ABC of clinical haematology

The future of haematology, molecular biology, and gene therapy

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This article will assess the impact of advances in science and technology on the practice of haematology and attempt to predict how haematology will change further over the next 10 to 15 years.

The major advances in scientific thought and technological development that have already changed the practice of modern haematology are likely to affect both laboratory diagnosis and treatment in the future. This article will address three specific areas of haematology—anaemia, haemophilia, and leukaemia—and explain how important innovations could be expected to change clinical practice in these areas. The article begins, however, with an overview of gene therapy, which, although currently some way from curing haematological diseases, is likely to have a role in most areas of haematological practice in the future.

Both diagnostically and therapeutically, the identification of the molecular pathology of the underlying disorder will continue to steer the future, but the ability to make more accurate diagnoses has not yet resulted in improved treatment.

Gene therapy

The term gene therapy is applied to any manoeuvre in which genes or genetically modified cells are introduced into a patient for therapeutic benefit. Gene therapy is still in its infancy, and despite the potential of the approach, clinical benefit has yet to be shown.

Successful gene therapy depends on the availability of reliable methods for delivering a gene into the nuclei of selected target cells and subsequently ensuring the regulation of gene expression. Haematological cells are readily accessible for manipulation and so can be genetically modified outside the body and reinfused. The aim in the future, however, will be to modify the target cells without first removing them from the patient. Genes that are to be delivered to cells must first be inserted into plasmids. These small circular molecules of double stranded DNA derived from bacteria can then be used to transfer therapeutic genes to cells by physical methods or by insertion into recombinant viruses.

The future of haematology—diagnosis and treatment

Diagnosis

- Increasing automation giving quicker and more reliable results—eg automated cross matching; automated diagnostic polymerase chain reaction
- More DNA/RNA based diagnosis, allowing increased diagnostic precision—eg precise definition of genetic abnormalities; diagnosis with polymerase chain reaction
- More “near patient” testing, allowing rapid screening—eg haemoglobinometers, monitoring of anticoagulant treatment

Treatment

- New drugs—eg tailored to molecular abnormalities
- New biological agents—eg thrombopoietin, which may speed platelet recovery after chemotherapy
- Transplantation across tissue barriers—eg cord blood transplantation
- Blood substitutes—eg recombinant haemoglobin
- Gene therapy—probably for many haematological disorders

Ex vivo and in vivo gene transfer strategies.

Gene therapy strategies

Strategy

Corrective replacement

Corrective gene addition

Corrective antisense treatment

Pharmacological

Cytotoxic

Prophylactic

Immunostimulatory

Potential application

Sickle cell disease—to replace the point mutation that causes the substitution of valine for glutamine on the sixth amino acid residue of the β globin chain

Haemophilia—to introduce a gene for missing coagulation protein

Low grade non-Hodgkin's lymphoma (NHL)—to introduce antisense oligonucleotides, preventing BCL2 overexpression, which is responsible for the failure of the lymphoma cells to undergo apoptosis

Continuous production of interferon alfa, erythropoietin, or other therapeutic proteins

Leukaemia—targeted delivery of cytotoxic proteins

Chemoprotection—drug resistance genes introduced into haemopoietic stem cells, conferring resistant phenotype, thus protecting against chemotherapeutic agents

Idiotypic vaccination—in B cell tumours such as NHL and myeloma the variable region sequences of the surface immunoglobulin of the tumour cell provide a tumour specific antigen against which an individualised vaccine for each patient can be produced

Whichever vector system is used, the barriers through which the therapeutic genes must be transported to reach their destination are the same. Many viral and non-viral vector systems are being developed to try to achieve the steps outlined here, and it is often difficult to choose the most appropriate vector for a particular application.

For gene therapy applications, where it is crucial to achieve gene expression in the progeny of the target (modified) cells, it is important to use a vector that stably inserts its genes into the chromosome of the host cell, and retrovirus vectors are the most suitable for this purpose. For direct in vivo gene delivery, vector attachment to a specific target cell is a vital additional requirement. Such vector targeting is at last beginning to look like a realistic possibility.

Anaemia

The move away from the main laboratory towards units nearer to patients—such as intensive care units, general practice surgeries—is likely to provide rapid and increasingly reliable testing of basic haematological variables outside the formal laboratory environment. A large haematology laboratory providing a centralised quality assured service will still be required, but along with the automated analysers, diagnostic tests based on polymerase chain reaction will be used increasingly.

With the identification of the precise mutations associated with many forms of hereditary anaemias, routine characterisation by nucleic acid sequence analysis will facilitate the use of disease specific diagnostic tests based on polymerase chain reaction. This will provide more precise prognostic information for affected individuals as well as accurate identification of affected embryos. As reliable prenatal diagnosis at an early stage will be available, so will an increasing range of prenatal treatment options.

Polymerase chain reaction can be carried out with a minimum of specialist equipment.

For patients affected by hereditary anaemias such as sickle cell disease and thalassaemia, advances in transplantation immunology may permit transplantation across tissue barriers, making bone marrow transplantation a treatment option for all affected patients. In addition, gene therapy for these disorders could provide another potentially curative strategy, although the genetic correction of hereditary anaemias still presents complex challenges. In sickle cell disease, for example, delivery of normal copies of the β globin gene to haemopoietic stem

ABC of gene therapy—A vector must be able to access the cells to be transduced and bind to and penetrate the membrane of the target cell. Once inside the nucleus, the exogenous DNA must be integrated into the cellular genome if stable expression is required. Gene expression must be at a high enough level and sufficiently regulated for clinical benefit.

Retroviral vectors used for gene therapy usually infect cells via a naturally occurring receptor on the surface of human cells. They can, however, be engineered to display on their surface various ligands that are capable of binding to their specific receptors on target cells. In vitro, infection can be shown by X gal staining (successfully infected cells turn blue).

Prenatal options from diagnosis based on polymerase chain reaction

<i>Tissue used</i>	<i>Strategies permitted</i>
Chorionic villus	Selective termination of affected fetus; in utero organ transplantation; in utero gene therapy
Polar body	Gamete selection (egg only, for in vitro fertilisation)
6-8 Cell embryo (after in vitro fertilisation)	Embryo selection
Blastocyst (after in vitro fertilisation)	Embryo selection

cells will be insufficient for cure. As continued Hb S production would be damaging, the mutated β globin genes must also be removed. Stem cell surgery techniques might be used to achieve such an aim.

Fully automated crossmatching procedures will soon be in place in all haematology laboratories, but in the longer term, genetically engineered recombinant haemoglobin is likely to replace transfusion with blood derived from donors. The use of artificial haemoglobin solutions has been associated with various toxicities including renal toxicity due to the renal filtration of haemoglobin dimers. Production of modified recombinant haemoglobin or in vitro modification of a recombinant product will hopefully overcome this problem.

Requirements for the production of recombinant haemoglobin

- A source of protoporphyrin IX (haem)—this prosthetic group is common to haem containing protein of many species and thus can be produced endogenously
- Synthesis of α and β globins
- A method of production that is suitable for large scale production and purification. This may be in microbial or mammalian cells or in transgenic animals. The former may result in structural and functional alterations as a result of expressing the proteins in bacteria or yeast

Haemophilia

As specific mutations have now been identified to account for a large proportion of patients with haemophilia, routine characterisation of all haemophilias by direct sequence analysis is likely. This will facilitate accurate carrier diagnosis and thus the use of disease specific diagnostic tests based on polymerase chain reaction for accurate identification of affected embryos. As for the inherited anaemias, several prenatal treatments are likely to become available.

Dependence on blood products with the attendant risk of viral transmission has been disastrous for many patients with haemophilia but is unlikely to be a problem in the future. Recombinant human factor VIII is already available, recombinant coagulation factor VIIa is improving the prospects for haemophilic patients with inhibitors to factor VIII, and the development of other recombinant coagulation factors will ultimately obviate the need to use fractionated human blood. Definitive gene therapy of haemophilia also has excellent prospects. Haemophilia is known to be curable by liver transplantation, indicating that it should also be curable by genetic modification of host cells to produce normal factor VIII. Regulation of gene expression is not thought to be a critical issue as a moderate excess of factor VIII has no known procoagulant effect. There are concerns that cells producing factor VIII might be recognised as "foreign" by the patient and rejected. A solution to this type of problem may lie in the recent demonstration that the rejection of foreign cells can be prevented when they are genetically modified to express Fas ligand, which triggers apoptotic cell death of cytotoxic T cells that enter the graft before they can cause any damage.

Leukaemia

An understanding of the molecular mechanism of malignant transformation in leukaemia forms the basis for improved diagnostic sensitivity and the monitoring of minimal residual disease and paves the way for more directed treatment interventions, including the eventual possibility of targeting the causative genetic defects. Assays based on polymerase chain

Stem cell surgery. Ideally, haemopoietic stem cells would be isolated from the bone marrow, followed by expansion of haemopoietic stem cells (HSCs) before purified cells are grown in culture and transfected with the DNA to correct the defect. The plasmid carrying the therapeutic DNA also carries a selectable marker (for example, an antibiotic resistance gene), which would allow colonies that contain the successfully integrated DNA to be selected.

Haemophilic patient with inhibitors and severe spontaneous bleeding. Currently the development of inhibitors represents one of the biggest problems facing patients with haemophilia.

The ability to culture mammalian cells in vitro has made a major contribution to research in many areas of modern haematology.

Scientific techniques and approaches that have made major contributions to modern haematology

Technique

Gene cloning and sequencing allow identification, characterisation, and manipulation of genes responsible for specific products or diseases

Polymerase chain reaction is a highly sensitive and versatile technique for amplifying very small quantities of DNA. Amplification of RNA molecules is possible after initial reverse transcription of RNA into DNA (RT-PCR)

Monoclonal antibodies allow immunohistochemistry of tissue and cells, analysis and cell sorting with fluorescence activated cell sorter (FACS), and cell purification

Mammalian tissue culture and gene transfer to mammalian cells, and creation of transgenic animals

Protein engineering and construction of recombinant proteins allow production of large quantities of human proteins

Applications in haematology

- Elucidation of the molecular pathology of disease and diagnostic tests based on the polymerase chain reaction
- Rapid diagnosis of infectious diseases (for example, hepatitis C); carrier detection and antenatal diagnosis in haemophilias and hereditary anaemias
- Increased diagnostic precision, "positive purging," ex vivo gene delivery, and expansion of progenitor cells
- Allows gene therapy, tissue engineering, and study of gene expression and function
- Recombinant drugs (for example, the haemopoietic growth factors), antibody engineering, recombinant blood products

reaction, which can detect one malignant cell in a population of 10^5 cells, are a highly sensitive diagnostic and monitoring tool, and the full implications of detection of minimal residual disease at various times during the treatment of leukaemia are not yet known. Assays based on polymerase chain reaction will also be used in conjunction with powerful immunophenotyping methods (that is, monoclonal antibodies capable of detecting specific cell surface molecules on malignant cells) to follow the fates of individual neoplastic subpopulations in response to different types of treatment.

In the treatment of acute leukaemia dose intensification with stem cell support seems to confer some benefit for patients—but at the expense of increased toxicity, so that overall survival seems not to have been improved by high dose strategies with autologous transplantation. So far, the use of blood rather than bone marrow as a source of haemopoietic stem cells, despite its advantages of faster haemopoietic recovery and economy, has not influenced treatment related mortality. It is possible that new haemopoietic growth factors, such as thrombopoietin, may speed engraftment sufficiently to have a beneficial effect. Although the freezer provides chemoprotection for haemopoietic stem cells, other normal tissues cannot be protected in this way, and methods for reducing extramedullary toxicity are likely to be developed.

The delineation of molecular defects should finally allow a more subtle approach towards the treatment of leukaemia. For example, it is already known that acute myeloid leukaemias associated with specific chromosomal rearrangements—such as inversion of chromosome 16, inv(16), or translocation of DNA between chromosomes 8 and 21, t(8;21)—are associated with a good prognosis and that their respective chromosomal translocations involve the opposite halves of a single heterodimeric transcription factor. Studies on the t(15;17) translocation in acute promyelocytic leukaemia pointed to deregulated expression of the translocated retinoic acid receptor gene as a retrospective explanation for the efficacy of retinoic acid in this disease, and new drugs directed at therapeutic targets such as the heterodimeric transcription factor described above are now likely to be developed.

Gene therapy strategies are also possible for leukaemia. Already the transfer of drug resistance genes as chemoprotection for normal haemopoietic stem cells during chemotherapy is being investigated clinically. Many other strategies are possible, such as the targeted delivery of cytotoxic proteins and the use of adoptive immunotherapy with genetically modified T cells.

Glossary

General molecular biology

- **Recombinant DNA**—Any DNA sequence that does not occur naturally but is formed by joining DNA segments from different sources
- **Polymerase chain reaction**—Process by which genes or gene segments can be rapidly, conveniently, and accurately copied, producing up to 10^{12} copies of the original sequence in a few hours
- **Reverse transcription**—Process by which RNA is used as a template for the production of a DNA copy
- **Transcription factor**—Protein that is able to bind to chromosomal DNA close to a gene and thereby regulates the expression of the gene

Haematology and immunology

- **Stem cell**—Pluripotent cell that has the ability to renew itself or differentiate. The haemopoietic stem cell gives rise to all lineages of haemopoietic cells
- **Minimal residual disease**—Cancer that is still present in the body after treatment but remains undetectable by conventional means—for example, light microscopy
- **Immunophenotype**—The cell surface markers on any given cell detected by the use of monoclonal antibodies
- **Chromosome translocations**—Areas of chromosomes may break and rejoin with other chromosomes (for example, t(8;21), where an area of chromosome 8 is abnormally fused to an area of chromosome 21) or break off and rejoin "back to front" (for example, inv16)
- **Fas ligand**—The ligand that binds to the cell surface molecule Fas (CD95) which is normally found on the surface of lymphocytes. When Fas ligand binds to its receptor, cell death (apoptosis) is triggered
- **Apoptosis**—Programmed cell death
- **Adoptive immunotherapy**—The transfer of immune cells for therapeutic benefit
- **Transgenic animals**—Animals with an intact or manipulated gene inserted into their germline

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