems in the blood**

Advances in recombinant DNA technology and in the understanding of the molecular biology of human disease have made the development of human gene therapy a realistic goal. A conference held at The Royal College of Physicians on 29 September 1994 provided fascinating insights into prospects and problems of gene therapy in haematological practice.

Gene therapy can be defined as the transfer of a genetic sequence into target cells in order to correct defective protein expression and production. Research in gene therapy originally concentrated on hereditary diseases caused by single-gene defects because they could theoretically be corrected by insertion of a functioning gene into the appropriate cells. In clotting factor deficiencies almost any somatic cell can be a target for gene therapy because the gene product is secreted and is required systemically. In other cases the gene must be delivered to a specific cell type, for example, haemopoietic stem cells in the haemoglobinopathies. In addition, gene therapy may also be useful for acquired disorders such as infectious diseases or haematological malignancies where, rather than the defective gene being inserted, a new functioning gene is introduced to enhance an anti-tumour response.

**Gene transfer techniques**

Most strategies for human gene therapy rest on the use of retroviral vectors which are highly efficient at introducing new genetic material into cells and stably integrating that information into a host cell chromosome. Other methods of gene transfer include adenovirus vectors and physical DNA transfer. *Professor D Onions* (University of Glasgow Veterinary School) summarised the advantages and disadvantages of the gene transfer vectors currently available for clinical use. The major advantages of adenovirus vectors are their potential to carry large segments of DNA (36 kilo base-pair genome), their ability to infect non-replicating cells and their suitability for infecting tissues *in situ*, especially the lungs. Major disadvantages include in many of the current vectors the presence of adenovirus genes that may stimulate immunity or have other adverse effects, and the potential instability of gene expression because the vector does not integrate into chromosomal DNA. The most appropriate setting for this vector is the delivery of the human cystic fibrosis gene to respiratory epithelium by inhalation.

The retrovirus is an attractive vehicle for the transfer of genetic material because an intact virion contains all the proteins required for the integration of the viral genome into the target cell genome. Retroviral vectors are retroviruses whose viral genes have been removed or altered so that no viral proteins are made in cells infected with the vector. Viral replicative functions are provided by the use of retroviral ‘packaging cells’ that produce all the viral proteins but do not produce infectious virus (Fig 1). Introduction of the DNA from a retroviral vector into packaging cells results in production of virions that carry vector RNA which can infect target cells, but no further virus replication and spread occurs. Most retroviral vectors for clinical use are derived from the Moloney murine leukaemia virus (MoMLV). The MoMLV is an enveloped, single-stranded RNA virus whose genome is composed of three regions. The first region, called the long terminal repeats (LTRs), contains the viral promoter and enhancer regions required for initiation and termination of transcription. The LTRs flank the viral genome and also contain segments required for viral integration into the target cell genome. The second region encodes the psi segment which is

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**Fig 1. Retrovirus packaging cell line. By kind permission of Q-ONE Biotech Ltd.**
required for efficient packaging of the RNA viral genome into viral particles. The third region contains the viral genes \textit{gag}, \textit{pol} and \textit{env} which encode for viral structural proteins, enzymatic proteins (including reverse transcriptase), and the viral envelope protein, respectively. Deletion of the viral genes and introduction of an exogenous gene(s) result in the formation of a retroviral vector. Since they lack the viral genes required for virion formation (\textit{gag}, \textit{pol} and \textit{env}), retroviral vectors cannot package themselves into virions. This led to the development of ‘packaging cell lines’ which contain a viral genome with intact \textit{gag}, \textit{pol} and \textit{env} genes but lacking the psi segment required for incorporation of the viral mRNA into virions. Introduction of the retroviral vector genome into the packaging cell line creates a ‘producer cell line’ which results in production of virions that carry vector RNA. These retroviral vector particles can infect target cells but are unable to replicate (Fig 2). Infection of appropriate target cells occurs either by co-cultivation with ‘producer cell lines’ or incubation with vector medium. This is initiated by the interaction of a viral coat protein with a cell surface molecule that acts as a receptor. Subsequently the retroviral genome, which is released into the cell after viral fusion with the cell membrane or after endocytosis, serves as a template for reverse transcriptase. This enzyme and cellular proteins then interact to convert the RNA genome into DNA with subsequent integration and expression.

**Quality control and safety aspects**

Professor Onions also spoke about the theoretical risks associated with retroviral-mediated gene transfer (RMGT). These include insertional mutagenesis, recombination with endogenous retroviral sequences, transfer of exogenous genetic material, and accidental exposure to replication-competent murine retroviruses. Since retroviral vectors insert randomly throughout the genome, insertional mutagenesis may occur when integration of a retroviral vector leads to abnormal regulation of cell growth by disruption or abnormal regulation of a gene. The risk of insertional mutagenesis varies depending on the cell population undergoing treatment.

The human genome contains many endogenous retroviral sequences (HERV) which are replication-defective due to multiple mutations. A theoretical concern is the restoration of replicative function by recombination with a retroviral vector. However, that risk appears to be very low, and the factors limiting this recombination also apply to human retroviruses such as the human immunodeficiency and human T cell leukaemia viruses.

Another rare event is the transfer of exogenous genetic material inadvertently packaged by virions. Endogenous mouse retroviral sequences contained in the vector packaging cell line, e.g virus-like 30s element (VL30), may also be integrated into cells transduced with the retroviral vector. The significance of VL30 sequences in mutagenesis is unknown and this raises the issue of using packaging cell lines from animal species other than murine.

Finally, of greatest concern is the contamination of retroviral vectors with a replication-competent murine retrovirus. This presumably arises by recombination with the retroviral vector donating its intact psi sequence to the psi-negative packaging cell genome. Recently, T cell lymphoma has been observed in primates participating in gene transfer experiments. In view of this, second and third generation packaging cell lines have been developed and retroviral vector constructs redesigned. One approach has been to place the \textit{gag} and \textit{pol} genes on a plasmid separate from the \textit{env} gene so that multiple recombinations between the vector and viral genes would be required before replicative function could be restored. Another approach has been to decrease homology between the vector and the packaging cell line’s viral genome.
Following on from this lecture, the question of quality control of the vector stock prior to patient exposure was discussed. Although still evolving, the regulatory procedures affecting products from human cell lines are founded on the principles developed for the production of vaccine viruses from cell lines. A fuller account of other regulations and safety aspects of gene therapy was given by Dr T Seddon (Imperial Cancer Research Fund). Three principles lie at the foundation of the development of safe products. First, the establishment of a master cell bank (MCB) under the conditions of good manufacturing practice (GMP); second, the demonstration that the cells do not contain adventitious agents by a laboratory accredited to conduct tests under good laboratory practice (GLP); finally, the evaluation of the vector in animal models to demonstrate safety and to provide data on the expression of the construct.

Specific problems of gene transfer in haematology

Dr J Apperley (Royal Postgraduate Medical School, London) described particular prospects and problems of gene therapy in haematological practice. Initially, gene therapy was directed towards correcting inherited disorders such as the haemoglobinopathies. More recently, haematological malignancies have been targeted by intervention of molecular events. For example, an in vivo trial in chronic myeloid leukaemia (CML) for purging autografts using oligonucleotides to the BCR-ABL gene is in progress. Other methods used include replacement of tumour suppressor genes, eg p53, which is mutated in a number of haematological malignancies, and inhibition of expression of drug-resistance genes, eg MDR gene. The immune response may also be enhanced in malignancies by expression of certain cytokines, eg interleukin-2 (IL-2), or boosted by plasmid vaccination. In the latter, a vaccine consisting of the polymerase chain reaction (PCR) product of the V region of the immunoglobulin protein on malignant cells may be beneficial. Manipulation of malignant cells, however, presents a number of serious issues such as whether a second hit may lead to more aggressive disease.

Other problems discussed included the suboptimal efficiency of transduction of haemopoietic stem cells. This is due to the fact that retroviral vectors require dividing cells for integration. Haemopoietic stem cells, however, are present in very small numbers and only a proportion are in division at any one time. There may also be failure to transcribe the exogenous gene, sustain expression or maintain levels of expression comparable to the endogenous gene. Another problem is the development of antibodies to the new protein product. Finally, since the size of the gene to be transfected is limited, cDNA is usually used; in these situations one has to rely on an exogenous promoter and that may explain why only suboptimal expression is obtained. The globin gene sequence, however, is small enough to allow both gene and promoter to be transfected. Nevertheless, the level of expression is low since in the case of β globin the locus control region (LCR) which is required for expression, is far upstream of the globin cluster. With some genes, eg factor VIII, even the cDNA is too large, leading to problems in expression.

In factor IX deficiency (haemophilia B), retrovirus-mediated gene transfer of factor IX into human fibroblasts has been shown to yield a biologically active product. These human factor IX secreting cells were either implanted under the skin of animals or were grown on collagen beads and injected intraperitoneally. Therapeutic levels were, however, not obtained and the duration of expression was short. Other target cells for factor IX gene transfer include keratinocytes, muscle cells and, more recently, hepatocytes. Whether such systems will be of practical use remains to be established.

Is gene therapy appropriate for diseases such as haemophilia where an existing effective therapy is already available? In these situations gene therapy might improve the quality of life, especially in patients who have developed inhibitors to factor IX concentrates.

Immunodeficiency disorders

Gene therapy in the treatment of primary immunodeficiency disorders such as severe combined immunodeficiency (SCID) was addressed by Dr G Morgan (Hospital for Sick Children, Great Ormond Street). SCID due to adenosine deaminase (ADA) deficiency results in a severe reduction of T and B lymphocytes and hence humoral and cellular immunodeficiency. Bone marrow transplantation (BMT) offers a 90% success rate in genotypically identical individuals but there remains a risk of death and morbidity associated with the conditioning regime, and graft-versus-host disease. The disease can be partially corrected by injections of bovine ADA, conjugated to polyethylene glycol (PEG) to increase its half-life in the circulation; but the treatment does not cure the disease, is immunogenic, expensive and requires repeated injections. In view of this, a number of gene therapy protocols for ADA deficiency have been investigated. The London UK gene therapy protocol involves harvesting bone marrow (BM) CD34+ cells, culturing them with the packaging cell line and vector in the presence of the growth factor IL-3 and then reinfusing them. Unfortunately, the bone marrow of a child so treated showed no evidence of ADA expression and the child now requires PEG-ADA treatment. Changes to the protocol are being investigated, including employing a different vector, omitting CD34+ cell selection in order to obtain a larger cell population, using other growth factors, and the use of patient conditioning to give advantage to the re-infused cells.
Hurler's syndrome

In this autosomal recessive lysosomal storage disorder defective α-L-iduronidase results in lysosomal accumulation of heparan and dermatan sulphate. Dr L Lashford (Christie Hospital, Manchester) informed us that in Hurler's syndrome enzyme replacement is not available, and allogeneic BMT is non-curative, since CNS impairment and skeletal abnormalities may occur post-BMT. Gene transfer experiments, however, have shown successful transfer and long-term expression of α-L-iduronidase in BM and cord blood cells with morphological correction of macrophages.

Haematological malignancies

Dr R Mertelsmann (Freiburg, Germany) discussed the investigative and therapeutic applications of gene therapy in haematological malignancies. He began by describing gene therapy as a tool in cell marking studies and then went on to talk about the use of cytokines in the treatment of neoplasia. Gene marking studies have provided valuable information about the source of relapse in patients undergoing autologous transplantation with BM or peripheral blood (PB) cells for malignant disease, e.g. acute myeloid (AML) and acute lymphoblastic leukaemia (ALL). Relapses result from residual disease in the PB or BM which has been re-infused into the patient. Methods to decrease this tumour load include in vivo purging with chemotherapy before and after harvesting the stem cells, using CD3+ cell selection, or using fewer cells for reconstitution, thereby reducing tumour cell contamination and expanding the autologous cells with cytokines prior to infusion. CD3+ cells selected from BM reconstitute, at the same rate as progenitor cells but whether long-term reconstitution is achievable is uncertain. This issue may be resolved by marking the cells.

The first experiments on gene transfer into human patients involved gene transfer into T lymphocytes using a retroviral vector. Tumour infiltrating lymphocytes (TIL) obtained from tumours of patients suffering from metastatic melanoma were infected with a retroviral vector in vitro, expanded in cell culture and then returned to patients in an attempt to destroy cancerous cells. The cells were marked by transfection so that their fate could be determined in vivo. The infected TILs were found to persist in the patients, particularly at tumour sites, and there were no adverse effects from the procedure. These results showed the feasibility of gene transfer in humans and the potential of modifying TILs to express proteins that assist in the destruction of tumour masses, e.g. tumour necrosis factor (TNF) and IL-2. Alternatively, autologous tumour cells could be cultured with autologous or allogeneic fibroblasts transfected with the IL-2 gene, followed by irradiation and the resultant cell medium used to vaccinate patients at regular intervals. So far, this has only been assessed in end stage patients who have shown no response. This may be due to the fact that local immunity is better than systemic but this form of therapy may be of benefit in minimal residual disease.

Vehicles for gene expression: progenitor cells

Most models of gene therapy have centred on haemopoietic stem cells as vehicles of gene expression because the transduction and transplantation of these cells would provide a means of ensuring a continuous supply of genetically modified haemopoietic cells during the lifetime of the patient. The transduction of stem cells, however, has proven to be quite difficult, mainly because they are found only in small numbers in the bone marrow and appear to be primarily quiescent. Efforts to optimise conditions for transduction have concentrated on examining ways to increase the proportion of stem cells. Dr Testa (Paterson Institute, UK) outlined the use of peripheral blood stem cells (PBSC) in transplantation. It has been known for many years that small numbers of primitive haemopoietic cells are present in the circulation of normal individuals. Such cell populations comprise stem cells which, when transplanted, reconstitute permanently the haemopoietic tissue of potentially lethally irradiated recipients, and progenitor cells which are able to expand and develop along multiple lineages to give rise to mature functional haemopoietic cells. Progenitor cells are quantified by their capacity to proliferate and give rise to colonies in vitro (hence their designation as colony-forming cells or CFC). Following treatment with myeloid growth factors (e.g. G-CSF), stem and progenitor cells are mobilised into the circulation in sufficient numbers for collection to ensure permanent regeneration of haemopoiesis when re-infused after ablative chemo-radiation. This effect may be increased when growth factors are used in conjunction with cytotoxic agents.

There is now considerable clinical experience in autologous PBSC transplantation. Studies have shown that PB mobilised progenitors have the same capacity as BM mononuclear cells for permanent haematological reconstitution after BM ablation. Furthermore, in vitro assays for BM repopulating (stem) cells in humans have demonstrated that both normal BM and PB cells, harvested by leukopheresis after chemotherapy and G-CSF, are equally able to produce long-term culture-initiating cells. This observation makes PBSC potential targets for gene therapy.

Haemoglobinopathies

Professor F G Grosveld (Erasmus University, Rotterdam) spoke on the control of globin synthesis. It has been shown that the locus control region of the β globin cluster is required for β globin gene expression. The gene order is important since different contigu-
ous genes appear to compete for regulation, dependent upon their positions relative to each other. In addition, the expression patterns of genes are altered by the stage of development, embryonic and adult genes responding differently to the same promoter regions.

Dr Swee Lay Thein (MRC Molecular Haematology Unit, Oxford) showed how knowledge of the molecular pathology of the thalassaemias has enabled the heterogeneous clinical phenotypes to be related to the underlying genotypes. In the β thalassaemias, mutations can either reduce or abolish production of β chains. The vast majority of mutations are due to point mutations, although deletions can occur. Also, defects which cause α thalassaemia range from deletions to point mutations although, unlike β thalassaemia, α thalassaemia results less commonly from point mutations. Studies of the molecular basis of the thalassaemias has thus provided a rationale for prenatal testing and genetic counselling as well as developing strategies for the management of severe forms of the disease.

Envoi

This was a stimulating conference, providing an update in the recent developments in genes and gene therapy in haematological practice. However, despite substantial progress, a number of important technical issues, particularly in areas of gene delivery and cell transplantation, still needs to be resolved before gene therapy can be effectively and safely applied in the clinic.

MANAGEMENT OF STABLE ANGINA

Edited by David de Bono and Anthony Hopkins

Angina is the symptom affecting 2% of the population aged over 30 years and nearly 5% of men aged between 40 and 65 years. The ischaemic heart disease which it reflects, is a major cause of morbidity and mortality. It is clear that there are widespread differences in the ways in which angina is investigated and treated. To facilitate the process of audit of care in this common condition, and as a step towards the establishment of clinical guidelines, the joint audit committee of the British Cardiac Society and the Royal College of Physicians of London set up a workshop to investigate clinical guidelines and audit points in the management of stable angina.

This book reflects the outcome of the workshop. It discusses both the pathophysiology of angina and its epidemiology and describes approaches to the investigation, management and treatment of stable angina. The different papers represent a wide variety of viewpoints, from general practice through district hospitals to teaching centres. Nevertheless, the summary indicates the considerable degree of uniformity which underlies the current approach to the management of angina, and is intended to facilitate local discussion and to establish unit based guidelines and audit standards.

Purchasers of health care, both fundholding general practitioners and district health authorities, will be specifying more precisely what services they expect their patients to receive. They will find this book valuable, as will cardiologists, general practitioners and those in training who will also find in its pages useful reviews of the effectiveness of their practice.

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