Molecular genetic approaches to understanding disease

Clinical review

Fortnightly review

Prophylaxis of venous thromboembolism

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Summary points

- Deep vein thrombosis is difficult to diagnose clinically and its frequency has therefore been underestimated
- The only effective treatment is prevention, and prophylaxis should be offered to all patients at risk
- Antithrombotic drugs are not appropriate for low risk patients; prevention should focus on elasticated stockings
- In medium risk patients mechanical methods should be combined with drug treatment
- In high risk patients the most effective drugs are low molecular weight heparins

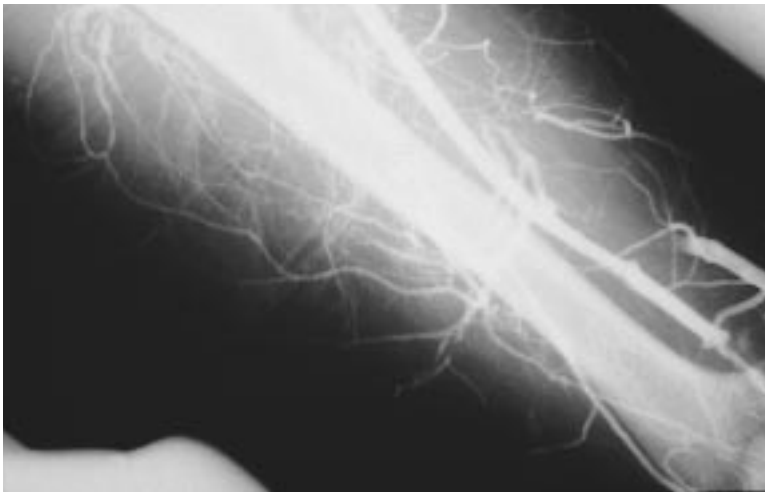
The incidence of deep vein thrombosis has for years been underrated because it is difficult to diagnose accurately by clinical history and physical examination. Minimal leg symptoms may be associated with extensive venous thrombosis whereas classic symptoms and signs of pain, tenderness, and swelling of the leg can be caused by non-thrombotic disorders. In patients without symptoms and signs of deep vein thrombosis, the diagnosis is most often revealed by a pulmonary embolism; indeed, less than 20% of patients with proved pulmonary embolism have clinical features compatible with venous thrombosis in the legs.[1] Thromboembolism in lower venous systems usually occurs as a complication of major surgery or of a serious illness. Without antithrombotic prophylaxis objective diagnostic measures have shown that 8-15% of patients develop venous thrombosis after major general (including abdominal) surgery, 36-60% after surgery for hip fracture, 47-57% after total hip replacement, and 40-80% after total knee replacement.[2]

The frequency of deep vein thrombosis is less well documented in medical patients. A recent study using colour Doppler imaging detected an unexpectedly high rate of deep vein thrombosis of 33% in patients in an intensive medical care unit; 48% of these cases were proximal leg thromboses.[3] The prevalence of deep vein thrombosis will probably increase in future because the average age of the population and the number of cancer patients is increasing, high age is becoming a lesser contraindication for major surgery, and many surgical patients, young and old, are being discharged from hospital before they are fully ambulant.

Thrombosis of deep leg veins is serious because proximal vein thrombosis is the cause of fatal pulmonary embolism in about 2 in 1000 postoperative patients each year. Most patients who die from pulmonary embolism do so within 30 minutes of the acute event, often too soon to make a correct diagnosis (because of the non-specificity of symptoms and signs) or start treatment. The only way to prevent fatal and non-fatal pulmonary embolism effectively is to prevent proximal vein thrombosis, particularly in patients in hospital, where the risk of fatal pulmonary embolism can increase up to 5%, for instance in patients at high thrombotic risk.[2] Deep vein thrombosis is also important because it can cause serious morbidity from chronic venous insufficiency. About 50-60% of patients with symptomatic proximal vein thrombosis and 30% with symptomatic calf vein thrombosis develop the post-phlebitic syndrome five to seven years later. As there is no satisfactory treatment for this late complication, prevention is the only approach medicine can offer.

Methods

This review of prophylaxis against deep vein thromboembolism is largely based on guidelines published by the Scottish Intercollegiate Guidelines Network and the Royal College of Physicians in Edinburgh.[4] Other recent reviews were published by the British Society of Haematology,[5] the THRIFT consensus group,[6] the European consensus group,[7] Pineo



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Venogram of deep vein embolism

Box 1—Risk factors for venous thromboembolism[5 7]

Background factors
- Age >40 years
- Severe obesity
- Immobility (bed rest >4 days)
- Pregnancy
- Puerperium
- High dose oestrogens
- Previous deep vein thrombosis or pulmonary embolism
- Thrombophilia:
  - Deficiency of antithrombin III, protein C, protein S
  - Activated protein C resistance
  - Phospholipid antibody or lupus anticoagulant
- Homocystinaemia

Disease or surgical procedure
- Trauma or surgery, especially of pelvis, hip, and lower limb
- Malignancy, especially pelvic or abdominal metastatic
- Heart failure
- Recent myocardial infarction
- Paralysis of lower limb(s)
- Severe infection
- Inflammatory bowel disease
- Nephrotic syndrome
- Polycythaemia
- Paraproteinaemia
- Behçet's disease
- Paroxysmal nocturnal haemoglobinuria

and Hull,[8] and Clagett *et al*.[2] Throughout the review I have used the definition of the type of evidence and the grading of recommendations described by Cook *et al*.[9]

Effective prevention

There are two strategies for effective prevention. Firstly, to institute primary prevention in all patients with a medium or high risk for deep vein thrombosis, and, secondly, to opt for secondary prevention by screening patients with a medium or a high probability of deep vein thrombosis with objective diagnostic tests. The second approach is expensive, time consuming, and can be applied to only a limited number of patients. Moreover, impedance plethysmography and duplex ultrasonography were shown to have only moderate sensitivity and positive predictive value in asymptomatic high risk patients.[10] Broad application of effective methods of prevention was shown to be more cost effective than selective, intensive surveillance.[11]

Several risk factors for deep vein thrombosis have been identified (box 1), and these can be used to distinguish groups of hospital patients with low, moderate, or high thrombotic risk (box 2).

Hospital patients at low risk

The general consensus is that patients at low thrombotic risk should not be exposed to the hazard and costs of routine prophylaxis with presently available antithrombotic drugs. As well as early ambulation the patients should wear graduated compression stockings, although their effectiveness is not based on evidence from medical trials.

Box 2—Classification of risk of deep vein thrombosis and pulmonary embolism for hospital patients

- Low risk (proximal vein thrombosis 0.4%, fatal pulmonary embolism <0.2%):
  - Patients <40 years undergoing major surgery (>30 minutes) with no other risk factors
  - Patients undergoing minor surgery (>30 minutes) with no other risk factors
  - Patients with minor trauma or illness with no thrombophilia but history of deep vein thrombosis or previous pulmonary embolism
- Medium risk (proximal vein thrombosis 2-4%, fatal pulmonary embolism 0.2-0.5%):
  - Major general, urological, gynaecological, cardiothoracic, vascular, or neurological surgery in patients >40 years or with one or more other risk factors
  - Major acute medical illness such as myocardial infarction, heart failure, chest infection, cancer, or inflammatory bowel disease
  - Major trauma
  - Minor surgery, trauma, or illness in patients with previous deep vein thrombosis, pulmonary embolism, or thrombophilia
  - Plastercast immobilisation of the leg in patients with minor injury
- High risk (proximal vein thrombosis 10-20%, fatal pulmonary embolism 1-5%):
  - Fracture or major orthopaedic surgery of pelvis, hip, or leg
  - Major pelvic or abdominal surgery for cancer
  - Major surgery, trauma, or illness in patients with previous deep vein thrombosis, pulmonary embolism, or thrombophilia
  - Leg paralysis
  - Critical leg ischaemia or major leg amputation

Hospital patients at medium risk

Graduated elastic compression stockings and intermittent pneumatic compression devices are effective after surgery, but it is unknown whether they significantly reduce fatal pulmonary embolism. Their effectiveness has not been evaluated in medical patients. There is insufficient evidence that the simultaneous use of both mechanical methods further reduces the frequency of venous thrombosis in this risk group. Elastic stockings should not be used on patients with severe leg ischaemia. Mechanical methods should, in principle, be combined with antithrombotic drugs. These include subcutaneous low dose unfractionated heparin (5000 IU, 12 hourly, with 5000 IU two hours before surgery), subcutaneous low molecular weight heparin or heparinoid (doses as indicated in table 1), or adjusted dose warfarin (international normalised ratio 2.0-2.5).

The various brands of low molecular weight heparins have different molecular weight distribution profiles, specific activities (anti-Xa to anti-IIa activities), and rates of plasma clearance, and each brand should therefore be considered as distinct and the doses set accordingly.[12 13] Indeed, antithrombotic potencies and potential bleeding effects of different low molecular weight heparins cannot be extrapolated from one product to another on the basis of weights in mg.

Table 1 Recommended doses of commercial low molecular weight heparins in prevention of deep vein thrombosis

Generic name	General surgery and other conditions of moderate thrombotic risk (anti-Xa IU)	Orthopaedic surgery and other conditions of high thrombotic risk (anti-Xa IU)
Ardeparin	—	50/kg twice daily
Certoparin	3000 once daily subcutaneously	3000 once daily subcutaneously
Dalteparin	2500 once daily	5000 once daily subcutaneously
Enoxaparin	2000 (20 mg) once daily subcutaneously	4000 (40 mg) once daily or 3000 twice daily subcutaneously
Nadroparin calcium	3075 once daily subcutaneously	60/kg once daily subcutaneously
Parnaparin	3200 once daily subcutaneously	6400 once daily subcutaneously
Reviparin	2500 units once daily subcutaneously	5000 units once or twice daily subcutaneously
Tinzaparin	3500 once daily subcutaneously	50/kg once daily subcutaneously
Danaparoid	750 twice daily subcutaneously	750 twice daily subcutaneously

These prophylactic recommendations are based on evidence from randomised trials with low false positive and false negative errors (grade A) for surgical patients and those in heart failure and chest infection. The supportive evidence for other patients at medium thrombotic risk is less rigorous (grade C), being based only on non-randomised concurrent cohort studies.[9]

Deep vein thrombosis is common after plastercast immobilisation for traumatic injury. In a randomised study the incidence of phlebographic deep vein thrombosis was significantly less in patients treated with one daily subcutaneous injection of a low molecular weight heparin (0%) compared with the untreated control group (4.3%).[14]

When anticoagulants are contraindicated—for example, in patients with a suspected or documented intracranial bleeding or in those who have sustained cerebral or spinal trauma— prophylaxis is limited to graduated elastic compression stockings or intermittent pneumatic compression. These should be used continuously until the patient is ambulant.

Hospital patients at high risk

Grade A randomised controlled trials have shown three methods to be effective in patients having hip surgery: adjusted dose subcutaneous unfractionated heparin (dosing to the upper limit of the normal range of the activated partial thromboplastin time); subcutaneous low molecular weight heparin or heparinoid (table 1), and adjusted moderate dose warfarin (initial dose the evening before surgery, postoperative international normalised ratio 2.0-3.0). Only low molecular weight heparins, intermittent pneumatic compression ,and adjusted dose warfarin were shown to be effective after knee surgery. All of these methods can be combined with graduated elastic stockings.

Intravenous dextran, a branched polysaccharide with a molecular weight of 40 000 or 70 000, has also been shown to be effective after major orthopaedic surgery. However, the use of dextran is cumbersome, expensive, and associated with rare anaphylactic responses, and it is contraindicated in patients with renal insufficiency and limited cardiac reserve.

Eriksson *et al* showed that recombinant hirudin (desirudin), started preoperatively, was more effective than unfractionated heparin after total hip replacement,[15] and this drug is undergoing further clinical evaluation. Bivalirudin (hirulog) was also found in a dose finding study to be more effective than unfractionated heparin after hip surgery.[16] In patients who have had major hip or knee surgery low molecular weight heparins were significantly more effective than unfractionated heparin (5000 U subcutaneously two or three times a day).[17 18] Low molecular weight heparins were also more effective than adjusted dose unfractionated heparin,[19] oral anticoagulants,[20 21] dextran, [22 23] and aspirin in such patients.

Patients having major pelvic or abdominal surgery for malignancy and those having any major surgery who have thrombophilia or a history of deep vein thrombosis should be given the same prophylaxis as recommended for those having major orthopaedic surgery. Grade A trials have also shown subcutaneous low dose unfractionated heparin (5000 IU, 8-hourly) to be effective in these patients.

In patients who have had a stroke and other high risk medical patients low molecular weight heparin reduces the risk of venous thrombosis by 60-90%.[24-26] Low molecular weight heparins are also more effective than unfractionated heparin in preventing deep vein thrombosis in patients with spinal cord injury .[27]

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Science, medicine, and the future

Molecular genetic approaches to understanding disease

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This is the second in a three part series on how basic science is transforming medicine.

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Abstract

Molecular genetics has greatly increased the understanding of diseases in which there is a single gene defect such as cystic fibrosis. Discovering the gene responsible and its function not only helps determine the pathogenesis of the disease but also offers a possible treatment—gene therapy. Polygenic disorders such as diabetes may soon yield their secrets to the same approach. Animal models of genetic diseases are proving useful research tools, and transgenesis has made xenografting possible. Furthermore, antisense technology allows specific inhibition of undesirably overexpressed genes such as those driving unwanted vascular cell proliferation and restenosis after angioplasty. The completion of the human genome project should make the search for "disease" genes much quicker and will increase still further the importance of these gene based approaches toward diseases.

Introduction

If you think that splicing is a carpentry technique or that a knockout is the end of a politically incorrect sporting contest you should read on. Indeed, even if you are one of the many doctors who knows that it is now routine to identify genes and manipulate their expression, the hazier details may be clarified by this article. My aim is to set out important genetic approaches toward understanding disease and introduce how advances in biomedical science may affect medicine in the next 15 years or so. Genetic terms are explained in the glossary. In my next article I will cover cellular approaches. The objective of subsequent articles in this series will be to foresee how modern science will impinge on the prevention, diagnosis, and treatment of common disease processes such as coronary heart disease, hypertension, and lung cancer.

Molecular genetics

Modern molecular techniques have revolutionised attempts to understand the pathogenesis of single gene disorders such as cystic fibrosis. The discovery of abnormal alleles seems almost routine, and the potential rewards of "finding the gene" are great. In cystic fibrosis the transmembrane conductance regulator (CFTR) is inactivated by various mutations. Discovering the gene gave an insight into pathogenesis—it was quickly realised that CFTR is an epithelial cell surface chloride transporter, which explains known defects in ion transport across epithelia in patients with cystic fibrosis. Secondly, there was a ready made candidate treatment—transfer to diseased tissue of the complementary DNA (cDNA) of the normal CFTR gene which can then be used by the cells to produce normal protein (see below) and reconstitute chloride transport.

Molecular geneticists have made remarkable contributions to the understanding of single gene disorders. Some studies have taken the candidate gene approach—scientists make an educated guess that a well characterised allele lies at or close to a disease locus. More usually, investigators undertake painstaking linkage analysis of DNA specimens from affected families. They seek to associate carefully documented cases of disease with particular chromosomal "landmarks" and then aim to find the gene in a relatively short segment of DNA. This positional cloning approach has been greatly facilitated by a comprehensive map of microsatellites—regions of DNA that vary greatly in their sequence between individuals but which occupy identical chromosomal positions.

Once a gene is mapped to a particular section of DNA various strategies can be used to speed the hunt for the gene among the millions of nucleotide bases in non-coding "junk" DNA, which constitutes up to 90% of the human genome. For example, help may come from the growing map of expressed sequence tags, a library of partially sequenced cDNAs obtained from unselected mRNAs. Furthermore, large chunks of chromosomal DNA can be handily stored, manipulated, and screened by being incorporated into a library of yeast artificial chromosomes that contain overlapping segments of human DNA—so called contigs (fig 1). However, positional cloning may still be difficult. It took years to find *PKD1*, the gene responsible for most cases of autosomal dominant polycystic kidney disease, because it was hidden in a cluster of similar genes on chromosome 16. Such difficulties may be removed by the mapping of the human genome.

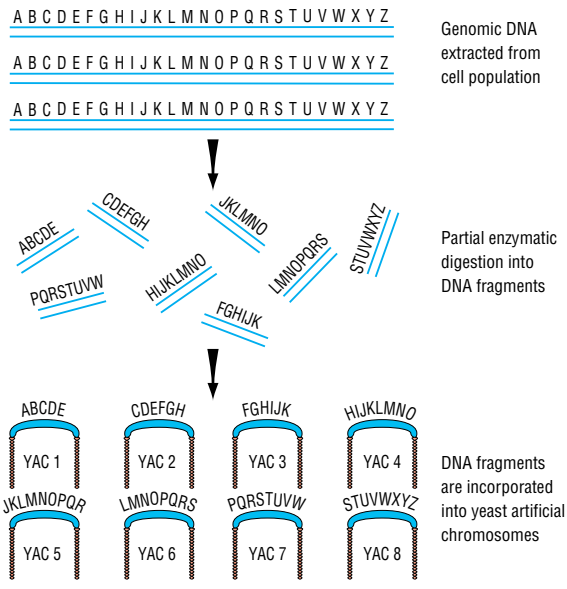

Fig 1 Assembling a contig of genomic DNA in a yeast artificial chromosome (YAC) library. Genes which map close to marker H may be found in YACs 2, 3, or 4

Bolstered by success with uncommon single gene disorders, molecular geneticists are turning to major causes of ill health and premature death such as coronary heart disease, hypertension, and diabetes mellitus. These disorders cluster in families but exhibit polygenic inheritance, expression of the disease phenotype apparently depending on the interaction of several genes, which may in turn interact with environmental factors. Genome screening is now feasible thanks to automated techniques and microsatellite markers, but such linkage studies require careful interpretation. One aim of linkage studies is to help determine pathogenesis by identifying gene products involved in disease. However, we will need a strong ethical framework with which to handle the knowledge that a particular gene is associated with increased risk of a serious disorder. Telling someone glibly that they have a fivefold increased risk of early death from coronary heart disease may not be ideal.

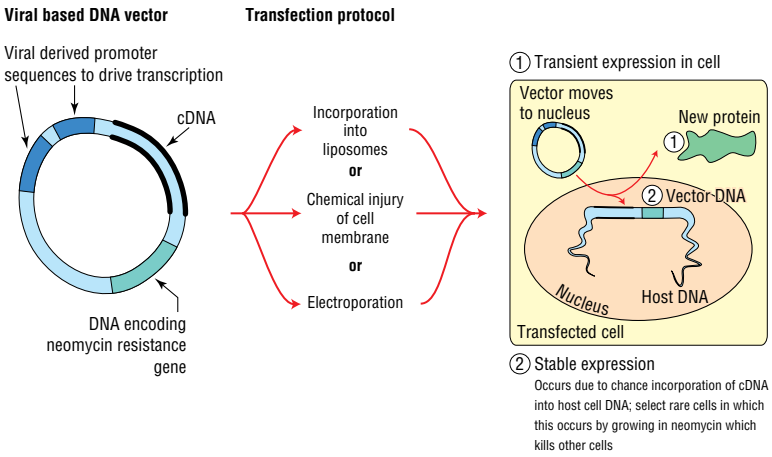

Fig 2 Principles of gene transfer. cDNA for a protein foreign to the host cell is incorporated into a DNA vector, which is then introduced or "transfected" into cells. The cDNA will be transiently expressed before being eliminated. However, occasionally vector DNA incorporates into cellular DNA, and these stably transfected cells can be selected in experimental settings

Gene transfer and gene therapy

Gene transfer is a simple and attractive concept (fig 2). All you need to do is take a piece of DNA that encodes for the desired protein and transfer it to a cell. The cell then transcribes the gene and translates the resulting mRNA to generate protein. It is easy to appreciate why gene transfer is causing so much excitement. Firstly, transferring human genes to simple organisms or cell lines allows production of large amounts of "therapeutic" proteins such as erythropoietin. Secondly, there is enthusiasm for using gene transfer to patients to treat disease—so called gene therapy. For example, it is hoped that normal epithelial function and defence against infection may be restored in the airways of patients with cystic fibrosis by transferring the normal CFTR gene.

However, behind the simple concept lies a large and incomplete body of knowledge. A functioning gene is not merely composed of the encoding DNA sequence (fig 3). Upstream of the site at which gene transcription is initiated there is a promoter region of DNA sequences that bind nuclear proteins called transcription factors. These factors regulate the initiation of transcription. Indeed successful elongation and processing of a gene transcript may require coordinated action of many pieces of genomic DNA which are not expressed in the final mRNA transcript. Therefore, although a cDNA can be "read" to generate a full length mRNA and protein, gene transfer will not work unless the cDNA is combined with elements designed to drive transcription. These are usually provided by incorporating the cDNA into a vector—a piece of DNA which is derived from a virus and which bears powerful promoter sequences which will bind host cell transcription factors and allow high level expression of the transferred cDNA.

Both the art and science of gene transfer revolve around which vectors to use and how to get them into the cells of interest. In gene therapy it is important that the vector must not cause adverse effects such as immune or inflammatory reactions, expression in the wrong tissue, or, worse, spread to other people.

Inhibiting gene expression may also be therapeutic

Techniques designed to transfer cDNA constructs into cells can also be exploited to interfere with gene expression by administering antisense oligonucleotides (fig 4). These consist of 20 or so nucleotides synthesised in a sequence which complements the mRNA of interest. The oligonucleotides bind the mRNA and prevent translation of the protein. Antisense oligonucleotides can be administered in vivo and have great promise in treating disorders of cell proliferation. For

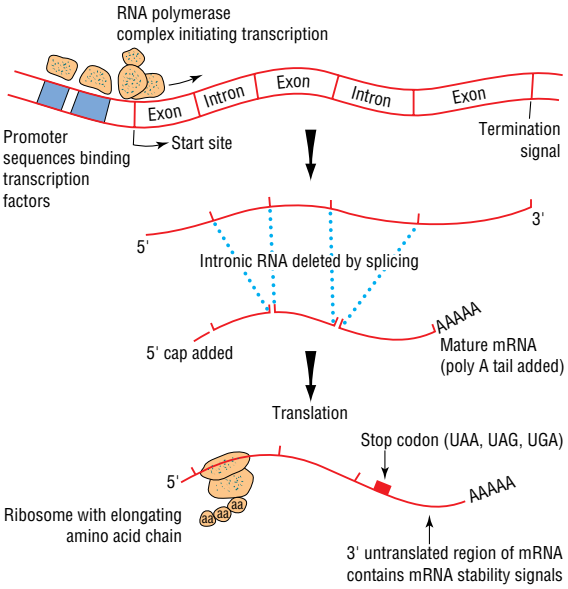

Fig 3 Control of gene expression. Transcription of DNA by a multiprotein complex with RNA polymerase activity is regulated by binding transcription factor proteins just upstream (or 5') of the open reading frame of the gene. RNA arising from introns is spliced out to yield mature mRNA which arises from the exons of the gene by means of the "cut and paste" process shown. The capacity of mRNA to be translated by ribosomes can be regulated by RNA sequences in the 3' untranslated region downstream of the trinucleotide stop codon which halts translation

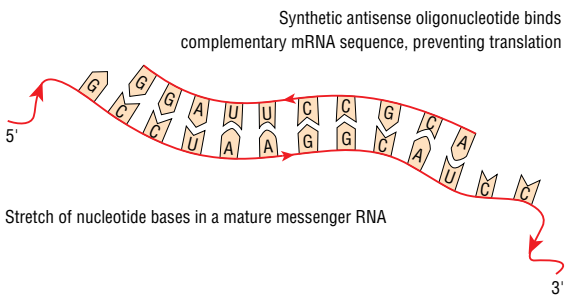

Fig 4 An antisense oligonucleotide prevents translation of an mRNA

example, the fibrocellular intimal hyperplasia underlying restenosis of atheromatous vessels after successful angioplasty can be blocked by antisense to the gene *c-myb*, which encodes a nuclear protein essential for cell division. However, because antisense strategies don't always work there is growing interest in using ribozymes, enzymes which specifically recognise and degrade particular mRNAs. Although antisense strategies might lack the glamour of gene therapy, the ability selectively to turn off gene expression may prove particularly important in medicine.

Knockout and transgenic technologies

Perhaps the best test of a gene function is provided by manipulating the genome of experimental animals. This is easiest in mice because scientists can now culture undifferentiated but pluripotential murine embryonic stem cells. Genetically altered embryonic stem cells can be microinjected into early embryos so that the manipulated cells contribute to all cell types of the resulting chimera, including germ line cells. Breeding these "founder" mice produces individuals carrying one copy of the altered gene and one normal copy (heterozygotes) or mice carrying two altered copies (homozygotes).

This technique has proved particularly useful in generating models of disease in which there is a genetic "loss of function." So called knockout mice are created by means of homologous recombination and targeted mutagenesis, techniques by which the gene of interest is inactivated by swapping the normal DNA for a similar DNA construct with a mutation. The DNA "plugged in" to the embryonic stem cell genome also contains a selection element such as a neomycin resistance gene so that cells bearing the desired mutation can be selected prior to injection into embryos because the knockout cells can propagate in otherwise toxic neomycin.

Knockout mice can also be used to model the consequences of somatic mutations which arise after birth and are believed to be particularly important in carcinogenesis. Mice deficient in the tumour suppressor gene *p53* in the germ line are models of human Li-Fraumeni syndrome, developing multiple tumours because of defects in deletion of cells in which ionising radiation causes somatic mutations which activate tumour promoting genes. Indeed, somatic mutations inactivating *p53* contribute to the pathogenesis of about a third of tumours. It will soon be possible to model such mutations by exploiting new techniques that allow you to switch on gene inactivation in particular cell lineages at will.

Transgenic technology, in which a normal gene is overexpressed for investigative purposes, preceded embryonic stem cell techniques and can be used in animals other than mice. However, transgenesis without embryonic stem cells is much more hit and miss because it relies on chance incorporation of DNA injected into early embryos. Nevertheless, the technique works, and the most dramatic practical application of transgenesis may prove to be in xenografts—transplants from animals to man. Normally, pig kidneys are rejected rapidly by primates because the recipient has high titres of antibodies which react with the glycoproteins on the surface of pig cells, especially endothelial cells lining the vasculature of the graft. Antibody

Glossary

Allele—One of two or more alternative forms of a gene which occupy the same locus (position) on a particular chromosome. Homozygotes bear identical alleles at two corresponding loci on a pair of chromosomes; heterozygotes have two different alleles.

Complementary DNA (cDNA)—A DNA copy made from a messenger RNA template which is transcribed in a "finish to start" orientation by a reverse transcriptase enzyme. When the cDNA is transcribed in the conventional orientation by an ordinary RNA polymerase enzyme a copy of the parent mRNA is produced. Consequently, cDNAs are valuable when one wants to mimic endogenous expression of a gene.

DNA construct—An engineered piece of DNA, usually designed for incorporation into a viral based vector for expression in other cells. For example, a construct might incorporate a deliberately mutated cDNA such that an abnormal form of the corresponding protein is ultimately produced.

Homologous recombination—Exchange of chromosomal DNA, by recombination or "crossing over" for another stretch of DNA which is homologous or similar. However, in gene manipulation experiments the replacement DNA has been subtly altered to affect the properties of the transcribed/translated product.

Positional cloning—Aims to identify the locus and the gene responsible for a particular disorder/protein relative to mapped markers, usually microsatellites. This is determined by linkage analysis which assesses the frequency with which a particular marker cosegregates with the disorder/protein in families. Because there may be crossing over of DNA between a pair of chromosomes the further a gene is from a marker the lower the chance of cosegregation. Cloning involves sequencing the gene in its entirety, usually in overlapping segments.

Targeted mutagenesis—A powerful technique in which particular bases in DNA are deliberately mutated to alter the properties of the mRNA resulting from DNA transcription by RNA polymerase. In turn this can alter the amino acids in the encoded protein, truncate the protein, or prevent protein expression altogether.

Transcription factors—Proteins which bind "promoter" regions of DNA upstream of the sequences which encode mRNA, thereby leading to assembly of an RNA polymerase and transcription of the gene.

Yeast artificial chromosomes (YACs)—Can accommodate long stretches of foreign DNA and act as storage vessels which can be propagated in yeast cells in culture.

fixation leads to complement activation, endothelial injury, thrombosis, and graft loss—so called hyperacute rejection. However, these phenomena are suppressed if the transplanted pig tissue overexpresses human cell surface proteins that regulate the activation of the complement system. Therefore pigs transgenic for complement regulatory proteins could be used as a source of organs for human transplantation. Nevertheless, apart from ethical and safety considerations, xenografts may prove susceptible to long term immunological injury by T cells rather than complement.

Molecular microbiology

Lastly, genetic approaches are proving important in infectious and parasitic diseases. By applying modern molecular genetic techniques to the study of microbial pathogenicity and to host resistance susceptibility, new insights are being gained into treatment and prevention, by vaccination and other strategies. For example, the particularly challenging problem of severe malaria due to *Plasmodium falciparum*, which resists both treatment and attempts at prevention by vaccination, is being addressed by a two pronged genetic approach. Firstly, a yeast artificial chromosome and expressed sequence tag map (see above) of the *P falciparum* genome is being constructed to speed up molecular analyses of potential targets in the parasite for treatment and vaccines. Secondly, attempts are being made to identify human genes which determine deleterious or advantageous responses to infection. Together these approaches could yield new vaccine candidates and new treatments.

Conclusions

The potential power of genetic approaches to disease is already great. My next article will deal with the rapidly evolving discipline of molecular cell biology. The tools described above are being used to investigate many different disorders. Furthermore, the importance of genetic approaches may redouble with the anticipated success of the human genome project. Watch this space.

Lesson of the week

Man's best friend: life threatening sepsis after minor dog bite

D J Mellor, Sunil Bhandari, K Kerr, A R Bodenham

Minor bites from domestic pets are common, usually causing no more than a superficial lesion. In an American series, however,[1] animal bites were the presenting complaint in about 1% of all referrals to emergency rooms. Although severe bite injuries usually present early, for management of soft tissue injury as well as prophylaxis against tetanus and rabies infection, apparently trivial injuries may present later due to insidiously developing, local or systemic infection. Catastrophic sepsis following late presentation after apparently trivial animal bites has been described before.[2,3] We describe a case of cardiovascular collapse, purpura fulminans, and acute renal failure in response to infection with capnocytophaga after an apparently trivial bite from a dog.

See editorial by Moore

Every animal bite should be regarded as potentially dangerous and thoroughly cleaned and treated with appropriate prophylaxis

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continued over

Case report

A 55 year old man who had been in good health was admitted to hospital with a 36 hour history of fever, rash, diarrhoea, and vomiting. Four days earlier he had been bitten by his pet dog. This hand wound apparently required no medical attention and did not look infected at any time. The history of his bite became apparent only retrospectively four days after admission, when the results of blood culture became available.

On admission he had an oral temperature of 39°C. Examination showed a widespread purpuric rash and poor peripheral perfusion. His blood pressure was 90/40 mm Hg and his pulse rate was 130 beats/minute. He was confused. Arterial blood gas concentrations showed a compensated metabolic acidosis with a pH of 7.38, a partial pressure of carbon of 2.6 kPa, a partial pressure of oxygen of 10.1 kPa, and a bicarbonate concentration of 11 mmol/l while he was breathing room air. His haemoglobin concentration was 131 g/l with a white cell count of 6.7×10^9/l. In support of the clinical signs, a haematological profile confirmed a picture of disseminated intravascular coagulation with a platelet count of 9×10^9/l and a prothrombin time of 36 s (normal 13 s), reduced fibrinogen concentration (0.002 g/l), and increased concentrations of fibrin degradation products (>0.016 g/l). The combination of purpuric rash and multiple organ system involvement in an acute septic process led to the provisional diagnosis of meningococcal septicaemia. After a full sepsis screen, including blood cultures, he received 1 g of intravenous cefotaxime.

Despite initial treatment his condition continued to deteriorate, and he required intensive care and mechanical ventilation owing to deteriorating gas exchange. Haemofiltration and haemodialysis were needed for oliguric acute renal failure (creatinine concentration 653 μmol/l, urea concentration 26 mmol/l), and he received inotropic support (adrenaline and dopexamine) for his circulation. His disseminated intravascular coagulation was supported with transfusions of platelets, fresh frozen plasma, and cryoprecipitate, as required. Benzyl penicillin and metronidazole were added to the cefotaxime antibiotic regimen.

Four days after admission, a Gram negative bacillus was detected in his initial blood cultures. This was later

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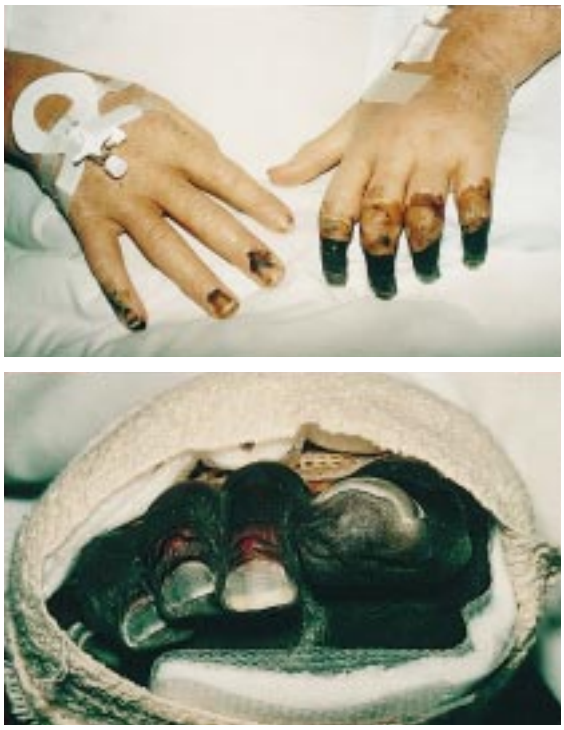

Fig 1 Peripheral ischaemic gangrene secondary to capnocytophaga septicaemia

shown to be *Capnocytophaga canimorsus*. He was discharged from the intensive care unit one month later. His (presumed) acute tubular necrosis had completely resolved. He underwent considerable peripheral amputation owing to ischaemic gangrene (fig 1), but subsequent reconstructive surgery to his feet allowed him to maintain independent mobility, and he was in good health one year later.

Discussion

Capnocytophaga spp are commensal bacteria in the mouths of dogs and cats.[2] Infection has been reported after bites from cats and dogs, but such catastrophic infection in previously healthy people is rare. Case reports suggest a higher risk in patients immunocompromised by haematological malignancy,[4] splenectomy,[5] or cirrhosis.[6]

Capnocytophaga spp are fastidious, capnophilic Gram negative rods. Growth in blood cultures is slow and a rapid provisional diagnosis may be made by Gram staining buffy coat preparations or material obtained from petechial lesions. Of the different species of *Capnocytophaga*, *C canimorsus* (formerly Centre for Disease Classification Dysgonic Fermentor-2 (CDC DF-2)) is the most sinister, with a high mortality. Like other common animal and human oral commensals responsible for infection from bites, *Pasteurella* spp, *Staphylococcus aureus*, and anaerobic organisms, *Capnocytophaga* spp are susceptible to co-amoxiclav as well as β-lactam antibiotics, erythromycin, chloramphenicol, and tetracycline.[7]

The management of bite injuries has been reviewed and the role of prophylactic antibiotics questioned.[8]

Our case suggests that no animal bite can be regarded as innocuous prospectively. The true cause of our patients' illnesses became clear only after capnocytophaga had been isolated from blood cultures, outlining the importance of taking an adequate history in these circumstances. The case shows the need for rigorous wound toilet and appropriate antibiotic prophylaxis to all animal bites regardless of extent.

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Comparing treatments

As the enuresis clinic wore on the avuncular paediatrician gave a reasoned opinion of its value to the two attendant medical students. He considered the changing fashions of his specialty and was equanimous that none of his colleagues would continue the clinic after his impending retirement.

A mother brought in her two sons. The younger was returning his enuresis alarm. Now dry at night, the boy demonstrated his star chart, complete in its latter stages. The alarm was redundant, the management strategy successful, and both mother and child were grateful. The lesson to the medical students was reinforced with cautionary examples of the adverse effects of tricyclic antidepressant drugs when prescribed to treat nocturnal enuresis, and their mortality in accidental overdose.

As the family rose to leave the paediatrician asked if the elder boy had suffered from enuresis. "Much worse than his brother" was the reply. "Did he use the alarm too?" asked the paediatrician. "Yes, we tried everything. Then one morning he wet the sheets when I'd no clean linen, it was pouring rain, and I was exhausted with the baby. I burst into tears, and before I knew it, I'd put him across my knee and given him a right good hiding. He never wet the bed again."

One condition and three treatments. The alarm may be overtaken by the natural history of the condition, the drugs are potentially lethal and the anecdotal report of the mother's effective treatment is difficult to reproduce and impossible to advocate. How much else in modern clinical practice is valiant but misguided, useless or harmful? What clinical condition does not have current treatments that are ineffective, dangerous, or both? Useful variables to compare treatments are difficult to find. We often measure what is easy to measure. Blood pressure and pulse are recorded when we would rather know cardiac output and tissue perfusion.

For enuresis, soaked sheets and pyjama trousers are obvious criteria to observe. Our 3 year old daughter is indignant when we confuse bed wetting with what she insists is unrelated and profuse sweating. When comparing treatments, if your seemingly suitable endpoint, easy to define and detect, is discovered to have multiple causes you are far from home and dry.

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